biomnis
SPECIALISED MEDICAL PATHOLOGY

Guide To Medical Pathology Testing

Specialised Pathology Tests

Translated from French

Biomnis

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SPECIALISED MEDICAL PATHOLOGY

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Specialised pathology holds a prestigious place in the continual advancement of medicine. It has contributed enormously to the development of medicine and the numbers of complex specialised tests are constantly increasing.

Today's pathologists are forever faced with not only the imperious necessity of exact measurements, which in itself imposes a knowledge of the sensitivity and the specificity of the methods used, but also the knowledge of their speciality along with the pathological result interpretation. This involves managing current data from both the physiological and medical pathological aspects.

The challenge undertaken by this book is to assemble more than 500 specialised pathology test forms in a clear and concise manner, with each one having the same layout. The layout will be comprised of definitions and synonyms, a reminder of the biological and pathological aspects of the test as well as the most appropriate clinical indications ("justified request") and the pre-analytical recommendations (whereby the non-compliance could put the quality of results and the usual values into question). Finally, each test form analyses the physiological and pathological variations and assists in the interpretation of results.

Written by specialists, who work with these tests on a daily basis, and using the feedback from their fellow clinicians, we would like to see this book become the best information tool available in pathology. Its objective is to update knowledge, to inform and to help in not only selecting test requests but also the diagnosis and monitoring of treatments. It will also therefore participate in modern medicine based on the consensus of experts.

We wanted its use to be simple, by filing the forms in alphabetical order. The majority of titles correspond to the title of the specialised tests while others are filed in relation to the assays.

A very detailed index at the end of this book allows items to be easily located.

We hope that this publication will provide precious help to the reader in his or her routine practice within the realms of specialised pathology or of medicine on a larger scale.

Professor Meyer Michel Samama

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CONTENTS

ACETONE

DEFINITION

Acetone is a colourless, highly volatile liquid which is miscible with water. It is in widespread use as a solvent in various industrial applications, notably in the paint, ink, varnish and glue industries.

Synonyms: dimethylketone - 2-propanone.

INTRODUCTION

Acetone is efficiently absorbed via the lungs (40-55% of the inhaled vapour being absorbed) but inefficiently through the skin. About 75% of the absorbed acetone is converted to 1-2 propanediol, formic acid and 2-propanol.

	Fate of acetone after absorption	Clearance half-life after absorption
Metabolised	75%	5 hours in the blood
Eliminated via the lungs	20%	16 hours
Eliminated in the urine	5%	3.5 hours

The main symptoms of acute and chronic acetone poisoning are local (irritation of the eyes, airways or skin), neurological (headache, lassitude, dizziness) and gastrointestinal (nausea and vomiting). With repeated or prolonged exposure, acetone depletes the skin of its lipid content, causing redness, peeling and cracks. Haematological problems have been reported, including increases in the counts of polymorphonuclear cells and erythrocytes. Acetone potentiates the toxicity of ethanol and chlorinated hydrocarbons.

INDICATIONS FOR MEASUREMENT

– Monitoring occupational exposure: the concentration of acetone in the blood correlates closely with the concentration of acetone in the air expired so this test gives a reliable measure of the amount of acetone in the body.

Acetone in the urine is the parameter that correlates best with the atmospheric concentration for a given work load. Assaying this parameter before and after a day's work, measures the extent of the worker's exposure that day.

Acetone may be detected in the urine after exposure to isopropyl alcohol.

– Decompensation in diabetes (with the risk of acidosis and coma): the body spontaneously generates acetone from acetoacetate which, together with beta-hydroxybutyrate, makes up ketone bodies. Large quantities of ketone bodies are produced when sugar cannot be consumed by cells and the body compensates by using lipid reserves as an energy source. The acetonuria can be measured using a reactive strip at the same time as testing for glycosuria.

INFORMATION

SAMPLE

– Whole blood sample with EDTA or heparin, at the end of the working day.

- Urine sample: Fill a plastic flask right up. Acetone is extremely volatile and it is important to minimise loss through evaporation and adsorption onto the vessel walls. Above all, the patient should not be fasting. Samples should be taken both before and after the working day.

QUESTIONS FOR THE PATIENT

Endogenous acetone production?

The amount of acetone in the urine correlates with the rate of fatty acid oxidation. It is important to check that none of the following pertain:

– fasting, alcohol consumption, repeated vomiting,

– prolonged exercise, exposure to low temperatures,

– diabetes mellitus.

Other factors that can affect the acetone concentration in the sample?

Ask the subject if he or she might have been exposed to isopropyl alcohol (the breakdown of which gives rise to acetone).

Smoking should be avoided because every cigarette contains 0.54 milligrams of acetone.

SAMPLE STORAGE AND TRANSPORT

Samples should be stored at a temperature of below 8 °C to cut down evaporation.

ASSAY METHODS

The assay is based on gas phase chromatography with flame ionisation detection, or coupled to mass spectrometry.

NORMAL EXPECTED VALUES

– Blood: in the general population: ≤ 2.3 mg/l

– Urine: in the general population: ≤ 2.3 mg/l.

PATHOPHYSIOLOGICAL VARIATIONS

At the end of the working day, in the urine of exposed subjects:

– Exposure Index (ACGIH): 50 mg/l

– German reference threshold (BAT): 80 mg/l

– Quebec reference threshold (IRSST): 100 mg/l.

FOR FURTHER INFORMATION

- INRS, *Dosage de l'acétone urinaire*; www.inrs.fr/htm/nature_du_dosage_acetone_urinaire.ht ml
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- Lacouture P.G., Heldreth D.D. et al., *The generation of acetonemia /acetonuria following ingestion of a subtoxic dose of isopropyl alcohol*, Am J Emerg Med., 1989; 7: 38-40.

(ACETYL) SALICYLIC ACID

DEFINITION

Salicylates are compounds derived from benzoic acid that were originally isolated from the bark of the willow tree. A number of derivatives are known, the main one being acetylsalicylic acid. The compound is mainly used for its analgesic, antipyretic and anti-inflammatory activities (at dosages of over 3 g/day), and to inhibit platelet aggregation (at a lower dosage).

Synonym: Aspirin.

METABOLISM

The compound activity sets in about 30 minutes after oral administration (tablets) and its concentration peaks in the blood after around two hours. About 80-90% of absorbed acetylsalicylic acid binds plasma protein. It is converted into salicylic acid within 15 minutes. About 10-30% is excreted in the urine as the parent compound, and the rest is conjugated in the liver with either glycine (generating salicyluric acid) or with glucuronic acid. Its half-life depends on the dose administered: it is usually about two hours but may rise to 40 hours after a very high dose. The clearance rate of acetylsalicylic acid and its metabolites is dependent on the pH of the urine, being four times faster at pH 8 than at pH 6.

INDICATIONS FOR MEASUREMENT

This test is sometimes ordered for therapeutic monitoring if there is evidence of toxicity. Too high a dose of salicylic acid can be extremely toxic, especially in babies and children. Severe poisoning may result with doses of 10 grams in adults and 100 milligrams per kilogram body weight in children.

An emergency assay of salicylic acid in the serum is usually carried out if acute poisoning has occurred or is suspected. Signs of such poisoning are:

– Central hyperventilation with sweet-smelling fruity acetone breath and, then, respiratory depression.

– Respiratory alkalosis followed by metabolic acidosis.

– Hyperthermia.

– Gastrointestinal problems (nausea and vomiting).

– Bronchospasm.

The interval between administration and the onset of symptoms varies between minutes and hours according to the pharmaceutical presentation.

INFORMATION

SAMPLE

– Serum, heparinised plasma or gastric fluid.

– For therapeutic monitoring, prepare a sample one hour after aspirin administration.

For poisoning, prepare the sample as soon as symptoms appear.

QUESTIONS FOR THE PATIENT

– Any request for a drug assay should include the following information:

The reason the drug was prescribed (for efficacy or toxicity evaluations); the time at which the test sample was taken; the date of the beginning of the course of treatment and/or dates on which the dosage was changed; dosing details (dose, frequency, route of administration) and whenever possible, the patients age, height and weight.

SAMPLE STORAGE AND TRANSPORT

Can be kept for 8 hours at room temperature or for 48 hours at +4°C (beyond that, store frozen at –20°C).

Transport at +4 °C or frozen.

ASSAY METHODS

The most commonly used assays are:

– Chemical: Trinder's test

– Immunological: FPIA

– Enzymatic: Salicylate mono-oxygenase.

NORMAL EXPECTED VALUES

Therapeutic windows in adults:

– Analgesic: 30-80 µg/ml

– Anti-inflammatory: 150-300 µg/ml

– Toxicity > 300 µg/ml.

– Conversion factor: 1 µg/ml (salicylic acid) = 138 mmol/l.

Symptoms are usually severe at blood salicylate concentrations of over 500 µg/ml. Above 900 µg/ml, dialysis should be considered.

In acute poisoning, salicylic acid should be assayed every 1-4 hours until the concentration has returned to normal. The results of serum salicylic acid tests should be interpreted in the light of the clinical picture as well as the interval between the time of administration and when the sample was taken.

FOR FURTHER INFORMATION

■ Manchon M., *Dosage des médicaments*, tome II, Cahier BIOFORMA N° 18, Paris, janvier 2000 ; chapitre aspirine: 22-29.

■ Dictionnaire Vidal®.

ACTH

DEFINITION

A peptide hormone of 4500 Da, secreted by the cells of the anterior pituitary from a precursor glycoprotein: pro-opiomelanocortin (POMC).

Synonyms: adrenocorticotropic hormone, corticotrophin, corticostimulin, corticotropic hormone.

INTRODUCTION

ACTH stimulates the biosynthesis and release of steroid hormones (cortisol, aldosterone, etc.) by the adrenal cortex. The regulation of ACTH secretion involves CRH (corticoliberin, a stimulant) and cortisol (negative feedback).

INDICATIONS FOR MEASUREMENT

An ACTH measurement helps in determining the cause of "hyper or "hypo" cortisolaemia (the ACTH and cortisol measurements must always be combined). Investigations other than basal measurement are sometimes required: these are dynamic tests (inhibition or stimulation) and medical imaging (scans, MRI or ultrasound).

INFORMATION

SAMPLE

Sampling in EDTA: Sample on a tube cooled to +4° C then centrifuge at +4° C immediately, separate and freeze the plasma immediately;

or

Sampling in EDTA + aprotinine: Centrifuge at +4° C and freeze the plasma.

Sample taken at 8 a.m. (± one hour) from a non-stressed subject.

ACTH is thermolabile and may be degraded by plasma proteases; numerous factors may increase its concentration, including pain, fever and stress.

QUESTIONS FOR THE PATIENT

Current treatment: Are you taking any glucocorticoids, etc?

SAMPLE STORAGE AND TRANSPORT

If analysis is deferred, freeze the plasma to -20° C without delay. The sample with then be stable for 2 months.

ASSAY METHODS

Immunometric sandwich assay method.

Basal measurement is sometimes completed by dynamic tests.

– Inhibition: Dexamethasone.
– Stimulation: Metopirone (inhibition of the 11β hydroxylase), CRH

NORMAL EXPECTED VALUES

Values may be expressed in ng/l or pg/ml or pmol/l (1 pmole = 4.5 ng). The normal values may vary according to the reagents used.

Indicative values: at 0800h: < 50 ng/l – at 1600h: < 23 ng/l, due to the existence of a circadian cycle in ACTH secretion, with a minimum towards midnight and a maximum between 6 and 8 a.m.

PATHOLOGICAL VARIATIONS

To be interpreted taking into consideration the values obtained for the cortisol levels.

HYPERCORTISOLEMIAS

a) Cushing's syndrome

– Cushing's disease (pituitary adenoma).
With normal or elevated ACTH and significantly elevated cortisol levels.
– Paraneoplasic syndromes (ectopic production of an ACTH-like substance by a tumour, often pulmonary). With very elevated ACTH and cortisol.

b) Adrenal tumour

Cortisol levels elevated significantly; ACTH depressed.

HYPOCORTISOLEMIAS

a) Addison's disease (primary adrenal insufficiency)

Cortisol depressed with ACTH highly elevated.

b) Secondary adrenal insufficiency (linked to deficiency of the pituitary gland or the hypothalamus)

Cortisol depressed with ACTH depressed (value of the CRH test).

FOR FURTHER INFORMATION

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ADENOVIRUS

DEFINITION

Human adenoviruses (HAdV) belong to the Adenoviridae family and the Mastadenovirus genus. They are non-enveloped, icosahedral-capsid DNA viruses with sizes ranging from 80 110 nm. The genome includes variable (E) genes and conserved (hexon) genes. There are approximately 50 different serotypes, classified in 7 species (A, B1, B2, C, D, E and F) according to their biological and structural characteristics. Each serotype or species possesses particular pathogenic properties: AdV serotypes 1, 2, 3, 5 and 7 are responsible for respiratory infections; serotypes 40 and 41 cause infantile gastroenteritis and serotypes 8, 19 and 37 produce ocular infections.

Adenoviruses cause benign pathologies in the majority of cases, although they may be of a serious nature in immunosuppressed subjects.

Human adenoviruses

A	12,18,31	- Intestinal infections in children
B	3,7,16 14,21 11,34,35	- Pharyngitis and pneumonia in children and young adults. - Respiratory Infections - Renal infections
C	1,2,5,6	50% of AdV infections - Respiratory and lymphoid tissue infections
D	8,9,37,10,13,15,17,19,20,22c, 30,32,33,36,38,39, 42c,47	- Keratoconjunctivitis - Asymptomatic infections
E	4	- Rare respiratory infections
F	40,41	- Infantile enteritis

according to F. Freymuth, Adénovirus. EMC.

INTRODUCTION

EPIDEMIOLOGY

AdV infections are very common in children during winter and spring and they are endemic for serotypes 1, 2, 5 and 6, and epidemic in the case of serotypes 3, 4, 7, 14 and 21. Half of these infections are asymptomatic, with the remainder comprising of respiratory, ocular and enteric infections. Transmission is faeco-oral for viruses of intestinal tropism, or by direct or indirect human-to-human contact in the case of viruses which cause respiratory infections. There are also very specific modes of transmission, such as swimming pool water in cases of conjunctivitis, as well as very rare cases of transmission by ophthalmological instruments. The virus penetrates via the pharynx or conjunctiva and travels to the respiratory system, where it replicates in epithelial tissues.

CLINICAL

Immunoincompetent patients:

Rhinopharyngitis in young children, sometimes with associated conjunctivitis producing Adenoidal-Pharyngeal-Conjunctival syndrome; pneumonia, sometimes with extremely serious bronchial pneumonia; ordinary conjunctivitis and more rarely epidemic keratoconjunctivitis; gastroenteritis.

In immunosuppressed subjects:

Latent infections are observed which, when reactivated, lead to very serious clinical forms with visceral damage, particularly involving the liver and lungs. Disseminated infections with relatively non-specific symptoms (fever, pneumonia or hepatitis) of which are fatal in 10 to 50% of cases.

SEARCH INDICATIONS

Diagnosis of an upper or lower respiratory infection.

Diagnosis of conjunctivitis or keratoconjunctivitis.

Diagnosis of a gastro-intestinal infection.

Diagnosis of possible dissemination in an immunosuppressed patient or of an infection with non-specific symptoms.

In a context of epidemiological research.

INFORMATION

SAMPLE

The sampling location is clinically determined:

– Respiratory conditions: Nasal or tracheobronchial secretions are collected.

– Ocular conditions: A conjunctival sample is taken.

– Gastroenteritis: A stool sample is taken.

– In immunosuppressed patients: A systemic investigation is carried out, involving various samples (stools, urine, ENT, etc.), combined if appropriate with a search for viraemia plus sampling of cerebrospinal fluid, or biopsies.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

Immunosuppression?

Current treatment?

SAMPLE STORAGE AND TRANSPORT

AdV's are relatively resistant, which simplifies the conditions of storage and transport.

Samples taken for the purposes of diagnostic orientation (pharyngeal, ocular or bronchoalveolar lavage) are stored at +4° C.

Smears are placed on slides and are fixed with acetone for 10 minutes and transported at room temperature.

Samples intended for cell culture should be stored at +4° C.

Swab samples require a viral transport medium.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

Antigen detection:

Techniques based on immunofluorescence (IF) or immunoenzymatic assay use group-specific anti-hexon monoclonal antibodies.

They are applied directly to smear samples of respiratory, nasal or conjunctival secretions.

Latex particle agglutination using the same group-specific antibodies is employed to find AdV's in stool samples (searches in parallel with rotaviruses).

These techniques are easy to use and provide rapid results, but their **sensitivity** differs between techniques (immunoenzymatic assay offers higher sensitivity than the others).

- **Cell culture:**

  This is the reference method, because it allows the identification of AdV species and serotypes. It is performed on diploid lines: MRC-5 or continuous human lines: HEp-2, HeLa and KB. The cytopathogenic effect appears at between 3 and 21 days and is characterised on continuous human cell lines by rounded refracting cells with retraction of the cellular layer.

  Serotypes 40 and 41 can only be cultivated on specific cells i.e. HEK cells converted by AdV5. After culture, confirmation that an AdV is involved is necessary by IF on cells from the culture, or identification of a precise AdV serotype by seroneutralisation using monoclonal or polyclonal antibodies.

- **Molecular biology:**

  PCR is used to detect viral DNA in samples (cerebrospinal fluid, biopsies, blood, etc.), but it is not yet routinely employed and its use is limited to disseminated infections in immunosuppressed subjects.

IINDIRECT DIAGNOSIS

Existing serological techniques use hexon, the group antigen common to all AdV's. The techniques are the complement-fixing reaction (CFR) and ELISA.

Techniques involving the inhibition of haemagglutination and seroneutralisation are only employed after cell culture. There is no cross-immunity between the various serotypes and re-infection by the same serotype is possible.

INTERPRETATION

The orientation diagnosis is easy to implement and is mainly used when searching for a respiratory infection, with or without an associated search for another virus.

It gives a rapid response but lacks sensitivity.

Methods of amplification (culture and PCR) of greater sensitivity confirm viral replication and allow the identification of small viral loads. They are very useful in epidemiological studies, because they enable the AdV strains to be characterised. Implementation, however, is more problematic and is thus reserved for specialised laboratories.

Serological diagnosis is difficult to interpret, due to the variable appearance of IgM and IgG and a lack of sensitivity in young children. It may be of retrospective diagnostic assistance in the context of an epidemic.

TREATMENT

At the present time, only cidofovir has been used with a certain degree of efficacy to treat serious AdV infections.

FOR FURTHER INFORMATION

- Freymuth F., *Adénovirus*, Encycl Med Biol (Elsevier Paris), 2003.
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ANTIDIURETIC HORMONE (ADH)

DEFINITION

Antidiuretic hormone (ADH), also known as Arginine Vasopressin (AVP), plays a role in controlling the flow rate of urine and in hydric metabolism. ADH deficiency or ineffectiveness leads to an insipid diet and dehydration. Conversely, excessive secretion of ADH results in hyperhydration.

INTRODUCTION

The volume and ionic composition of the various compartments of the body depend on exchange of free water and electrolytes, with water circulating freely through cell membranes. The hypothalamus controls both water intake, by inducing the sensation of thirst, and water loss. The secretion of ADH, by increasing the permeability of the collector tubes, causes passive reabsorption of water and so minimises loss.

ADH is a peptide hormone composed of 9 amino acids. It is secreted in the supraoptic and paraventricular nuclei of the hypothalamus, under the control of the osmotic pressure of the extracellular liquids. ADH is then transported along the neurons of the hypothalamo-hypophyseal tract and stored in the anterior pituitary. Its secretion follows a circadian rhythm (with the maximum during the night) and its half-life is 5 to 10 minutes. ADH is released as a result of certain stimuli. The carotid and aortic baroreceptors, and the voloreceptors of the left atrium, constantly supply information on plasmatic volume. ADH secretion is only stimulated, however, when rapid changes of blood volume (more than 10% – 20%) occur. Furthermore, the concentration of ADH in the blood is closely correlated with the blood osmolarity, which is communicated to the hypothalamic osmoreceptors. However, sensitivity to variations in blood osmolarity, and therefore the amplitude of ADH secretion vary greatly from one individual to another. In addition, at extreme ages the ability to adapt to water/electrolyte imbalances is less effective. Other mechanisms, physiological and otherwise may also influence the secretion or effectiveness of ADH:

	Increased ADH secretion or renal hypersensitivity	Inhibited ADH secretion or renal insensitivity
Physiological stimuli	Nausea reflex Stress	
Medicines	Arginine-vasopressin DDAVP (desmopressin) Carbamazepine Diuretics Morphine Chlorpromazine Clofibrate	Demeclocycline Lithium Central anaesthetics Tricyclic antidepressants Specific inhibitors of serotonin re-uptake Chlorpromazine Phenytoin Non-steroid anti-inflammatories
Toxins	Nicotine Caffeine, theine, theobromine	Alcohol

ADH is recognised by two specific types of receptor, V1 and V2. These are transmembrane receptors coupled with G proteins. V1 receptors are situated on the surface of vascular smooth muscle cells and their action induces vasoconstriction. Activation of V2 receptors increases the number of hydric channels (aquaporins) expressed at the endoluminal (urinary) pole, thus increasing the kidney's capacity to reabsorb water.

INDICATIONS FOR MEASUREMENT

Declaring the existence of inappropriate ADH secretion requires **knowledge** of the clinical context (arterial tension, weight, presence of oedemas, signs of dehydration, measurement of intake and loss), creatinine clearance values, blood and urine ionograms, the plasma/urinary osmolarity ratio, calcaemia and calciuria, as well as glycaemia and glycosuria. This allows the exclusion of conditions including osmotic polyuria (glycosuria, hypercalcaemia and hypokalaemia). Measurements must if possible be made in the absence of diuretic treatment or any other medication which alters the hydrosodium metabolism (anti-epileptics, centrally-acting anti-hypertensives, etc.).

■ ADH HYPOSECRETION

Insufficient secretion of ADH is observed when there are lesions of the central nervous system, hypothalamus and pituitary gland. In a majority of cases, this involves cranial trauma, tumours, cerebral irradiation and cerebrovascular accidents. In all the central forms, the concentration of ADH is not appropriate for the plasma/urinary osmolarity ratio. Plasmatic osmolarity is normal (water loss compensated by increased intake), but the urine is diluted. In a water restriction test, concentration of urine is only observed after the administration of ADH.

In a large majority of situations, ADH measurement is only requested at a secondary stage. The clinical context is particularly indicative in cases of chronic alcoholic intoxication, the prescription of centrally-acting drugs (see table above), or potomania (water intoxication). It should be emphasised that true potomania is exceptional: the clinical diagnosis is usually obvious and does not endanger the patient. If the urine dilution capacity is intact, the patient would have to drink at least 30 litres of water per day to suffer hyponatraemia. Psychogenic polydipsia is characterised by plasma-urine iso-osmolarity and, in a hydric restriction test, the ADH concentration is appropriate to the urinary osmolarity. In a few cases, during treatment and/or chronic alcoholic intoxication, ADH secretion is inhibited and the hydric restriction test may be difficult to interpret.

■ ADH HYPERSECRETION

■ Renal insensitivity to ADH (or nephrogenic diabetes insipidus)

Insensitivity of the collector tubes to the action of ADH is usually acquired *(see table above)*. The diagnosis is supported by the absence of urine concentration in a hydric restriction test and the ineffectiveness of administering AVP. Congenital forms are rare and transmitted in an X-linked recessive mode. The symptoms are present at birth, with subsequent mental retardation and episodes of dehydration and hypernatraemia associated with hyperthermia. If the mutation is identified, investigation of the family is possible (diagnosis of carrier mothers and perinatal diagnosis). The early treatment of children has completely changed the prognosis for this pathology.

Inappropriate ADH secretions

There are numerous situations in which ADH secretion is excessive i.e. inappropriate for the requirements of hydric homeostasis (syndrome of inappropriate ADH secretion or SIADH). The diagnosis is suggested in cases of hyponatraemia associated with urinary hyperosmolarity. It is only confirmed in the absence of hyperglycaemia, sodium intake restriction, adrenal cortex insufficiency, hypothyroidism and diuretic treatment (thiazide). Urinary osmolarity must always be interpreted according to hydroelectrolytic intake and diuresis.

Neurosurgery patients often present SIADH (trauma, infections, benign or malignant tumours, abscesses). SIADH is frequent in pneumopathies (streptococcal pneumonia, tuberculosis, asthma, mucoviscidosis, trauma). Paraneoplasic forms are rare in comparison and associated with smoker's pulmonary carcinoma or small-cell pulmonary carcinoma).

INFORMATION

SAMPLE

1 ml of plasma EDTA in a pre-cooled sample tube: Patient preferably fasting.

Please ensure that sample is non-haemolysed, non-icteric and non-lipaemic.

Urine sample: 2nd urination of the morning.

QUESTIONS FOR THE PATIENT

Current treatment?

SAMPLE STORAGE AND TRANSPORT

After collection, the plasma sample can be kept for a maximum of 1 hour at +4° C before being centrifuged (15 minutes at 2000 g and at +4° C) then decanted and rapidly frozen to -20° C.

If analysis is deferred, urine samples must be frozen within 4 hours of collection.

Before the measurement, samples must be thawed in melting ice.

ASSAY METHODS

Measurement is performed using a radioimmunoassay technique, with prior extraction.

REFERENCE VALUES

In adults:

Plasma ADH < 8 pg/ml

Urine ADH < 112 ng/l.

HYDRIC RESTRICTION TEST

The test is only carried out in a hospital environment. The patient is subjected to total hydric restriction for at least 3 hours while being under strict clinical monitoring (weight, temperature, arterial tension and pulse) throughout the duration of the test. The test must be halted in the event of fever or intolerance (loss of weight, haemoconcentration, accelerated pulse or extreme thirst). Plasma and urine samples are collected every hour for measurements of ADH and plasma and urinary osmolarities. At the end of the test, if the urine has not concentrated, AVP or DAVP is administered and the measurements repeated.

In normal subjects, urinary osmolarity reaches 1000 mOsm/kg at the 20th hour and the administration of AVP induces no further concentration of urine.

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ALBUMIN

DEFINITION

Albumin is a protein with a molecular weight of 69 kDa and is synthesised by hepatocytes. It is distributed approximately 60% in interstitial areas and 40% in the vascular system, where it represents two thirds of the circulating proteins. Its half-life in plasma is approximately 20 days.

INTRODUCTION

Albumin essentially contributes to maintaining vascular oncotic pressure (hypoalbuminaemia encourages the development of oedemas) and plays a fundamental role as a protein carrying numerous substances in a biologically-inactive but rapidly-mobile form. These may be endogenous substances such as hormones, amino acids, fatty acids, trace elements, vitamins, etc, or medicines (diminution of the link between the drug and plasma albumin leads to a rise in the active drug's free fraction, with a risk of overdose).

Hypoalbuminaemia is encountered in many pathological situations, including malnutrition, inflammation, hepatocellular insufficiency, glomerular protein leakage plus digestive and cutaneous disorders. Hyperalbuminaemia is rare and usually associated with haemoconcentration.

INDICATIONS FOR MEASUREMENT

– Diagnosis and monitoring of pathologies with protein leakage:
- Digestive: Exudative enteropathies
- Renal: Nephrotic syndrome etc

– Diagnosis and monitoring of chronic inflammatory syndromes

– Assessment of chronic malnutrition:

In most cases Albumin is measured with prealbumin, CRP and orosomucoid in the context of a "nutritional profile", in order to take the inflammatory component into account, this often being present in cases of malnutrition. It also contributes to the calculation of PINI (Prognostic Inflammatory and Nutritional Index), a nutritional index particularly used in geriatrics.

PINI = [CRP (mg/l) x orosomucoid (mg/l)]/ [albumin (g/l) x prealbumin (mg/l)].

INFORMATION

SAMPLE

Serum is preferable to plasma (plasma samples may interfere with the assay). Ensure samples are not haeomolysed or lipaemic.

If Phlebotomy Cuff is attached for longer than 5 minutes then the sample albumin levels may rise by approximately 15%.

QUESTIONS FOR THE PATIENT

Are you taking any of the following medicines?: Treatment with oestrogens may slightly lower the serum albumin concentration: the same effect may be produced by dextran perfusions and treatment by dapsone or L-asparaginase.

Albumin perfusions increase the serum albumin concentration.

SAMPLE STORAGE AND TRANSPORT

Viability of serum: 1 week at +4° C and several months at -20° C.

Transport: +4° C within one week or frozen if more than one week or if sample has already been frozen.

ASSAY METHODS

Immunochemical methods: Immunoturbidimetry and immunonephelometry.

Colorimetric methods: Bromocresol Green.

Electrophoresis methods.

NORMAL EXPECTED VALUES

Newborn babies: 36 to 54 g/l.

Babies of 1 to 3 months: 27 to 41 g/l.

Babies 3 months to 2 years: 30 to 42 g/l.

Adults: 35 to 50 g/l.

Since 1994, there has been an international reference material for immunochemical measurements. This material is CRM 470, certified for 14 proteins including albumin.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Age-dependent:

see above.

Sex-dependent:

Values are slightly higher in men than in women, but become equal for both sexes after 65 years of age.

During pregnancy:

Oestrogens have the effect of reducing albumin synthesis slightly (by approximately 20%) and the concentration of albumin by haemodilution.

PATHOLOGICAL VARIATIONS

Hypoalbuminaemia is observed in various pathological situations:

– Hepatocellular insufficiency: Acute (viral, drug-induced or toxic hepatitis) or chronic (cirrhosis etc.)

– Acute or sub-acute inflammation but above all chronic inflammation: The decrease in serum albumin can reach 50% in cases of persistent severe inflammation.

– Severe chronic malnutrition and/or increase in energy expenditure (mental anorexia or chronic alcoholism). Due to its long half life (20 days), albumin does not allow acute nutritional variations to be detected. It is however used to evaluate chronic

malnutrition and its long-term evolution. Measurement is often carried out at the same time as measurements of orosomucoid and prealbumin, in the context of a nutritional protein profile.

Evaluation of nutritional status using PINI

(according to Ingelbleek Y and coll. Int J Vit Nutr Res 1985; 55: 91-101

PINI < 1: absence of risk
PINI between 1 and 10: low risk
PINI between 11 and 20: moderate risk
PINI between 21 and 30: high risk
PINI > 30: critical risk

(NB: according to M. Ferry, *Ann. Biol. Clin*., 1990, 48: 303-308: critical risk if PINI > 25).

– Nephrotic syndrome: Glomerular protein leakage (if proteinuria is > 3 g/24 hours) produces hypoalbuminaemia

– Digestive leakage in exudative gastroenteropathies:

- Through increased permeability of mucosae to proteins: Crohn's disease, enterocolitis, coeliac disease, gastroenteritis, intolerance to cows' milk proteins, etc.

- Through abnormal lymph flow: Primary or acquired intestinal lymphangiectasia (due to venous hyperpressure or organic obstruction: Budd-Chiari syndrome, lymphoma, etc,).

– Cutaneous loss: Eschars, extensive burns, etc.

– Plasma extravasation: Shock, formation of ascites or significant pleural effusion.

– Monoclonal hypergammaglobulinaemia involving IgG or IgA: Reduced synthesis of albumin to maintain the balance of oncotic pressure.

– Congenital analbuminaemia: Very rare.

■ **Hyperalbuminaemias** are rare:

It usually reflects haemoconcentration by extracellular or overall dehydration. The other causes are iatrogenic (albumin perfusion) or artefactual (extended cuff attachment time or analysis error).

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ALDOLASE

DEFINITION

The enzyme known as aldolase is involved in biochemical mechanisms of hepatic and muscular glycolysis. It cleaves fructose-1 phosphate and fructose 1-6 bisphosphate respectively into dihydroxyacetone phosphate and glyceraldehyde or glyceraldehyde phosphate. Three isoenzymes of aldolase can be found in humans:

– Aldolase A is essentially present in muscles and in erythrocytes. Its preferential substrate is fructose 1-6 bisphosphate. This is the form which predominates in serum.

– Aldolase B is found almost exclusively in the liver, is involved in neoglucogenesis. A mutation in the gene for this enzyme causes fructose-1-phosphate to accumulate in tissues and is responsible for the hereditary condition known as fructose intolerance.

– Aldolase C is essentially present in the brain. Its physiological role is poorly understood.

INTRODUCTION

The serum and/or erythrocyte activity of aldolase rises in certain muscle conditions such as Duchenne muscular dystrophy, polymyositis and dermatomyositis. It is therefore a marker for muscular distress in the same way as creatine kinase (CK), of which the measurement is more sensitive and more widely used. Aldolase levels may be increased in those situations where CK is normal.

A mutation involving aldolase B is responsible for hereditary intolerance to fructose, a disease in which the ingestion of fructose or its derivatives by affected children causes digestive disorders, hepatic abnormalities and a reduction in glycaemia. Treatment relies on eliminating fructose from the diet: failure to carry out these dietary measures may lead to death. When this disease is present, aldolase activity in the liver is reduced, but remains normal in muscles (the serum activity of aldolase is slightly reduced). At present, however, diagnosis of this condition is based on using molecular biology to detect the A149P mutation (responsible for a large majority of cases in Europe) on the aldolase B gene.

INDICATIONS FOR MEASUREMENT

In practice, serum aldolase analysis has now become obsolete. There no real routine indications for the measurement. The only cases where it may be of interest are:

– Suspected muscular lysis with normal CK activity.

– Suspected inherited intolerance to fructose (but looking for the A149P mutation of the gene is preferred in diagnosing the disease).

INFORMATION

SAMPLE

Plasma preferably collected in EDTA, oxalate, fluoride or heparin, or serum (collected in a dry tube). Reject haemolysed samples (presence of a large quantity of aldolase in erythrocytes). Erythrocyte aldolase is measured in a non-centrifuged haemolysate.

It is advisable to collect the sample after a rest of approximately 30 minutes in order to avoid any interference due to muscle activity.

QUESTIONS FOR THE PATIENT

Are you being treated with corticoids or an ACTH analogue? Do you suffer from hyperadrenocorticism? These situations can lead to an increase in plasma aldolase activity, in the order of 10 to 20 UI/l. However, oestrogen may reduce aldolase levels.

SAMPLE STORAGE AND TRANSPORT

Storage of plasma or serum: 48 hours at 20° C; 4 days at +4° C or several months at -20° C.

Transport: recommended to take place at +4° C (if < 4 days).

ASSAY METHODS

Fixed-time colourimetric method (37° C) or kinetic method by measuring the consumption of NADH, H+ (37° C).

NORMAL EXPECTED VALUES

In adults: plasma (or serum) aldolase: 2 to 7.6 UI/l (technique at 37° C); erythrocyte aldolase: 2.33 to 4.05 UI/g (method recommended by the *International Council for Standardization in Haematology*).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

The plasma aldolase level varies with age. In newborn babies, the normal values are approximately twice as high as those in adults. Levels rise in childhood before diminishing in a hyperbolic curve to reach adults values at about 17 to 20 years of age. Indicative values: normal values before 3 years of age: 10 to 25 U/l; 3 to 20 years: 3 to 15 U/l.

PATHOLOGICAL VARIATIONS

Increased aldolase activity

Aldolase activity in the plasma generally rises in all situations of acute cellular distress.

Pathologies of skeletal muscles: as in the case of CK, aldolase rises early and significantly (up to 50 times the upper limit of the reference interval) in the course of progressive muscular dystrophies (Duchenne's is the most common and most serious of these conditions), as well in tetanus, muscular glycogenesis, dermatomyositis and polymyositis. In contrast, aldolase activity is normal (along with that of CK) in multiple sclerosis, poliomyelitis and other muscular conditions of neurological origin.

Hepatic pathologies: epatoma and acute viral hepatitis (rising to 7 to 20 the upper limit of the reference interval, in parallel with a rise in ALAT); normalisation in 15 to 20 days. NB: plasma aldolase is normal in chronic hepatitis, cirrhosis and cholestasis.

Cardiac pathologies: Rise in aldolase during myocardial infarction (in parallel with ASAT) and myocardial conditions in general.

Other pathologies: Hyperadrenocorticism, trichinosis, cancer of the prostate, breast, cervix, stomach, colon, œsophagus, etc, malignant melanoma, hypothyroidism, acute pancreatitis, megaloblastic anaemia (10 to 13 times the upper limit of normal values), etc.

NB: Erythrocyte aldolase activity rises in subjects with regenerative anaemia.

- **Reduced aldolase activity**

  In hereditary intolerance to fructose: this recessive autosomal condition is due to a deficiency of aldolase B. Plasma aldolase activity is only slightly reduced; its measurement is not sufficient to diagnose the disease. Diagnosis relies on the use of molecular biology to search for the A149P mutation.

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ALDOSTERONE

DEFINITION

Aldosterone, the main mineralocorticoid hormone, is produced in the zona glomerulosa of the adrenal cortex. In this zone, progesterone is converted by 21-hydroxylase into 11-deoxycorticosterone (DOC) which then undergoes 11-hydroxylation to generate corticosterone. The corticosterone is converted into 18-hydroxy-corticosterone which is then oxidised to generate aldosterone. In an alternative pathway, DOC is acted on by 18-hydroxylase and then 11β-hydroxylase. In any case, the three steps involved in converting DOC into aldosterone, i.e. 11β-hydroxylation, 18-hydroxylation and 18-methyl-oxidation, are all catalysed by the same enzyme, namely CYP11B2 or aldosterone synthase, which is only found in the zona glomerulosa of the adrenal cortex.

It should be noted that both DOC and corticosterone can be synthesised in two other parts of the adrenal cortex: the zona fasciculata and the zona reticularis, although most 18-hydroxycorticosterone is generated in the zona glomerulosa. The rate of 18-hydroxycorticosterone production correlates closely with that of aldosterone.

INTRODUCTION

Aldosterone secretion is regulated by a number of factors, the most important of which are the renin-angiotensin system and kaliaemia *(see Renin)*. Thus, in normal physiological conditions, the concentrations of renin and aldosterone are closely related. Hyperkalaemia stimulates aldosterone secretion and hypokaliaemia inhibits it. Other factors such as ACTH, POMC-derived peptides, sodium, vasopressin, serotonin, β-adrenergic agents, ANP and somatostatin act either on the initial step of steroidogenesis (the conversion of cholesterol into pregnenolone), the final step of aldosterone synthesis (catalysed by CYP11B2 or aldosterone synthase) or both. ACTH stimulates "acute" aldosterone secretion but not "chronic" secretion.

In the plasma, a small fraction of aldosterone (20%) is bound to corticosteroid binding globulin (CBG) and a larger fraction (40%) is bound to albumin; the rest is considered as unbound or free.

Aldosterone is the main mineralocorticoid hormone and it has a high affinity for mineralocorticoid receptors in target tissues, notably in the kidney, the colon and the salivary glands. It stimulates distal sodium reabsorption and, through ion exchange, promotes the excretion of potassium and H+ ion via the urine. Its precursors, DOC, corticosterone and 18-hydroxy-corticosterone, also have mineralocorticoid activity but are not as potent as aldosterone.

Like cortisol, aldosterone is broken down in the liver by successive reduction reactions. The main metabolite is the 5β-reduced, 3α-hydroxylated derivative, tetrahydroaldosterone (THALDO), which represents 35-40% of urinary aldosterone metabolites. THALDO is conjugated with glucuronic acid at C3 before excretion in the urine. Another conjugate is generated by the conjugation of unreduced aldosterone with glucuronic acid, aldosterone-18-glucuronide, which represents about 10% of urinary metabolites.

INDICATIONS FOR MEASUREMENT

Quantitative assays for aldosterone in the plasma and for tetrahydroaldosterone (its aldosterone glucuronide metabolite) in the urine are recommended for the investigation of arterial hypertension when adrenal adenoma is suspected; removal of the adenoma would resolve the hypertension.

INFORMATION

SAMPLE

Aldosterone can be assayed in both serum and plasma but, since the same sample is usually used for a parallel renin assay, only EDTA plasma samples are accepted (because the other types of sample are not suitable for assaying renin). The sample is centrifuged at room temperature and then the plasma is drawn off and frozen at –20°C *(see Renin)*.

Many drugs used to manage arterial hypertension act on the renin-angiotensin-aldosterone system (RAAS), at the level of angiotensinogen, renin or aldosterone. This can affect the aldosterone/renin ratio, a parameter used in the diagnosis of primary aldosteronism. Before any investigation of the RAAS, such drugs should therefore be withdrawn long enough beforehand to guarantee reliable results. The main groups of medicinal products that can interfere with the RAAS are listed in the Table below (together with the recommended interval between drug withdrawal and testing, to be observed prior to RAAS investigation).

Medicinal product	Effect	Time of withdrawal prior to RAAS investigation
Spironolactone	Increased renin	6 weeks
Oestrogens	Increased angiotensinogen	6 weeks
Angiotensin-converting enzyme inhibitors	Increased renin and reduced aldosterone	2 weeks
Diuretics (other than spironolactone)	Increased renin	3 weeks
Non-steroid anti-inflammatory drugs	Reduced renin	2 weeks
β-adrenergic inhibitors	Reduced renin	2 weeks
Calcium channel blockers	Reduced aldosterone	2 weeks

If the course of anti-hypertensive treatment cannot be interrupted, α-blocking agents can be administered.

ESSENTIAL INFORMATION

Information must be provided about age, the patient's position at the time of blood drawing (sitting or lying down) and the time the sample was drawn, for women of child-bearing age, the phase of the menstrual cycle; it is also important to say if the patient is on a low-sodium diet and provide details of any concomitant drugs. Finally, it should be stipulated if the assay is in the context of suppression or a stimulation test.

SAMPLE STORAGE

Frozen plasma samples can be stored for about six months.

ASSAY METHODS

Aldosterone is usually assayed using a radio-immunological assay. The antisera used today are specific enough for the direct assaying of plasma aliquot. Recently, immunological assays using anisotopic tracers have been developed, and these can be run on analysers.

DYNAMIC TESTS

■ STIMULATION TESTS

Stimulation tests involve stimulating the secondary RAAS by inducing effective hypovolaemia and/or sodium depletion.

■ Orthostatism

The simplest test involves passage of the subject from the lying position to a standing one. The first sample is drawn after eight hours in bed and the second after one hour of walking around. The aldosterone concentration should double between the two time points.

■ Sodium depletion

Sodium depletion is a potent stimulator of the RAAS. It is induced by the oral administration of furosemide, a sulfamine diuretic that inhibits the reabsorption of sodium and chloride (mainly in Henle's loop).

■ ACTH stimulation test

Aldosterone peaks 30 minutes after the injection of ACTH.

■ SUPPRESSION TESTS

■ Sodium loading test

A sodium load induces hypervolaemia resulting in the inhibition of aldosterone secretion by the renin-angiotensin system.

■ Captopril suppression test

Captopril inhibits the enzyme that converts angiotensin I into angiotensin II and thereby reduces the concentration of aldosterone and increases that of renin. The absence of such responses would point to primary [hyper] aldosteronism.

ALDOSTERONE/RENIN RATIO

In primary hyperaldosteronism, aldosterone and renin levels fluctuate in opposite directions so the simplest way of detecting the uncoupling of aldosterone production (as in primary hyperaldosteronism syndrome) is analysing the aldosterone/renin ratio. However, no threshold value has yet been established for this ratio because it varies according to the assay method and the units in which the two hormones are expressed. Moreover, some studies have been based on plasma renin activity and others on active renin.

Proposed thresholds have varied from one study to another: some have used a figure of 23 (aldosterone RIA and renin IRMA in pg/ml) whereas others have used 30 or even 50. When the aldosterone concentration is expressed in ng/dl and that of renin in µU/ml (aldosterone RIA and renin IRMA), the threshold value is 3. Finally, for non-radioactive methods (chemiluminescence, LIA), the threshold has been set at 71 (aldosterone in pmol/l and renin in mU/ml) or 43 (aldosterone and renin both in pg/ml). This is summarised in the following Table.

Aldosterone (units) (assay method)	Active renin (units) (assay method)	Threshold value for the aldosterone/renin ratio
pg/ml or ng/l (RIA)*	pg/ml or ng/l (IRMA*)	23
ng/dl (RIA)	µ U/ml (IRMA)	3
pg/ml or ng/l(LIA)*	pg/ml or ng/l (LIA)	43
pmol/l (LIA)	mU/l (LIA)	71

RIA: radioimmunoassay; IRMA: immunoradiometric assay; LIA: chemiluminescence.

To enhance the ratio specificity in the diagnosis of primary hyperaldosteronism, some experts add a second criterion, namely an aldosterone concentration over 200 pg/ml.

USUAL VALUES

Usual values will vary according to the assay method used. For reference purposes, those observed using a chemiluminescence assay in adults are provided in the following Table.

Aldosterone	pg/ml	pmol/l
Lying down	20 - 140	56 - 388
Standing up	30 - 220	83 - 611

Reference values for aldosterone concentrations in the plasma of adults with normal sodium intake

In children, the aldosterone concentration varies with age.

To convert pg/ml into pmol/l use the conversion factor of 2.774.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

RAAS activity depends on age, gender, circadian rhythm, hormonal status, endocrine activity and sodium intake. Aldosterone and renin concentrations are high in new-borns and babies of less than one year of age. They subsequently wane steadily with age due to increased sodium intake and maturation of renal tubule function. Nevertheless, concentrations remain higher in children than in adults. In women of child-bearing age, the rate of aldosterone secretion rises during the luteal phase of the menstrual cycle in response to the increase in renin induced by the anti-natriuretic activity of the progesterone secreted in this phase of the cycle.

In a normal pregnancy, plasma volume rises due to increases in cardiac output, blood flow through the kidneys and the rate of glomerular filtration. Despite this increase in volume, the RAAS is stimulated by prostacyclin and prostaglandin E2, as well as through direct effects of oestrogen and the anti-natriuretic activity of progesterone.

The aldosterone concentration drops in adults between the third and seventh decades.

■ PATHOLOGICAL VARIATIONS

■ High concentrations

<u>Primary hyperaldosteronism</u>

Primary hyperaldosteronism should be suspected in a patient presenting high blood pressure coupled with spontaneous hypokalaemia. In some cases, the hypokalaemia may cause symptoms such as frontal headache, muscular weakness, flaccid paralysis or polyuria. This disease is more common in women with a peak of incidence between 30 and 50 years of age.

The relevant diagnostic tests focus on increases in the concentrations of aldosterone in the plasma and of its metabolites in the urine (aldosterone glucuronides and tetrahydroaldosterone) associated with the suppression of renin, as evidenced by the aldosterone/renin ratio which is the most commonly used diagnostic parameter. It is important to emphasise that any agent that might affect the RAAS should be avoided (see Information). Dynamic tests might be indicated to investigate the independence of aldosterone secretion. Neither sodium loading nor fludrocortisone or Captopril® suppression induce a decrease in aldosterone.

Primary aldosteronism is seen in 2-10% of subjects with high blood pressure. Two-thirds of these will have an adenoma of the adrenal cortex and the others will have adrenal hyperplasia.

a) Primary aldosteronism due to a tumour, Conn's syndrome

Hyperaldosteronism usually results from the presence of a unilateral adenoma which is most often benign. Its cells derive from the zona glomerulosa or reticulata. There may be more than one adenoma and both sides may be involved; situated in a adrenal gland where the zona glomerulosa is hyperplastic.

b) Bilateral hyperplasia of the zona glomerulosa (bilateral idiopathic aldosteronism)

The zona glomerulosa of both adrenal glands is focally or diffusely hyperplastic. The pathogenic mechanism is unknown. The manifestations of idiopathic aldosteronism are less severe than those associated with an adenoma. The aldosterone concentration tends to be less elevated and both the hypokalaemia and renin suppression less marked.

c) Dexamethasone-sensitive primary aldosteronism

This condition is characterised by its hereditary transmission as a dominant autosomal trait. The rate of production of 18-hydroxylated steroids is elevated and both blood pressure and aldosterone levels are normalised by low-dose dexamethasone.

Identification of the molecular lesion explained the disease's pathogenic mechanism: unequal recombination between the CYP11B1 and CYP11B2 genes (which encode 11β-hydroxylase and aldosterone synthase) creates a chimeric gene with a sequence encoding a protein that can synthesise aldosterone together with the promoter sequence of the 11 β-hydroxylase gene which renders its expression sensitive to ACTH in the zona fasciculata.

Secondary hyperaldosteronism

Aldosterone levels rise in all pathological situations which cause oedema, including heart failure, cirrhosis of the liver and nephrotic syndrome. This is because the rate of aldosterone secretion rises in response to the increase in renin induced by a reduction in the effective volume of circulating blood, secondary to reduced cardiac output or the transudation of fluid out of the vessels into the tissue space.

Secondary hyperaldosteronism is also seen with tumours that secrete renin (reninoma), and in stenosis of the renal artery or kidney problems which cause salt loss.

Bartter's syndrome

Bartter's syndrome is a rare condition characterised by hyperreninaemia, hyperaldosteronism, hypokalaemia and alkalosis without either hypertension or œdema. This onset of disease occurs during childhood, manifesting as muscular weakness, cramps, polyuria, retarded growth and learning difficulties. There is hyperplasia of the juxtaglomerular cells and an increase in the amount of prostaglandin E2 secreted in the urine. Nephrocalcinosis and kidney stones are common. The concentration of calcium ions in the blood is reduced, that of parathyroid hormone is increased, and bone mass is lost.

Pseudohypoaldosteronism

This childhood salt depletion syndrome is very rare which has been attributed to insensitivity of the renal tubule to the mineralocorticoid activity of aldosterone. It can be transmitted as either a dominant or recessive autosomal trait. In the first case, aldosterone resistance is restricted to the renal tubule whereas, in the second, more severe form, the salivary and sudoriferous glands and colon are also affected.

This syndrome may be symptomless or may present as hypoaldosteronism with low blood sodium, high blood potassium and renal sodium loss. In all cases, both aldosterone and renin are found at very high concentrations. Obviously, there is no response to exogenously administered mineralocorticoids.

Low concentrations

Primary adrenocortical failure (Addison's disease)

In primary adrenocortical failure (Addison's disease), all three zones of the adrenal gland are damaged by the pathological process and the secretion of all adrenal steroid hormones including the glucocorticoids, mineralocorticoids and androgens are compromised. However, symptoms only become evident by the time 90% of the adrenal cortex has been destroyed.

Addison's disease is characterised by a combination of physical and psychological lassitude, loss of libido, pigmentation of the skin and mucosa, weight loss, gastrointestinal problems and low blood pressure. Signs of hypoglycaemia may develop due to the lack of cortisol. Diagnostic tests focus on documenting low concentrations of cortisol, aldosterone and DHEA sulphate. ACTH and renin levels tend to be elevated. Interpreting cortisol results is problematic although a concentration of less than 30 ng/ml points to a positive diagnosis whereas one of over 190 ng/ml rules Addison's disease out; anything in between is uncertain and a dynamic test (e.g. an ACTH stimulation test) is indicated.

It should be noted that, in adrenocortical failure secondary to corticotrophin deficiency, mineralocorticoid functions are unchanged since the renin-angiotensin system is not affected.

Isolated mineralocorticoid deficiency

Hypoaldosteronism should be suspected in any subject with unexplained hyperkalaemia. Hypoaldosteronism may be primary or secondary to a problem with the renin-angiotensin system.

A) Primary hypoaldosteronism

– Congenital forms

a. CYP11B2 (aldosterone synthase) deficiency

This rare condition is transmitted as a recessive autosomal trait. In clinical terms, affected children present with severe salt depletion associated with retarded growth. It is due to the lack of an enzymatic activity in the aldosterone biosynthesis pathway. The enzyme concerned is aldosterone synthase (CYP11B2) which has two distinct activities:

corticosterone 18-methyloxydase I (CMO I) which hydroxylates corticosterone; and CMO II which converts the 18-hydroxyl group into an aldehyde group.

In COM I deficiency, aldosterone cannot be detected and the level of 18-hydroxycorticosterone (18OHB) is very low. In COM II deficiency, 18-OHB levels are very high and aldosterone is detectable. These two types can be distinguished on the basis of the ratio of 18-OHB to aldosterone: in COM I deficiency, this ratio is below 10 whereas, in COM II deficiency, it is over 100.

b. CYP21A2 (21-hydroxylase) deficiency

Deficiency in 21-hydroxylase is the most common enzyme deficiency, accounting for more than 90% of cases of congenital adrenal hyperplasia.

Classic forms manifest as two clinical pictures, one characterised by simple virilism and the other by virilisation due to mineralocorticoid deficiency. In the latter form, there is salt depletion coupled with hyponatraemia, hyperkalaemia, hypovolaemia and increased blood renin (as a result of the hypoaldosteronism).

– Acquired forms

Heparin can have a directly toxic effect in the zona glomerulosa and can induce hypoaldosteronism. Acquired primary hypoaldosteronism can also occur in certain severe conditions and certain forms of adrenal metastasis with isolated mineralocorticoid involvement.

B) Secondary hypoaldosteronism or hyporeninaemic hypoaldosteronism

Hyporeninaemic hypoaldosteronism is characterised by unexplained, asymptomatic chronic hyperkalaemia associated with moderately impaired kidney function and tubular acidosis. In a subject with unexplained chronic hyperkalaemia, this diagnosis is confirmed by the detection of very low levels of aldosterone in the blood and urine combined with a low renin concentration. Hyporeninaemic hypoaldosteronism is most common in patients with diabetic nephropathy or chronic interstitial nephritis but it can also occur in other diseases, such as systemic lupus erythematosus, multiple myeloma and HIV infection.

Transient hyporeninaemic hypoaldosteronism can also be induced by certain drugs, notably non-steroid anti-inflammatory drugs, cyclosporine and mitomycin C.

C) Endogenous hypermineralocorticism

– Tumour-induced hypermineralocorticism

The secretion of mineralocorticoids other than aldosterone—DOC or corticosterone—by a tumour can cause hypertension and hypokalaemia, but the concentrations of both aldosterone and renin remain low.

– Hypermineralocorticism due to 11β-hydroxylase or 17α-hydroxylase deficiency.

Both of these rare deficiencies are autosomal recessive traits that cause high blood pressure. 11β-hydroxylase deficiency is also associated with excessive androgen production whereas, in 17α-hydroxylase deficiency, neither androgens nor oestrogens can be synthesised. In both cases, aldosterone and renin concentrations are low.

From a pathogenic point of view, cortisol synthesis is inhibited in 11β-hydroxylase deficiency, leading to the over-secretion of ACTH which causes build-up of the precursor immediately upstream of the blockage point, namely deoxycortisol. In parallel, corticosterone cannot be synthesised and it is DOC that builds up; since this intermediate has mineralocorticoid activity, it inhibits renin production. In contrast, adrenal androgens are over-produced and this induces virilisation in girls and pseudo-puberty in boys.

In 17α-hydroxylase deficiency, cortisol cannot be synthesised and the hyper-secretion of ACTH results in stimulation of the production of all precursors upstream of the blockage point, i.e. progesterone, pregnenolone and steroids in the mineralocorticoid pathway (DOC and corticosterone). This deficiency also affects androgen and oestrogen synthesis, leading to primary amenorrhoea in girls and pseudo-hermaphroditism in boys.

D) Pseudohyperaldosteronism

1. Apparent mineralocorticoid excess syndrome (Type I AME)

This is a rare cause of hypertension in children, very few of whom survive into adulthood. Its prevalence is low and hereditary forms are transmitted as a recessive autosomal trait.

From a pathogenic point of view, this syndrome is due to a deficiency in 11β-hydroxysteroid dehydrogenase (11HSD). In the kidney, aldosterone and cortisol have the same affinity for the mineralocorticoid receptor in the distal tubule, the activation of which induces the reabsorption of sodium and the excretion of potassium. However, this receptor is not usually activated by cortisol even though its concentration is 100-1000 times higher than that of aldosterone: this is because 11HSD converts cortisol into inactive cortisone. When this enzyme is deficient, cortisol builds up locally and mediates mineralocorticoid effects.

The body contains two different isoforms of 11β-hydroxysteroid dehydrogenase, one in the kidneys and the other in the liver.

Type I AME is due to deficiency in the renal isoform and its differential diagnosis depends on a combination of hypokalaemia, low concentrations of both renin and aldosterone, a slight elevation in free cortisol in the urine and, most importantly, an increase in the urine in the ratio of metabolites of cortisol to those of cortisone.

2. Apparent mineralocorticoid excess (Type II AME)

Type II AME is even rarer and has a similar clinical picture although the ratio of cortisol metabolites to cortisone metabolites is normal. The deficiency must concern both isoforms of the enzyme.

3. Excessive liquorice consumption

The same enzyme, 11β-hydroxysteroid dehydrogenase, can also become inhibited following excessive intake of glycyrrhizinic acid due to the over-consumption of liquorice-containing foodstuffs and drinks. The clinical picture is the same as that of Type I AME.

4. Relative 11HSD deficiency

In ectopic ACTH syndrome, the over-production ACTH by a non-pituitary tumour (such as a small-cell lung carcinoma) induces excessive cortisol production and Cushing's syndrome. The hypermineralocorticism observed in these patients is due to a relative deficiency of 11HSD as a result of excess cortisol.

Similarly, hypersecretion of cortisol by tumours of the adrenal cortex can induce relative 11HSD deficiency.

5. Liddle syndrome

   This hereditary disease which is characterised by high blood pressure, hypokalaemia, hyporeninism and hypoaldosteronism, is due to hyperpermeability of the amiloride-sensitive calcium channel.

In conclusion, investigation of the RAAS should always include assays for both aldosterone and renin. For diagnosis of a problem affecting the RAAS, the first step is to compare the results of these two tests.

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ALKALINE PHOSPHATASE AND ITS ISOENZYMES

DEFINITION AND SYNONYMS

The alkaline phosphatases or ALP (orthophosphoric monoester phosphorylase, EC 3.1.3.1) are membrane glycoproteins which are found widely in human tissues. They are 170 kDa zinc-containing molecules, which catalyse alkaline pH hydrolysis of monophosphoric esters, releasing phosphate and therefore enabling metabolites to pass across cell membranes.

The alkaline phosphatase isoenzymes are a group of glycoproteins of heterogeneous composition but the same activity. They have different genetic origins and are more or less specific for a tissue or organ group, such as the liver, kidney, bone, intestine, lung, testes, ovary and placenta. Some "abnormal" isoenzymes, Nagao, Regan and Kasahara, are associated with malignancy.

INTRODUCTION

Enzymes located on the cell membrane are released into the systemic circulation when the membrane is damaged. Their tissue distribution varies, in decreasing order of concentration from placenta, intestine, renal cells, osteoblasts to liver cells.

INDICATIONS FOR MEASUREMENT

In cholestatic hepato-biliary diseases.

In bone diseases with osteoblast regeneration.

In pregnancy, for cholestasis of pregnancy.

In some bowel diseases.

In monitoring some malignancies, such as primary or metastatic bone tumours and primary and secondary liver tumours.

INFORMATION

SAMPLE

Serum or plasma obtained from venous blood, collected into a dry or heparinised tube. Oxalate, citrate, fluoride and EDTA anticoagulants must not be used as these complex metal co-factors of the enzyme (zinc and magnesium).

The patient should preferably have been fasting for 12 hours to avoid raised intestinal ALP. Avoid haemolysed samples.

QUESTIONS FOR THE PATIENT

Age and sex?

Ethnic origin?

Are you pregnant?

Are you taking medicinal products which could influence serum ALP concentrations?

Consolidating recent fracture?

SAMPLE STORAGE AND TRANSPORT

The sample must be centrifuged and separated promptly. Plasma or serum can be stored for 7 days at room temperature or at + 4°C or for 3 months at –20°C.

ASSAY METHODS

TOTAL ALP

Measurement of serum ALP concentration uses a colourimetric method with a substrate, which releases a coloured substance on dephosphorylation. Degradation of para-nitrophenylphosphate and release of para-nitrophenol are read in a spectrophotometer at 405 nm.

ALP ISOENZYMES

Isoenzymes are separated and identified by electrophoresis. Several types of gel exist:

- **Alkaline pH agarose gel electrophoresis** distinguishes all of the isoenzymes apart from the liver and bone fractions, which have similar electrophoretic migration.
- **Affinity gel electrophoresis** is used for automated separation and quantification of the different ALP isoenzymes without pre-treatment. This demonstrates 6 fractions: two hepatic, H1 and H2; three intestinal: I1, I2, I3 and one bone. The liver and bone isoenzymes can be separated because of the lectin contained in the gel which has high affinity for the sialic acid on the bone form. Bone ALP can be measured in isolation by immunoenzymatic or radio immunological methods.

NORMAL EXPECTED VALUES

TOTAL ALP

For reference, usual values in adults are between ***50 and 136 I/UI*** at 37°C (on the Dade-Behring Dimension™).

ALP ISOENZYMES

ISOENZYME	% SERUM ALP
bone	20 to 75 %
hepatic H1	15 to 72 %
hepatic H2	1 to 14 %
intestinal (I1 + I2 + I3)	0 to 14 %

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL AND IATROGENIC VARIATIONS

- **Age:** the bone isoenzyme fraction depends on age and is associated with growth. It is raised in children and adolescents (in which values may reach 4 times those in adults). Adult values are reached by the age of 20 years old, when concentrations remain stable between 20 and 50 years old, then increasing in post-menopausal women.
- **Pregnancy:** serum ALP associated with expression of placental forms of the enzyme increase from the 20th week of pregnancy reaching values of 2 to 3 times normal at childbirth.
- **Dietary status:** ALP is increased in the obese, particularly in women. It falls in malnutrition.

- **Drugs:** many medical drugs influence ALP activity:

  Increased activity: oral anticoagulants, antiepileptics, oral hypoglycaemic agents, erythromycin, cyclosporin and phenothiazines.

  Reduced activity: lipid lowering drugs, systemic corticosteroids, oestrogens.

PATHOLOGICAL VARIATIONS

Investigation of ALP isoenzymes discriminates between some human diseases, particularly bone and hepatobiliary.

- **In bone diseases:** ALP concentrations are raised in bone diseases involving osteoblast regeneration, such as acromegaly, osteogenic sarcoma, osteomalacia, rickets, hypervitaminosis D, Paget's disease, osteitis deformans and hyperthyroidism.
- **In liver diseases:** the rise in serum ALP is an early indicator of mostly cholestatic hepatic disease, particularly obstructive jaundice. It is raised in chronic alcoholism, constitutional liver diseases such as primary biliary cirrhosis and Gilbert's syndrome, acute viral hepatitis (HBV, HAV and HCV), infectious mononucleosis and cytomegalovirus infection, drug-induced hepatitis, sarcoidosis, tuberculosis, amyloidosis and liver abscess.
- **In bowel diseases:** damage to bowel tissue causes a release of intestinal ALP into serum. Values are increased in peptic ulceration, bowel ulceration, ulcerative colitis and coeliac sprue.
- **Endocrine and auto-immune diseases:** ALP concentrations are raised in hyperthyroidism and hyperparathyroidism. Some authors report changes in serum ALP in auto-immune diseases such as insulin-dependent diabetes, rheumatoid arthritis and multiple sclerosis.
- **Haematological disease:** serum ALP concentrations are raised in leukaemia and myelofibrosis in parallel with bone changes. Conversely, concentrations are reduced in pernicious anaemia.
- **Oncological diseases:** some tumours produce "abnormal" isoenzymes. The Regan "placental-like" isoenzyme is similar to placental ALP and is present in lung, uterine and ovarian cancers. The Kasahara *"intestinal foetal-like"* isoenzyme is produced by liver metastases from bowel tumours and the Nagao *"placental-like"* isoenzyme is present in testicular seminomas and thyroid tumours.

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ALPHA-1 ANTITRYPSIN

(Faecal clearance)

DEFINITION

A measurement of alpha-1 antitrypsin (AAT) clearance can be used to investigate suspected protein-losing syndrome. Alpha-1 antitrypsin, one of the main protease inhibitors in the serum, is mainly synthesised in the liver. It is neither absorbed nor secreted in the gut and, unlike other plasma proteins, is resistant to digestive and bacterial proteases in the intestine. For this reason, it is an ideal marker for gastrointestinal protein loss. Although its blood concentration is usually relatively stable over the short term, its rate of production can rise in response to inflammation so, to avoid possible distortion, the parameter used in preference is faecal alpha-1 antitrypsin clearance rather than its absolute concentration.

$$\text{AAT clearance} = \frac{\text{Faecal AAT (g/l)} \times \text{Mean weight of faeces passed over 24 h (g)}}{\text{Serum AAT (g/l)}} \text{ (ml/24h)}$$

INTRODUCTION

Protein-losing syndrome is characterised by the excessive loss of plasma protein into the gut lumen and is due to enhanced intestinal permeability to proteins resulting from either pathological involvement of the mucosa or sub-mucosa (eosinophilic gastroenteritis, coeliac disease, sprue, milk protein intolerance, chronic inflammatory bowel disease, etc.) or abnormal lymph flow due to obstruction of lymphatic drainage (primary or acquired intestinal lymphangiectasia). Although methods based on radio-labelled compounds such as albumin have shown the gastrointestinal aetiology of "idiopathic hypoproteinaemia", these radioactive markers are unsuitable for routine use. To overcome this problem, faecal alpha-1 antitrypsin clearance can be measured.

INDICATIONS FOR MEASUREMENT

This test is indicated in a patient with oedema and/or hypoproteinaemia coupled with hypoalbuminaemia and hypogammaglobulinaemia, in the absence of proteinuria, inflammatory syndrome or liver failure, i.e. in the context of a work-up to investigate suspected protein-losing gastro-enteropathy.

INFORMATION

■ SAMPLE

An AAT clearance assay can only be performed if all the faeces passed during a given period of time have been collected in a special recipient (taking care not to mix faeces and urine). For the sake of convenience and hygiene, the faeces passed per 24 hour period are collected into one or more non-transparent recipients of adequate capacity (usually one litre) and kept in a refrigerator. Each recipient should be marked with the date so that the total weight passed each day can be recorded and, subsequently, the mean daily weight over the collection period can be calculated. All the faeces passed over three successive days are collected, making sure not to forget those passed at night. A single day of collection is probably insufficient but could be considered for subjects with slight spontaneous variations in the rate of transit and in young children. A fasting blood sample is taken on one of the three faeces collection days for a serum AAT determination.

No radiological examinations with contrast medium or preparation for colonoscopy (which alter the gut ecosystem) should be performed in the eight days leading up to and during collection. During the collection period, it is important to avoid products that could distort the result, such as laxatives (e.g. paraffin oil), bowel cleansers or anti-motility drugs.

Faecal clearance test results are difficult to perform and interpret in children, and even more so in babies, because of age-related variability in daily evacuation rates coupled with problems with 24-hour faeces collection (for which, to prevent the absorption of faecal fluid, diapers cannot be used). For these reasons, a simple quantitative determination is usually performed with the result expressed with respect to dry faecal material (necessitating measurement of the dry weight).

A patient with suspected protein-losing gastropathy should take an antacid for five days before and during collection because AAT is destroyed at pH values of below 3.

This test is not applicable in subjects with congenital serum alpha-1 antitrypsin deficiency.

■ SAMPLE STORAGE AND TRANSPORT

Total faeces (24, 48 or 72 hours) or an aliquot of the mixed 24-hour samples (with details of daily evacuation rates) together with the serum sample (drawn into a dry tube) can be kept at +4°C if the tests are to be performed within one week of collection; otherwise, if the test is to be performed later than that, the sample should be frozen to –20°C within 24 hours of sampling.

ASSAY METHODS

In serum, alpha-1 antitrypsin is assayed by immunonephelometry. In faecal material, the assay is based on immunonephelometry or radial immunodiffusion.

NORMAL EXPECTED VALUES

Serum alpha-1 antitrypsin: 0.9-2.1 g/l

Faecal alpha-1 antitrypsin: < 0.15 g/l

Faecal alpha-1 antitrypsin: < 1.25 mg/g of dry material.

$$\text{AAT concentration in the dry material} = \frac{\text{AAT faecal (g/l)} \times 1\,000}{\text{Dry weight (\%)}} \text{ (mg/g)}$$

Alpha-1 antitrypsin clearance: < 20 ml/24h.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Physiological variability in intestinal transit rates justifies the collection of total faeces over a three-day period. All the collected faeces are then thoroughly mixed and the test

performed on an aliquot of this mixture. The result is expressed as the 24-hour mean.

In babies, the faecal AAT concentration reaches the same level as in healthy adults by the age of six days.

PATHOLOGICAL VARIATIONS

An AAT clearance rate of over 20 ml/day should be considered as abnormal; this is to avoid misinterpreting increases due to diarrhoea with temporary acceleration of transit. AAT clearance rates of 20-30 ml/day are considered as corresponding to minor protein loss; rates of 31-70 ml/day as moderate; and rates of over 70 ml/day as severe.

Since faecal AAT excretion is increased in gastrointestinal bleeding, interpretation of the AAT assay result may be complemented if the clinical picture is not informative, by a parallel assay for blood in the faeces (a human haemoglobin test).

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ALPHA-1 ANTITRYPSIN

DEFINITION

Alpha-1 antitrypsin (AAT) is a small glycoprotein (51 kDa) produced in the liver. It accounts for 90% of the alpha-1 globulin fraction in protein electrophoresis. In plasma, it inhibits serine proteases, which are a family of proteolytic enzymes that includes elastase, trypsin, chymotrypsin, plasmin and thrombin. Alpha-1 antitrypsin deficiency is a genetic disease which is transmitted as a recessive, autosomal trait. In children, deficiency is associated with impaired liver function, and in adults with impaired lung function. Alpha-1 antitrypsin deficiency is common in populations from northern Europe.

Synonyms: α-1antitrypsin (AAT); alpha 1- proteinase or protease inhibitor (α1-Pi); Prolastin; anti-elastase; Trypsin proteinase or protease inhibitor; serpin protease inhibitor; AAT gene or α1AT gene; serpin A1.

INTRODUCTION

PHYSIOLOGICAL ROLE

Alpha-1 antitrypsin is a plasma glycoprotein that is mostly produced in the liver and, to a lesser extent, by pulmonary epithelial cells and monocytes. Alpha-1 antitrypsin belongs to the family of serpins (serine proteinase inhibitors) which inhibit the catalytic activities of various serine proteases (enzymes released by a variety of cells in inflammatory processes, notably by neutrophils and macrophages). The function of alpha-1 antitrypsin is to protect lung tissue against the catalytic activity of human neutrophil elastase. When this glycoprotein is deficient, these tissues are unprotected and this elastase causes damage; constitutional deficiency can thus lead to lung emphysema or progressive cirrhosis of the liver.

THE GENE AND ITS VARIANTS

The alpha-1 antitrypsin gene is highly polymorphic with more than 100 known alleles. Most of these alleles encode protein variants with normal biological activity that are synthesised at a normal rate. These variants were initially distinguished on the basis of differences in electrophoretic mobility on isoelectric focusing. A letter from the beginning of the alphabet was chosen for variants that migrate towards the anode, and a letter from the end of the alphabet was chosen for variants that migrate towards the cathode: M for medium (normal), F for fast, S for slow, and Z for very slow). For the normal phenotype (M), 8 variants have been characterised (M1 to M8), none of which have any clinical consequences. The variants that are associated with alpha-1 antitrypsin deficiency are the S, Z and null variants *(Table 1)*.

The gene that encodes alpha-1 antitrypsin—the Pi (for proteinase inhibitor) locus—is located on the long arm of chromosome 14 at 14q32.1. It contains seven exons (Ia, Ib, Ic, II, III, IV, V) stretching over a sequence of about 12.2 kb. Its product is a 52 kDa protein containing 394 amino acids (Figure 1). The protein is secreted into the plasma in which its concentration is between 1.32 and 2.5 g/l. Alpha-1 antitrypsin is the main protein in the α1 globulin fraction seen when serum is analysed by gel electrophoresis.

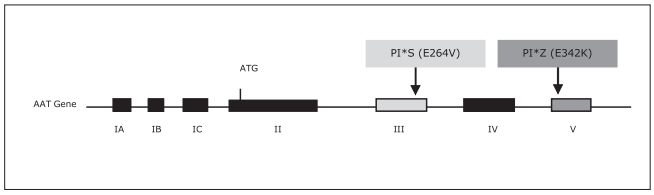

Figure 1. The α1-antitrypsin gene and the positions of the mutations in the S and Z variants (14q32.1).

In any individual, both AAT alleles are expressed, i.e. they are co-dominant. Genetic transmission of alpha-1 antitrypsin deficiency is autosomal and co-dominant. A person with normal alpha-1 antitrypsin (genotype: PI*MM) inherited one normal M allele from each parent. Hereditary alpha-1 antitrypsin deficiency results from a mutation which either prevents transcription of the gene (no synthesis, PI*null-null variants) or inhibits liver cell secretion of alpha-1 antitrypsin into the plasma (PI*ZZ variant). Deficiency arises when both parents pass on a null or Z allele to their offspring (PI*null-null or PI*ZZ homozygotes). Depending on the subject's genotype, alpha-1 antitrypsin deficiency may be mild, moderate or severe, and this explains the variability in the disease's symptoms.

Allele variants

Z variant

This is the most common variant in populations from northern and western Europe where its frequency is between 1% and 3%.

In Caucasians, the prevalence of homozygous PI*ZZ alpha-1 antitrypsin deficiency is between 1/2000 and 1/4000, with a decreasing gradient going from the north-west to the south-east. The Z variant is due to a point mutation (E342K) in exon 5 of the gene which leads to substitution in the protein of glutamic acid (E) at position 342 by lysine (K). This substitution interferes with the folding of the protein resulting in its retention in the endoplasmic reticulum of the liver cells that synthesise it. Retention and build-up of the defective alpha-1 antitrypsin lead to deficiency in the plasma and cause liver damage in the new-born baby. In subjects with the PI*ZZ genotype, the plasma alpha-1 antitrypsin concentration is of the order of 10-15% of that found in normal subjects (genotype: PI*MM).

The estimated frequency of heterozygous carriers of the Z allele (genotype: PI*MZ) is 3-5%. PI*MZ heterozygotes have a plasma alpha-1 antitrypsin concentration of 50-60% of that found in normal PI*MM subjects, and this is not associated with symptoms.

S variant

The S allele is most common in southern European populations with the number of carriers decreasing as one goes from the south-west to the north-east. It is most common in Spain and Portugal, with up to 28% of the subject carriers. The S allele is due to a point mutation (E264V) in exon 3 which leads to substitution in the protein of glutamic acid (E) at position 264 by valine (V). Between 5% and 10% of Caucasian subjects carry the S allele (genotype: PI*MS). Homozygous PI*SS subjects have a plasma alpha-1 antitrypsin concentration of 50-60% of that

found in normal PI*MM subjects. No symptoms are observed in either PI*SS homozygotes or PI*MS heterozygotes but composite PI*SZ heterozygotes—whose plasma alpha-1 antitrypsin concentration is 30%-35% of that found in normal PI*MM subjects and may have clinical manifestations (although this is controversial).

Other variants

Two other variants (apart from Z variant) are associated with alpha-1 antitrypsin deficiency. The Siiyama variant (S53F—substitution of serine at position 53 by phenylalanine) is the most frequent variant in Japan; and the Mmalton (or Mnichinan or Mcagliari) variant (delF52—deletion of the phenylalanine at position 52) which is common in Sardinia.

Null variants

A number of null variants have been identified although these are rare in Caucasians. They result from deletions, microdeletions or point mutations within the gene which cause the appearance of a premature stop codon: PI* null Granite Falls Y160X; PI*null Bellingham L217X; PI*null Procida 17Kbdel. In PI* null-null homozygotes, deficiency is total and no alpha-1 antitrypsin at all is synthesised by the liver cells.

Variant	Molecular lesion	Alpha-1 antitrypsin/ normal subject %	Problems in the homozygote	Estimated frequency %
M1 (Ala^{213})		100	none	20-23
M1 (Val^{213})	A237 V	100	none	44-49
M2 (His^{101})	R101 H	100	none	10-11
M3 (Asp^{213})	E376 D	100	none	14-19
F	R223 C	-	none	rare
$P_{St.\ Albans}$	D341 N	100	none	rare
S	E264 V	40-70	pulmonary	2-4
Z	E342 K	<15	pulmonary and hepatic	1-2
M_{malton}	delF52	-	hepatic	Sardinia
$M_{procida}$	L41P	-	pulmonary	rare
$M_{heerlen}$	P369 L	2-5	pulmonary	rare
AAT Siiyama	S53F	<15	pulmonary and hepatic	Japan
null	Deletions and nonsense mutations	0	pulmonary and/or hepatic	rare

Table 1. Prevalence of α1-antitrypsin alleles and concentrations in homozygotes (white United States population).

- **In new-born babies** with severe deficiency, impaired liver function leads to neonatal cholestatic hepatitis accompanied by hypertrophy of the liver and spleen, pruritus, jaundice and abnormal liver function test results (elevated transaminase activities). A liver biopsy shows aggregates of polymerised alpha-1 antitrypsin. If liver function is only moderately abnormal, the problem resolves within 6-12 months although cirrhosis may develop before the age of 20.

- **In adults,** impaired lung function leading to emphysema is the most common manifestation (the severity depending on the level of the α-1 antitrypsin deficiency). The risk of developing emphysema is exacerbated by smoking, asthma and lung infection.

Lung	**Adult with symptoms** – bronchodilator-refractory asthma – emphysema – chronic bronchopneumopathy – unexplained bronchiectasis – ANCA-associated vasculitis **Adult without symptoms** – unexplained impairment of lung function – pulmonary sensitivity associated with chronic disease
Liver	Idiopathic liver involvement Hepatic sensitivity associated with chronic disease **Child with symptoms:** neonatal cholestatic hepatitis
Family history	Family history of AAT deficiency Family history of liver or lung disorders Prenatal work-up for parents with known AAT deficiency

INDICATIONS FOR MEASUREMENT AND FOR DEFICIENCY TESTING

– AAT is a positive marker for inflammation (hepatic synthesis) and an assay can be considered in this context.

– Suspected AAT deficiency and/or a low serum AAT concentration.

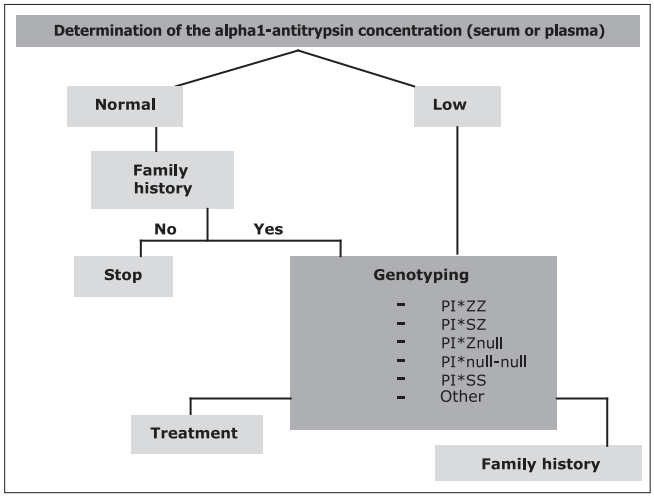

Table 2. Diagnosing alpha-1 antitrypsin deficiency.

INFORMATION

SAMPLE

- **AAT assay: Serum** (Dry tube). Quantitative assay must be avoided in subjects on lipid-based parenteral alimentation.
- **Phenotyping:** 5 ml of blood in EDTA. Ideally, the subject should be fasting to minimise hyperlipidaemia, especially if there is a history of hypercholesterolaemia or hypertriglyceridaemia. Serum should be quickly decanted off to minimise *in vitro* haemolysis.

Order form

Provide information about why the genetic test is being ordered, notably the clinical observations and test results that point to alpha-1 antitrypsin deficiency and, if the index case is identified, genealogical details.

QUESTIONS FOR THE PATIENT

Deficiency can be masked in certain situations in which the AAT concentration tends to be elevated. Ask women about pregnancy (during which the concentration doubles) and use of oral or steroid contraceptives (elevation). Ask all patients about infectious and inflammatory syndromes (elevation), recent surgery (elevation), and cancer of the liver or pancreas (variations independent of constitutional synthesis). Sampling should be postponed if the patient is suffering from seasonal allergic asthma (elevation) or immediately after physical exercise (elevation).

It is important to know about these situations for heterozygote screening and in the event of inflammation with a high CRP concentration, the AAT assay should be postponed for 10-14 days; in homozygotes, the AAT concentration is unaffected.

SAMPLE STORAGE AND TRANSPORT

Serum samples are stable for 7 days at 20-25 °C, and for 3 months at 2-8 °C. Whole blood can be kept at room temperature for up to 24 hours: beyond that, store at 2-8°C.

ASSAY METHODS

– AAT assay: Immunonephelemetry. The method based on the evaluation of the inhibition of serum proteolytic activity is no longer used.

– AAT phenotyping: Starch gel immunoelectrophoresis.

Many methods have been developed to characterise S and Z variants, based on polymerase chain reaction (PCR) amplification of part of the AAT gene containing the mutation. There are a number of ways of characterising the mutation from the PCR product, including:

– Treatment of the amplified product by restriction endonucleases (the PCR-restriction fragment length polymorphism method).

– Amplification of wild-type and mutated allele sequences using specific primers (the PCR sequence-specific primers method) or modified primers (the PCR amplification-refractory mutation system method).

– Hybridisation of the amplified product with probes specific to the wild-type and mutated allele sequences (the PCR allele-specific oligonucleotide method).

– Real-time PCR based on detection of a fluorescent signal (hydrolysis probes, tandem probes, molecular beacons, scorpion probes, fluorescent primers).

– Treatment of the amplified product by primer extension (minisequencing or SNaPshot, Single base extension ELISA).

– Sequencing of the amplified products.

NORMAL EXPECTED VALUES

For reference purposes:

Adults:	0.9 - 2.1 g/l
Children:	New-born: 1.25 - 2.5 g/l
	1 month - 15 years: 0.98 - 1.96 g/l

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

The serum AAT concentration rises during pregnancy and on an oestrogen-containing oral contraceptive.

PATHOLOGICAL VARIATIONS

An electrophoretic protein profile is a good way of investigating AAT deficiency because AAT is the main protein in the alpha-1 globulin peak. A concentration of less than 0.7 g/l is strongly indicative of homozygote deficiency.

Immunoelectrophoresis gives the patient's definitive phenotype.

The AAT concentration rises in active inflammatory processes, cancer of the pancreas and liver, acute hepatitis, haematological disorders and allergic asthma.

TREATMENT OF DEFICIENCY

If the disease leads to liver failure, the only effective treatment is liver transplantation. The management of impaired lung function is based on the infusion of exogenous alpha-1 antitrypsin protein.

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ALPHA-1 MICROGLOBULIN

DEFINITION

Alpha-1 microglobulin (α1M) is a small molecular weight protein (33 kDa) which acts as a stable, sensitive urinary marker for disease involving the renal tubules. Normally, its concentration in the urine is very low.

INTRODUCTION

In tubular disease, small proteins (<40 kDa) that would normally be re-absorbed in the proximal tubule are re-absorbed inefficiently and pass into the urine. These proteins are commonly referred to as microproteins because of their small molecular weight and the fact that their physiological concentration in the urine is low. These proteins are alpha-1 microglobulin, β-2 microglobulin, retinol binding protein and free immunoglobulin light chains. If sustained proteinuria (>150 mg/24h) has been detected, the proteins involved should be identified and quantified. Specific protein assays make it possible to make a quick diagnosis of tubular problems. The ideal marker in this context is α1M which is commonly used to investigate impaired tubule function following exposure to certain toxins (e.g. trichloroethylene) or heavy metals (e.g. cadmium).

INDICATIONS FOR MEASUREMENT

Investigation of tubule disease: Diagnosis and monitoring of disease.

Proposed as a predictive marker of chronic rejection in kidney transplantation.

INFORMATION

■ SAMPLE

24-hour urine collection: Stipulate the 24 hours urine volume and the subject's age and gender.

■ QUESTIONS FOR THE PATIENT

Are you being treated for cancer? A recent report showed increases in the concentration of α1M in the urine of children being administered cytostatic drugs to treat leukaemia and lymphoma.

■ SAMPLE STORAGE AND TRANSPORT

Store and transport at +4°C.

ASSAY METHODS

Immunonephelemetry.

NORMAL EXPECTED VALUES

Normal values vary according to the assay method. For reference purposes, <10 mg/24 hours in adults.

PATHOLOGICAL VARIATIONS

– Alpha-1 microglobulin is the ideal marker for early diagnosis of tubule disease. It has a number of advantages over the two other laboratory markers routinely used to detect impaired tubular function, namely β_2-microglobulin and RBP: α1M is sensitive and is stable, even if the pH of the urine is low; the rate of its excretion rises rapidly with tubular involvement; its concentration rises to a very high level; and it is very little affected by extrarenal factors (although a rise in urinary α1M was recently reported in children infected with hepatitis C virus). In contrast, β_2-microglobulin is unstable at low pH (< 5.5) and its concentration in the urine can rise in various other circumstances, notably kidney failure, immunologic disease and neoplasia; RBP is less sensitive than α1M and the amplitude of its response is smaller.

Interpretation of the main changes in the urinary concentrations of protein markers for impaired tubule function *(taken from Le Carrer)*.

Alpha-1 microglobulin	Normal	Elevated	Elevated	Elevated
Beta-2 microglobulin	Normal	Elevated	Normal	Elevated
Retinol Binding Protein	Normal	Elevated	Elevated	Normal
Interpretation	***Normal tubular function***	***Tubule disease certain***	***Impaired tubule function very likely*** (urine pH < 5.5)	***tubule disease likely*** (limited sensitivity of the RBP assay)

– Kidney transplantation

A Finnish group recently showed that an increase in the ratio of α1M to creatinine in the urine is an early, sensitive marker for poor long-term prognosis in kidney transplant patients.

– Toxicology

α1M has been proposed as a marker for monitoring the renal toxicity of trichloroethylene following exposure. It would also be a better marker than β_2-microglobulin for cadmium-induced tubule damage.

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ALPHA-2 MACROGLOBULIN

DEFINITION

Alpha-2 macroglobulin (A2M) is a 720 kDa glycoprotein produced in the liver, and by macrophages and fibroblasts at sites of inflammation. It consists of four identical polypeptide sub-units connected by disulphide bridges, linked together by carbohydrates. It has a half-life of five days.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

A2M is a marker for fibrosis used to monitor chronic liver disease. In patients with alcoholic cirrhosis, it is monitored in parallel to prothrombin time, gammaglutamyl transpeptidase (GGT) and apolipoprotein A1). In patients with hepatitis C it is followed in parallel to the other parameters of the Fibrotest® (haptoglobin, bilirubin, apolipoprotein A1 and GGT). In all these patients, inflammation and fibrosis activate a cytokine, hepatocyte growth factor, which stimulates A2M synthesis.

A2M is at a high concentration in the serum, accounting for 80% of the alpha-2 globulin fraction. It is the main protease inhibitor in plasma and inhibits most proteolytic enzymes, including trypsin, plasmin, thrombin and the metalloproteinases which break down the extracellular matrix. A2M inhibits proteases by forming a complex with them. The resultant complexes are rapidly broken down by the reticuloendothelial system. A2M also binds and transports numerous species, including growth factors, cytokines and hormones.

INFORMATION

SAMPLE

Serum or plasma: Drawn into heparin, preferably from a fasting subject.

Reject haemolysed samples and hyperlipaemic samples.

QUESTIONS FOR THE PATIENT

Stipulate the patient's age and gender.

SAMPLE STORAGE AND TRANSPORT

Storage of serum or plasma: 1 week at +4°C or several months at –20°C.

ASSAY METHODSS

Immunochemistry: Immunonephelemetry, immunoturbidimetry.

Reference substance: CRM470.

Precautions: Cloudy serum samples and thawed samples should be centrifuged for ten minutes at 1,500 g before testing.

NORMAL EXPECTED VALUES

Adults: 1.3-3 g/l. Values are 10% higher in women and during pregnancy.

Children: values are 50% higher and remain higher than those in adults until the age of 16.

For reference:

Children < 2 months	2.7-5.3 g/l
Children of between 2 months and 10 years	2.8-5.6 g/l
Children of 11-14	2.6-5.2 g/l
From 15-16	2.0-3.9 g/l
Adults	1.3-3.0 g/l

PATHOLOGICAL VARIATIONS

- **Liver disease**

Hepatic fibrosis and hepatitis C:

Assaying A2M together with the other Fibrotest® markers (haptoglobin, bilirubin, apolipoprotein A1 and GGT) yields a fibrosis index which is used as an alternative to liver biopsy analysis.

Alcoholic cirrhosis:Parallel assays of prothrombin time (P), GGT (G), apolipoprotein A1 (A) and A2M (A) yield the PGAA index which can be used to define whether an asymptomatic chronic alcoholic patient has cirrhosis or not.

- **Kidney disease**

In nephrotic syndrome, a drop in albumin is associated with an increase in the concentration of high molecular weight proteins, including A2M.

A2M is a marker for impaired glomerular function because, being so big, it is not normally filtered by the glomeruli.

- **In inflammatory syndrome,** the increase in the concentration of A2M is far less marked than that of CRP (C-reactive protein), orosomucoid or haptoglobin.
- **Reduced alpha-2 macroglobulin**

Reductions, usually modest in the concentration of alpha-2 macroglobulin, are observed in pneumonia and pleurisy, gallstones and kidney stones, gastroduodenal ulcers, gastritis and some forms of cancer.

FOR FURTHER INFORMATION

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ALPHA-2 ANTIPLASMIN

DEFINITION

Alpha-2 antiplasmin (2-AP) is a glycoprotein which is produced in the liver. It specifically inhibits plasmin in the blood, immediately and irreversibly, thereby regulating fibrinolysis. Its half-life in plasma is close to three hours.

Synonym: 2-AP.

INTRODUCTION

Fibrinolysis is a physiological phenomenon in which fibrin clots are solubilised by plasmin, an enzyme generated from plasminogen, which is bound to and absorbed by fibrin.

In this system, alpha-2 antiplasmin mediates three main functions. It inhibits plasmin, blocks the adsorption of plasminogen onto fibrin and it binds to the alpha-chain of fibrin.

In normal physiological conditions, the plasmin formed when plasminogen comes into contact with a thrombus is initially neutralised by alpha-2 antiplasmin covalently bound to the fibrin through factor XIIIa. This prevents lysis of the haemostatic clot occurring to early. Subsequently, once the plasmin-inhibiting capacities of the alpha-2 antiplasmin bound to the clot have been saturated, excess plasmin begins to break the fibrin down. If alpha-2 antiplasmin is deficient or non-functional, premature lysis of fibrin clots can lead to haemorrhagic syndrome.

INDICATIONS FOR MEASUREMENT

This is a second-line test ordered to investigate unexplained haemorrhagic syndrome. Only a few specialised laboratories can perform this test.

INFORMATION

SAMPLE

Draw the blood into citrate at a proportion of 10% (v/v, i.e. 0.5 ml for 4.5 ml blood) citrate solution (3.2%, 0.109 M). Citrate tubes (3.8%, 0.129 M) are also accepted. The blood can also be collected on CTAD (citrate, theophylline, adenine, and dipyridamole) which ensures better conservation of the sample; this collection system should be used if the sample is not to be dispatched within two hours. No other anticoagulant is suitable.

Ideally the patient should give blood on an empty stomach and he/she should have been at rest for at least ten minutes.

For further information, refer to the «Conditions préanalytiques générales en hémostase» ("General pre-test information for clotting tests") sheet.

Transport the sample to the laboratory immediately after drawing the blood from the patient. The sample must platelet depleted and frozen within 2 hours from the point the sample is taken.

QUESTIONS FOR THE PATIENT

Are you taking any drugs? Alpha-2 antiplasmin levels drop in response to fibrinolytic drugs such as streptokinase, urokinase, rt-PA and tenecteplase.

Do you have any bleeding-related symptoms?

SAMPLE STORAGE AND TRANSPORT

The test is carried out on platelet-depleted plasma obtained by centrifugation (15 minutes at 2000-2500 g, at a temperature of 10-20°C). If the test is to be carried out later, the plasma should be decanted off into a polyproplylene or PET tube and re-centrifuged before freezing.

Separated plasma can be kept for one month at –20°C, and for about three months at –80°C.

Transport the sample to the reference laboratory in its frozen state.

ASSAY METHODS

Amidolytic chromogenic method: The principle underlying the test method is the rapid inhibition of plasmin. After the addition of a defined amount of plasmin, residual plasmin is quantitatively determined using a specific chromogenic substrate.

REFERENCE VALUES

Results are usually expressed as the percentage of the normal value or in IU/ml (1 IU/ml = 100%). Reference values in babies (of over one month of age), children and adults: 80-120%.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

In new-born babies (the first month), reference values are lower: 55-115%.

In pregnant women, the reference value is between 80% and 150%.

PATHOLOGICAL VARIATIONS

Alpha-2 antiplasmin activity is decreased in liver failure and a few cases of congenital deficiency, associated with haemorrhagic syndrome, have been reported.

A constitutional deficiency can only be definitively diagnosed after a repeat test conducted after a lapse of time has given the same result, and once the possibility of acquired deficiency (which is far more likely) has been ruled out and an enquiry into the family history has been made.

FOR FURTHER INFORMATION

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- Aillaud M.F., *Alpha-2 antiplasmine*, Encycl Méd Biol (Elsevier, Paris), 2003.
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ALPHA FOETOPROTEIN

DEFINITION

Alpha foetoprotein (AFP) is a glycoprotein produced in the fœtal yolk sac and, later, in the fœtal liver and gut. Its production rate falls off after delivery but it is still found at low concentrations in adult blood. It has a half-life of 3.5 - 6 days.

INTRODUCTION

In fœtal blood, AFP may act as a multi-purpose carrier, like albumin in adults. In adults, the serum AFP concentration is normally low and a rise may point to a neoplastic process involving de-differentiated or undifferentiated cells exhibiting some of the properties of foetal cells.

INDICATIONS FOR MEASUREMENT

In oncology, in conjunction with an hCG test, blood AFP is used as a marker for hepatocellular carcinoma and non-seminomatous germ cell tumours (testicular cancer).

In gynaecology and obstetrics, it is mainly used to screen for chromosomal abnormalities or open neural tube defects in the second trimester of pregnancy.

AFP can also be assayed in various types of sample, including aspiration biopsy fluids and amniotic fluid.

INFORMATION

Haemolysis or hyperlipidaemia can interfere with certain assay methods.

■ SAMPLE

Serum or plasma: Refer to the manufacturer's instructions, depending on the method.

The test can also be carried out on various types of sample (aspiration biopsy fluid, etc.).

No circadian rhythm reported.

It is not essential that the subject be fasting when the sample is taken.

■ QUESTIONS FOR THE PATIENT

In oncology: Stipulate the disease and any treatment underway (modalities and dates).

In pregnant women: Give exact dates concerning the pregnancy.

■ SAMPLE STORAGE AND TRANSPORT

Can be kept for 4 days at between 2 and 8°C, or for 2 years at –20°C.

Transport at +4°C (serum, plasma or aspiration biopsy fluid).

ASSAY METHODS

Immunometric sandwich method.

NORMAL EXPECTED VALUES

The usual serum concentration is < 10 ng/ml but this may vary according to the assay method used.

– International standard: CRM 486 (100 micrograms of purified AFP per vial)

– Conversion factor: 1 IU = 1.126 ng

– BRAHMS KRYPTOR (cancer marker): 1 ng CIS = 1 IU 1st IRP WHO 72/225

– PERKIN ELMER Autodelfia (HT21): KU*0,826 = µg/l or µg/l*1.21 = KU IRP 72/22

In pregnancy: The results of the test are usually expressed as multiples of the median which are obtained by dividing the observed value by the median value for that term of the pregnancy.

Mean target value at Week 16: 35 ng/ml (to be adjusted according to the mother's weight and, if relevant, if the pregnancy is multiple or the mother suffers from intercurrent Type-1 diabetes).

Between Weeks 15 and 18: a "normal" value will fall between 34 and 58 ng/ml (28 and 48 IU/ml).

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

In the pregnant woman, the serum AFP concentration begins to rise around Week 12 and continues on up through Week 30. It is affected by a range of different factors, both physiological and pathological, including the mother's weight, the presence of more than one foetus, intercurrent Type-1 diabetes, and foetal malformations and/or kidney problems. After delivery, values rapidly drop back down to normal.

In the new-born baby, the serum concentration of AFP remains high throughout the first few months of life, especially in premature babies. It subsequently steadily drops, reaching adult values by about 8 months.

AFP (ng/ml)	
Premature baby	95 000 – 175000
New-born baby	13000 – 83000
2 weeks	500 – 66000
2 weeks	20 – 19000
2 weeks – 1 month	20 – 19000
1 month	20 – 5600
2 months	20 – 600
3 months	10 – 180
4 months	10 – 130
5 months	10 – 70
6 months	0 – 20
7 months	0 – 17
8 months	0 - 15

Serum AFP concentrations at different ages *(taken from S. Loric, Cahier de Formation Biochimie (Biochemistry Training Manual), Volume IV,* Bioforma 1999).

PATHOLOGICAL VARIATIONS

Non-specific rises in serum AFP concentration

– Liver disease: Acute or chronic hepatitis, cirrhosis and during liver regeneration (e.g. following hepatectomy or shared liver transplantation)

– Intestinal disorders: Crohn's disease, polyps

– Dialysis.

Rises in serum AFP concentration due to cancer

Hepatocarcinoma:

The serum AFP concentration increases, sometimes to extremely high levels in 70-90% of patients. This test can be used to detect hepatocellular carcinoma (HCC) in at-risk populations such as chronic carriers of the HBs antigen or cirrhosis sufferers (in whom a test is recommended every six months). The threshold value pointing to HCC in these populations is estimated to 200-400 ng/ml.

AFP testing is also useful for monitoring disease progression after surgical excision; a rise allows for the screening of local or metastatic recrudescence.

Non-seminomatous testicular tumours

An AFP test affords a sensitivity of between 60% and 90% (depending on the expert). Its serum concentration seems to correlate with the grade of the disease. To monitor patients, testing for both AFP and hCG is recommended every two months for the first six months, every three months for another six months, and once every four months for one year thereafter. After total surgical excision, AFP levels return to normal within less than a month. During a course of chemotherapy, serum AFP levels correlate with the clinical response; a drop of more than 50% predicts a positive outcome while an increase during monitoring is suggestive of recrudescence or metastasis.

Increase in serum AFP concentrations in obstetrics

Between Weeks 15 and 18, a drop in the maternal blood AFP concentration of the order of 30% points to a chromosomal abnormality. The mean AFP value drops to 0.7 multiples of the median in trisomy 21, and to 0.6 multiples of the median in trisomy 18.

A high AFP concentration (≥ 2.5 times the median) in the second trimester of pregnancy may point to an open neural tube defect in the foetus (spina bifida, anencephaly), an abdominal wall problem (omphalocoele, laparoschisis), a kidney malformation or a leakage of foetal blood into the mother's circulation (retroplacental haematoma).

Other factors may influence the serum AFP concentration in the pregnant mother's blood, including the presence of a placental angioma and molar pregnancy as well as a number of other foetal defects. Abnormal values with respect to those expected given the term of the pregnancy: if it is high, an ultrasound examination should be ordered to investigate closure of the neural tube and the abdominal wall; and if there is a risk of foetal trisomy 21, amniocentesis should be considered.

FOR FURTHER INFORMATION

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ALPHA SUBUNIT

DEFINITION – INTRODUCTION

Together with human chorionic gonadotropin (hCG) the three pituitary glycoprotein hormones; LH, FSH and TSH are formed from two α and β subunits bound non-covalently. Whereas the β subunit is specific to each hormone, all 4 hormones have the same common α subunit. As the free subunits are not biologically active they need to be bound to the α and β subunits to produce the specific hormone action.

The α subunit is completely identical in the same species in all three pituitary glycoprotein hormones and chorionic gonadotropin. It is formed from 92 amino acids and is coded for by a single gene.

The α subunit is secreted in a pulsatile manner by anterior pituitary thyrotropic and gonadotropic cells and possibly by lactotropic and somatotropic adenoma cells. Under physiological conditions examination of its pulsatility has revealed episodic secretion coincident with fluctuations in LH, suggesting that LHRH has a stimulatory effect. Physiological variations and pharmacological stimulation or inhibition of the pituitary glycoprotein hormones are therefore accompanied by parallel changes in the α subunit. There is however one exception to this rule, which is observed during long-term treatment with LHRH agonists, where α subunit concentrations remain high, whereas the LH and FSH concentrations are very low. The stimulatory effect of TRH in normal people is less spectacular.

The α subunit does not appear to have biological activity.

INDICATIONS FOR MEASUREMENT

α subunit measurement is used in the investigation of the anterior pituitary when disease due to an adenoma may be responsible.

It can be used in hypo and hyperthyroidism with inappropriate TSH secretion to differentiate pituitary thyroid hormone resistance from thyrotropic adenomas. It is also used for post-treatment monitoring of thyrotropic adenomas.

α subunit measurement is required in the investigation of gonadotropin pituitary adenomas and particularly so-called non-functional pituitary adenomas as α subunit secretion is not associated with specific endocrine signs as it has no biological activity.

INFORMATION

SAMPLE

The subunit is measured in heparinised plasma or serum.

QUESTIONS FOR THE PATIENT

Clinical diagnosis for which the α subunit is being measured must be reported: thyrotropic, gonadotropic or silent adenomas. Similarly, the request should state whether measurement is part of a TRH or LHRH stimulation test.

SAMPLE STORAGE AND TRANSPORT

Separated plasma or serum must be stored at –20°C within 4 hours of sampling and transported at this temperature to the laboratory. Samples can be stored for approximately two months, frozen at – 20°C.

ASSAY METHOD

The α subunit is currently measured using an immunoradiometric technique (IRMA) with two monoclonal antibodies. Standards are calibrated against the international reference preparation (1st WHO IRP 75/569) for the hCG α subunit.

This method is very specific, as a cross-reaction with all of the glycoprotein hormones is less than 0.1% and also highly sensitive as the limit of detection is extremely low.

USUAL VALUES

Peripheral blood α subunit concentrations are very low in people without any endocrine disease. Concentrations reflect an imbalance between α sub-unit and β sub-unit production. Usual adult values are shown in the table blow.

The kit standards used were calibrated against the international standard WHO 75/569: 1 µg of α-hCG = 1 IU 1st IR.

	α subunit (mIU/ml)
Non-menopausal women	0.02 – 0.9
Post-menopausal women	0.02 – 1.6
Men	0.02 - 0.8

DYNAMIC TESTS

The TRH test only produces a small rise in the α subunit in normal adults. The α subunit, however, increases significantly in response to TRH in pre-pubertal boys and girls.

The rise in the α subunit varies in magnitude depending on the stage of puberty in the LHRH test.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

α subunit concentrations are low in the pre-pubertal period and rise at puberty.

Pubertal stage and (age)	α subunit (mIU/ml)
Usual values in boys	
Pre-pubertal boys (3 to 12 years old)	< 0.10 to 0.40
Puberty stage 2 (11 to 15 years old)	< 0.10 to 0.50
Puberty stage 3 or 4 (13 to 18 years old)	0.20 to 1.00
Usual values in girls	
Pre-pubertal girls (3 to 11 years old)	< 0.10 to 0.60
Puberty stage 2 (9 to 13 years old)	< 0.10 to 0.60
Puberty stage 3 or 4 (10 to 15 years old)	0.10 to 0.80

No differences are seen between average concentrations in men or women with ovarian activity in adults. After the menopause, however, average concentrations rise in women, although in most cases concentrations remain within the range seen in women with ovarian activity.

PATHOLOGICAL VARIATIONS

The α subunit is a marker of pituitary tumours used in some cases for diagnosis or to monitor treatment.

Pituitary tumours

Gonadotropic adenomas

These are more common in men than in women and are generally seen after the age of 50 years old.

Gonadotropin oversecretion by these adenomas, if present, does not cause any clinical features. These adenomas are then discovered from the mass effect of the tumour or from pituitary insufficiency. The diagnosis is occasionally made fortuitously following a neuroradiological investigation requested for another purpose.

Gonadal over-stimulation resulting in clinical features of ovarian or testicular hyperstimulation is rare.

Biologically, gonadotropins, particularly FSH and their subunits, are secreted in excess. FSH over-secretion, however, is difficult to identify in post-menopausal women, although the association of a normally raised FSH in post-menopausal woman associated with a very low LH suggests a diagnosis of gonadotropic adenoma. α subunit concentrations are often raised and may be associated with over secretion of FSH or LH, although may also occur in isolation. It is difficult to interpret a rise in α subunit concentration in post-menopausal women as it increases physiologically after the menopause in parallel to the gonadotropins.

The LHRH test usually stimulates the gonadotropins, occasionally with an explosive and prolonged response. The TRH test which does not stimulate LH or FSH in normal people produces a response in approximately half of patients, both on the gonadotropins and on the LH α and β subunits. When this response occurs it supports the diagnosis of gonadotropic adenoma.

Prolactin adenomas

α subunit concentrations are only significantly raised in a small proportion (10 to 30%) of patients.

Non-secretory pituitary adenomas

The terms non-functional or clinically silent adenomas appear to be appropriate. Symptoms are due to the mass effect of the pituitary tumour. Excessive α subunit secretion is found in approximately a quarter of these patients – the α subunit is then the best marker to monitor treatment.

Primary thyrotropic adenomas

These are the rarest of the pituitary adenomas. They secrete TSH, and in two thirds of cases, also secrete the α subunit. Other pituitary hormones are relatively often secreted. The diagnosis is made in three situations: hyperthyroidism with goitre but no exophthalmia, tumour mass effect with headaches or even signs of raised intracranial pressure and visual field loss and acromegaly and amenorrhoea with or without galactorrhoea.

Biologically, inappropriate secretion of TSH occurs. Serum concentrations of free thyroid hormones (T4 and/or T3) are raised with an inadequately suppressed TSH. Pro-secretion of the α subunit occurs in two thirds of cases but the molar ratio of the α subunit to TSH is over 1 in only 80% of cases. The ratio is calculated as the concentration of the α subunit (in ng/ml) multiplied by 10 and divided by the TSH concentration (in µU/ml). The molar ratio is not therefore a reliable criterion for diagnosis particularly as the α subunit is increased in post-menopausal women and in primary hypogonadism. The α subunit is therefore relatively difficult to interpret as sex and age of the person and their TSH, FSH and LH concentrations all need to be considered at the same time.

The TRH test only produces a TSH response in pituitary thyroid hormone resistance. Similarly, dopamine antagonists (metoclopramide) do not produce a rise in TSH.

Other diseases

Moles and choriocarcinoma

The α subunit increases along with chorionic gonadotropin (hCG) and the free hCG β subunit in moles and choriocarcinomas. It can therefore be used to monitor and detect recurrence of metastasis of trophoblast tumours.

Malignant non-trophoblast tumours

Cancer of the pancreas, liver or gastro-intestinal tract.

Lung cancer.

Carcinoid tumours.

Non-tumoural diseases

Primary hypothyroidism.

Primary hypogonadism.

Chronic renal insufficiency.

Inflammatory bowel diseases.

Administration of GnRH or its analogues.

POUR EN SAVOIR PLUS

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ALPHAVIRUS

DEFINITION

Formerly classified as Type "A" arboviruses, alphaviruses belong to the Togaviridae family. They are spherical with a diameter of about 70 nanometers with positive-sense RNA. They are contained inside an envelope derived from the membrane of the infected cell. This membrane carries two types of glycoprotein which project outwards from the surface and mediate the agglutination of red blood cells from diverse animal species, including geese and hens. Alphaviruses can be grown in cell lines derived from mammals (BHK-21, Vero) and mosquitoes. In the laboratory, new-born mice are highly susceptible to alphavirus infection.

INTRODUCTION

EPIDEMIOLOGY

Alphaviruses are transmitted by mosquitoes. They can multiply in both mammalian and avian reservoirs as well as in the active vector. The natural reservoir is wild animals, notably rodents and monkeys. Alphavirus infections are primarily zoonoses and human infection is an accidental biological dead-end. They cause the greatest problems in the tropics during the rainy season when mosquitoes are proliferating. Some alphaviruses are endemic in the USA and Europe.

SYMPTOMS

Most human alphavirus infections are asymptomatic or mild (short-lived fever) but, depending on the species, there may be joint or cerebral involvement *(see Table)*.

Virus	Vector	Reservoir	Human pathology	Region
Chikungunya	Mosquito	Monkeys	joint involvement	Africa, Asia, Indian Ocean
O'Nyong-nyong	Mosquito	?	joint involvement	Africa
Ross River	Mosquito	Rodents Pets Kangaroo	joint involvement	Australia-Oceania
Sindbis	Mosquito	Birds	fever	Africa, Asia, Europe, Australia
Eastern Equine Encephalitis (EEE)	Mosquito	Birds	encephalomyelitis	Eastern USA, Canada, Amazonia
Western Equine Encephalitis (WEE)	Mosquito	Birds	encephalomyelitis	Western USA, Canada, South America
Venezuelan Equine Encephalitis (VEE)	Mosquito	Rodents	encephalomyelitis	Central America, South America

Fever and joint pain

The chikungunya (CHIK - meaning "bent in two" in Swahili), O'Nyong Nyong (ONN – "joint-breaker" in Acholi), Ross River (RRV – epidemic polyarthritis) viruses and, to a lesser degree the Sindbis (SIN) virus, cause sudden-onset fever accompanied by migratory polyarthralgia of joint systems (notably the fingers, knee, elbow and vertebrae), which can be debilitating in some cases. After a short remission, the fever returns together with a maculopapular rash. The usual outcome is a full and complete recovery but sometimes asthenia and joint pain persist.

Encephalitis

Alphaviral encephalitis is referred to as "equine encephalitis" because horses are susceptible and can sustain serious nervous system damage. Like the human infection, this represents a biological dead-end for the virus. The Eastern Equine Encephalitis (EEE) virus is the most highly neuropathogenic with the initial fever giving way to encephalitis in 5-10% of cases. Nerve cells are killed as a result of both direct viral cytotoxicity and the associated inflammation (vasculitis). Mortality rates are high, especially in babies (over 50%) and survivors often suffer major sequelae. The Western (WEE) and Venezuelan Equine Encephalitis (VEE) viruses are less invasive in the nervous system and mortality rates do not exceed 2%.

SEARCH INDICATIONS

Diagnosis in a patient with fever, joint pain or encephalomyelitis, in the epidemic season in an endemic region.

INFORMATION

SAMPLE

Whole blood or CSF for direct detection of the virus.

Serum samples 7-10 days apart for antibody assays.

QUESTIONS FOR THE PATIENT

What are the symptoms?

Have you visited an endemic region?

SAMPLE STORAGE AND TRANSPORT

Blood should be drawn within four days of the onset of fever.

If possible, CSF should be sampled at the beginning of the neurological phase. If the test cannot be performed immediately, send the sample frozen at –20°C.

Non-haemolysed serum should be stored at +4°C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

The testing can only be performed at reference centres and specialist laboratories.

The virus can be detected in peripheral blood during the primary febrile phase but it is only usually present for a very short window of time.

At the beginning of the nervous phase, the virus can also be detected in CSF.

- **Isolation:** The virus can be isolated by intra-cerebral inoculation into a new-born mouse or, more commonly, in a culture of VeroE6 cells (a monkey cell line).
- **Detection of viral genetic material:** Viral genetic material is detected by RT-PCR. Sequencing allows high-resolution analysis of viral strains.

■ IINDIRECT DIAGNOSIS

Alphavirus-specific antibodies can be detected by methods based on complement fixation, neutralisation, inhibition of haemagglutination and, most commonly, by ELISA (in which IgM antibodies are detected after immunocapture). In France, the reagents are not available commercially.

INTERPRETATION

Detection of either the virus or its genome confirms the diagnosis. It is important to emphasise that the sample should be taken as soon as possible since the virus is only present for a short period of time.

More often, the diagnosis is made on the basis of the detection of IgM antibodies which usually appear on the fourth or fifth day after onset of the fever. Their levels rise rapidly and the antibodies persist for 3-6 months. IgG antibodies appear towards the tenth day, peak after 4-6 weeks, and persist indefinitely after cure. The presence of isolated IgG antibodies points to immunity against this virus. Different alphaviruses are often cross-reactive.

TRAITEMENT

■ CURATIVE

Flu-like syndromes do not usually require any treatment. For forms in which there is joint pain or neurological involvement, only the symptoms can be treated since there is currently no effective antiviral drug available.

■ PREVENTION

Prevention depends essentially on mosquito control in endemic regions, notably the destruction of larvae in breeding sites near inhabited places. Individuals can be protected by wearing suitable clothing and by using mosquito nets and insect-repellent. Laboratory workers ought to take special precautions because some alphaviruses (notably VEE) can be transmitted in aerosols.

For people at very high risk of exposure and horses, there are formol-inactivated vaccines against EEE, WEE and VEE. There is also an attenuated vaccine against VEE (strain TC-83) which is used to control transmission of the virus in horse-breeding units.

FOR FURTHER INFORMATION

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ALUMINIUM

DEFINITION

Aluminium is a metal found throughout the Earth's crust and is an important element in clays. Its atomic mass is 27 and its atomic number is 13. It is a light metal which conducts both heat and electricity well, and resists corrosion in air. Aluminium is used in many alloys. In medicine, it is used as an antacid and cytoprotectant. In terminal renal failure, it is used to complex phosphate in the intestine, thereby inhibiting its absorption and reducing its concentration in the blood, which if abnormally high, can lead to hyperparathyroidism, a common complication of renal insufficiency.

INTRODUCTION

Assaying aluminium became widespread in the 1980's, when its toxicity in patients with renal failure or on parenteral alimentation emerged. It may also be implicated in the pathogenesis of Alzheimer's disease.

■ WHEN ALUMINIUM POISONING CAN OCCUR

– By inhalation of dusts containing very small particles of the metal, in industrial settings (notably in the production of cellar concrete and other industrial applications), of fumes generated during aluminium welding and the production of aluminium by electrolyse, aluminium fluoride and zinc/aluminium chloride (which is used in military smoke bombs), or in farming (aluminium phosphide is used as a pesticide).

– Via the digestive tract, which is the most common form of poisoning and can occur if foodstuffs, drinks and/or medicinal products (antacids containing aluminium hydroxide or carbonate taken over the long term, or aluminium-based additives) containing a total of 10-100 milligrams of aluminium are ingested on a daily basis.

Aluminium absorption is stimulated by the concomitant oral consumption of citrate or alpha-hydroxylated derivatives of vitamin D 1 as well as low intestinal pH.

Aluminium tends to build up in the body when renal function is impaired. However, cases of acute poisoning have been reported in patients in intensive care being administered prophylactic antacids to prevent ulcers, and also by dialysis centres in Africa, southern Europe and the Caribbean in times of drought (when large amounts of aluminium sulphate have to be used in water purification processes).

Today, aluminium poisoning due to parenteral alimentation or contaminated dialysis solutions has practically disappeared.

■ METABOLISM

Orally administered aluminium is absorbed inefficiently. Once in the blood, 80% binds to transferrin before being distributed around many tissues, notably the liver, brain, parathyroid gland and, above all, the bones. Most is cleared via the kidneys.

■ MECHANISM OF ACTION

Aluminium is involved in many enzyme systems. Because of its affinity for phosphate, it can interfere with intracellular calcium-dependent processes and, by binding with the phosphate groups and bases of DNA, with nuclear processes.

For more than 30 years, it has been known that aluminium can cause encephalitis in dialysis patients. More recently, it has been suggested that aluminium from drinking water may be involved in Alzheimer's disease (because the metal is detected in the brains of affected patients); however, no definite relationship has been established between levels of aluminium in drinking water and the prevalence of Alzheimer's disease.

■ POISONING SYMPTOMS

Acute poisoning is rare but can occur if aluminium phosphide is ingested. This causes gastrointestinal symptoms resembling food poisoning, neurological symptoms (excitation then depression and paralysis), hepatitis and renal involvement, followed by coma and, in the event of shock, death.

Chronic poisoning manifests as:

– Neurological symptoms: Myoclonic encephalopathy with dysarthria and in dialysis patients, encepalopathy sets in after about three years and regresses after kidney transplantation.

– Bone involvement: Osteomalacia or calcitriol-refractory adynamic osteodystrophy associated with hypercalcaemia, hyperphosphataemia and normal alkaline phosphatase levels.

– Haematological symptoms: Microcytic anaemia without iron deficiency.

– Pulmonary fibrosis: Due to exposure to aluminium-laden dust in an industrial situation.

INDICATIONS FOR MEASUREMENT

To investigate aluminium toxicity in subjects who are at risk or in the above-listed situations. Patients with impaired renal function should be tested for aluminium at least twice a year, especially if they are on a long-term course of aluminium salts and are being dialysed with water that has not been osmotically purified (or if they live in a drought-prone region). Patients on long-term parenteral alimentation should also be regularly tested.

INFORMATION

■ SAMPLE

Most commonly, serum or plasma drawn into heparin or EDTA. Avoid using glass tubes or plastic stoppers that might leach aluminium. Choose tubes with plastic stoppers that are guaranteed to be free of aluminium.

– Urine: 24-hour urine collection acidified with nitric acid, in a polystyrene or polypropylene recipient.

– Water: Collect the sample into an acid-washed plastic tube with a stopper guaranteed free of aluminium.

■ SAMPLE STORAGE AND TRANSPORT

Store and transport at a temperature of no more than 4°C.

ASSAY METHODS

Atomic absorption spectrophotometry with electromagnetic correction (Zeeman), or a coupled plasma torch method with an optical detection system.

NORMAL EXPECTED VALUES

For reference purposes, normal expected values (in unexposed subjects) are:

– Serum: 0.06-0.55 µmol/l

– Plasma: 0.03-0.41 µmol/l

– Whole blood: <0.35 µmol/l

– Urine: 0.08-0.26 µmol/l.

Aluminium in the urine:

At the end of the shift (German reference): 200 µg/l

The Monday after the last shift (Finland): 160 µg/l

– Conversion factor: 1 µmole = 27 µg

Threshold in drinking water: In France, 0.2 mg/l.

Threshold in dialysis water: 0.010 mg/l (European Pharmacopoeia).

PATHOLOGICAL VARIATIONS

Interpreting the results of a blood aluminium assay depends on the patient having a normal blood iron concentration, i.e. a blood ferritin concentration of between 100 and 500 ng/ml. This is because the binding of aluminium to transferrin is increased when ferric ion is deficient, and reduced when it is in excess.

In aluminium poisoning, the toxin partitions equally between red cells and serum.

– A serum aluminium concentration of >2.2 µmol/l (60 µg/l) defines major exposure.

– A serum aluminium concentration of 3.7 µmol/l (100 µg/l) is the toxicity threshold in patients on dialysis.

– Plasma concentrations of 113-490 µg/l, have been reported in poisoned dialysis patients who experienced convulsions but survived. Concentrations of 300-1200 µg/l have been detected in cases of fatal poisoning.

For patients on dialysis who have taken or are taking aluminium/phosphorus complexes, an elevation of blood aluminium > 27 µg /l (1 µmol/l) ought to be taken into account. Such an elevation may lead to aluminium-induced adynamic hypercalcaemic osteodystrophy with a risk of fracture within five years. Nevertheless, prior overload of the body cannot be formally ruled out on the basis of the absence of any obvious increase in the blood aluminium concentration (>1 µmol/l) if exposure ended some months beforehand; in this case, such overload could be diagnosed by means of a blood aluminium test following a deferoxamine challenge.

■ POISONING TREATMENT

Parenteral deferoxamine: Deferoxamine is an iron chelator which is usually used to treat iron overload but it can also mobilise other trivalent metals in the body, including aluminium.

FOR FURTHER INFORMATION

■ Goullé J.-P., Droy J.M., Leroy J.-P., *Métaux*, Encycl Med Biol, Elsevier Ed, Paris, 2003.

■ Morinière P., Oprisiu R., Leflon P., El Esper N., Fournier A., *Aluminium dans les liquides et tissus humains*. In: Kamoun P., Fréjaville J.-P., Guide des examens de laboratoire, 4e Ed. Médecine-Sciences Flammarion, Paris 2002; 1218-21.

■ Pepin D., *Toxiques chimiques dans l'eau d'alimentation*, Encycl Med Biol, Elsevier Ed. Paris, 2003.

AMIKACIN

DEFINITION

Amikacin is an aminoglycoside antibiotic with bactericidal activity against many species, both Gram-positive and Gram-negative. It is practically exclusively used in combination therapy, notably together with a β-lactam antibiotic for Gram-negative bacilli plus for targeting, staphylococci and streptococci. However, like other aminoglycosides, it has significant toxicity, potentially causing hearing loss and kidney damage.

PHARMACOKINETICS

Amikacin is always administered by injection after which it is 100% bioavailable. Its concentration usually peaks in the serum 30 minutes after intravenous injection, and 90 minutes after intramuscular injection. Amikacin is not broken down in the body and most (90%) is eliminated in the urine as the biologically active parent compound. In plasma, it has an average half-life of 2-2.5 hours but this varies substantially with age, efficiency of kidney function and body mass index.

Aminoglycoside antibiotic activity is dose-dependent, and is characterised by a post-antibiotic effect so the interval between successive doses can be extended without risking bacterial regrowth.

INDICATIONS FOR MEASUREMENT

Amikacin is administered daily in either two injections of 7.5 mg/kg, or a single injection of 15 mg/kg. Because of its low therapeutic index, amikacin levels are usually closely monitored during a course of treatment and, given the drug's toxicity (a risk of hearing loss and kidney damage) coupled with the severity of its indications, the results of these tests are used to check that an effective blood concentration has been obtained without any risk of toxic effects.

With "classic" administration (twice-daily injection), two assays have to be performed:

– At "peak" (the maximum concentration), to check efficacy and make sure that concentration is not above the toxicity threshold.

– The "trough" concentration to check that there is no build-up of the antibiotic.

If the drug is being administered once-daily, the "peak" concentration alone will usually suffice.

INFORMATION

■ SAMPLE

Serum or plasma with heparin or EDTA. Do not use gel-containing tubes (because of a risk of adsorption).

Tests have to be carried out at the beginning of the course of treatment, once the steady state has been reached (i.e. 24 hours after the first dose), and whenever the dosage is modified.

–Before the next injection (to test the trough concentration).

– 30 minutes after the end of IV infusion (the blood should be drawn from the other arm) or one hour after IM injection (to establish the peak concentration).

■ QUESTIONS FOR THE PATIENT

– Any request for a drug assay should always include.

- The reason the drug was prescribed (for efficacy or toxicity evaluations).
- The time at which the test sample was taken.
- The date of the beginning of the course of treatment and/or dates on which the dosage was changed.
- Dosing details (administered dose, frequency, route of administration).

The patient's age, height and weight.

Concomitant medications: Notably other drugs that can cause hearing loss and kidney damage.

■ SAMPLE STORAGE AND TRANSPORT

Samples can be kept for a matter of days at +4°C; beyond that, freeze to –20°C.

Transport at + 4 °C or frozen of the sample has already been frozen.

ASSAY METHODS

Usually immunological methods: FPIA, EMIT or immunoturbidimetry.

NORMAL EXPECTED VALUES

– Classic administration (twice-daily injection):

Trough concentration: <5 µg/ml

Peak concentration: 15-30 µg/ml

Toxicity threshold: Peak concentration >40 µg/ml and/or trough concentration >10 µg/ml

– Single daily dose:

Trough concentration <3 µg/ml

Peak concentration: 30-60 µg/ml

– Conversion factor:

1 µg/ml = 0.585 µmol/l

FOR FURTHER INFORMATION

■ Meley R., *Dosage des médicaments*, tome II, Cahier BIOFORMA N° 18. Paris, janvier 2000, chapitre amikacin: 35-42.

■ Dictionnaire Vidal®.

AMINO ACIDS

DEFINITION

Amino acids are fundamental components in the body that carry a carboxylic acid group and an amino group on the same carbon atom. They have three functions; they are the building blocks of proteins; they are important metabolic intermediates; and they act as precursors for biogenic amines. Some amino acids exist only in a free form. A distinction is made between two different types of amino acid; the essential amino acids which can only be obtained from the diet since the body is incapable of synthesising them; and the non-essential amino acids which the body can itself synthesise from nitrogen- and carbon-containing precursors in enzyme-catalysed pathways.

INTRODUCTION

Hereditary metabolic diseases involving amino acids (aminoacidopathies) can be divided between two categories: (i) diseases due to defective membrane transport mechanisms involving either the cell's plasma membrane (affecting the cells of the renal tubules and/or intestinal epithelial cells) or intracellular membranes (mitochondria, lysosomes); and (ii) enzymopathies involved in the catabolism of amine-derived nitrogen (urea genesis) or a step in the catabolism of the carbon fraction of amino acids. If the defect affects one of the early steps in the catabolism of an amino acid, the amino acid in question will build up (like the accumulation of phenylalanine in phenylketonuria). But if the defect involves one of the late stages of the metabolic pathway, the compound that will build up will be an organic acid. The usual treatment strategy in all aminoacidopathies is drastic restriction of the intake of endogenous proteins coupled with dietary supplementation with mixtures of amino acids (containing all the essential amino acids but not the amino acid whose metabolism is compromised).

INDICATIONS FOR MEASUREMENT

Analysing amino acids in diverse biological fluids (blood, urine, and cerebrospinal fluid) may be useful in various situations:

– Diagnosis of aminoacidopathy: The assays of amino acids in amniotic fluid may also be ordered in the context of the prenatal diagnosis of deficiencies in argininosuccinate synthetase, argininosuccinate lyase and sulphite oxidase.

– Monitoring and prevention of aminoacidopathy and in this context, the analysis of amino acids in plasma has two purposes: (i) to check the concentration of the amino acid whose metabolism is compromised; and (ii) to check the subject's overall nutritional status in terms of the concentrations of the essential amino acids.

– Amino acid assays may also provide useful information in certain kidney, liver and bone problems as well as following trauma and in infections.

INFORMATION

■ SAMPLE – SAMPLE STORAGE AND TRANSPORT

– Blood amino acid tests are carried out on heparinised plasma samples (because in serum, concentrations are too variable).

– CSF should be collected into a dry tube.

– Blood samples should be drawn from the elbow fold, in the morning with the subject fasting. The sample should be rapidly (within half an hour) transported to the test laboratory at a temperature of + 4°C. Alternatively, the sample should be centrifuged and the plasma decanted and frozen within one hour of drawing; the sample should be sent to the laboratory frozen.

– Assays of urinary amino acids are usually performed on a sample (10 ml) of the first urine passed by a fasting patient in the morning, collected into a suitable recipient and either rapidly transported to the laboratory on ice or, alternatively, immediately frozen and sent to the laboratory. When daily diuresis has to be quantified (e.g. for therapeutic monitoring in cystinuria), collected urine should be stored in a refrigerator.

– Cerebrospinal fluid samples (1 ml of non-haemorrhagic CSF) are collected into a dry tube and either sent immediately to the laboratory at +4°C, or frozen and later transported at –20°C.

The sample preparation instructions above must be rigorously followed because they minimise the breakdown of unstable amino acids and the disappearance of sulphur-containing amino acids (all of which may be important for the diagnosis). Samples should be frozen to at least –20°C and –80°C is even better. In these conditions, all amino acids are stable apart from glutamine which is steadily converted to glutamic acid at –20°C.

■ QUESTIONS FOR THE PATIENT

– Are you taking any medications?

Many medicinal products contain amino acids in their composition and others interfere with the metabolism of certain amino acids, e.g. treatment with valproic acid can induce hyperglycinaemia, hyperglycinuria and hyperalaninuria.

ASSAY METHODS

– Samples should be depleted of protein using sulphosalicylic acid.

– The reference method for assaying amino acids in biological fluids is ion-exchange chromatography coupled with colorimetric detection at two wavelengths (570 and 440 nm) after reacting with ninhydrin. Apart from proline and hydroxyproline (which give a yellow colour detected at 440 nm), all the other amino acids give a violet colour detected at 570 nm. The intensity of the colouration is proportional to the amount of amino acid present in the reaction mixture. In fixed conditions, the 570 nm/440 nm optical density ratio is characteristic for each amino acid. The sensitivity of this reaction is about 50 pmol/l. This method allows determining the concentrations of 25 amino acids in routinely tested biological fluids.

– Ninhydrin can be replaced with orthophthalaldehyde which, when it reacts with a primary amine, yields a fluorescent compound. The detection threshold for this reaction is about 1 pmol/l. It permits the resolution and therefore the quantification of about 50 compounds—amino acids and ninhydrin-positive derivatives.

– Methods based on gas-phase and reverse-phase chromatography with derivation of the amino acids prior to analysis have also been developed; these high-performance methods are useful when particularly high sensitivity is required.

NORMAL EXPECTED VALUES

– Plasma and CSF: Usual values are presented in Table I.

– Urine: In contrast to plasma, there is marked age-related variability (Table II).

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

A number of physiological factors can induce departure from the reference values, including fasting for more than 12 hours (↘ alanine, branched amino acids ↗), eating (↗ all amino acids or almost all), genetic factors and transient neonatal fluctuations due to the immaturity of certain enzyme systems and renal transport mechanisms (hypertyrosinaemia, iminoglycinuria, cystinuria, lysinuria).

■ PATHOLOGICAL VALUES

– Table III summarises typical changes in amino acid levels observed in the main aminoacidopathies.

– Above and beyond the hereditary aminoacidopathies, certain pathological conditions induce changes in the concentrations of amino acids in the plasma and urine: hyperammonaemia (glutamine ↗, glutamic acid ↗, alanine and lysine ± ↗), hyperlactacidaemia (alanine ↗, proline ± ↗), kidney failure (citrulline, cystine, 3-methylhistidine: ↗), liver failure (tyrosine, methionine: ↗, branched amino acids ↘), malnutrition (generalised hyperaminoaciduria apart from glycine), Fanconi's syndrome (hyperaminoaciduria), hypercatabolism (generalised hyperaminoacidaemia), hyperinsulinism (branched-chain amino acids ↘), and a low-protein diet (essential amino acids ↘).

– About a dozen atypical amino acids can be found in certain pathological situations, including sulphocysteine, hawkinsine, aspartylglucosamine, phosphoethanolamine, pipecolic acid, saccharopine, homocitrulline (HCi), alloisoleucine (alle), cystathionine, cysteine disulphide homocysteine (Cys-HCy), argininosuccinic acid (Asa), homocystine (HCy) and hydroxylysine.

Table I. Reference values for amino acids in plasma and CSF. Results expressed in µmol/l (mean ± SD)

Amino acid		Plasma	CSF
Taurine	**Tau**	82 ± 45	5 ± 2
Aspartic acid	**Asp**	-	3 ± 2
Aspartic acid + Asparagine	**Asp + Asn**	59 ± 16	-
Hydroxyproline	**Hyp**	23 ± 12	-
Threonine	**Thr**	124 ± 39 27 ± 9	
Serine	**Ser**	137 ± 31	34 ± 10
Asparagine	**Asn**	-	5 ± 2
Glutamic acid	**Glu**	-	15 ± 13
Glutamine	**Gln**	-	614 ± 241
Glutamic acid + Glutamine	**Glu + Gln**	578 ± 92	-
Proline	**Pro**	180 ± 47	-
Glycine	**Gly**	229 ± 44	9 ± 2
Alanine	**Ala**	312 ± 78	28 ± 10
Citrulline	**Cit**	26 ± 7	2 ± 1
α-amino-butyric acid	**Abu**	19 ± 10	3 ± 1
Valine	**Val**	230 ± 44	14 ± 4
Cysteine	**Cys**	83 ± 18	-
Methionine	**Met**	27 ± 7	3 ± 1
Isoleucine	**Ile**	64 ± 15	5 ± 1
Leucine	**Leu**	123 ± 27	12 ± 3
Tyrosine	**Tyr**	60 ± 28	8 ± 2
Phenylalanine	**Phe**	55 ± 11	8 ± 2
Ornithine	**Orn**	72 ± 25	8 ± 2
Lysine	**Lys**	183 ± 39	19 ± 6
Histidine	**His**	81 ± 15	11 ± 3
Methylhistidine	**His (tMe)**	< 6	14 ± 7
Arginine	**Arg**	77 ± 20	14 ± 7

Table II. Amino acid concentrations in the urine at different ages (mmol/mmol of creatinine). The results are expressed as range (mean).

Amino acid	0-1 month	1-6 months	6-12 months	1-2 years	2-4 years	4-7 years	7-10 years	10-13 years	>13 years
Tau	8-226 (49)	6-89(22)	9-123(35)	12-159(69)	13-200(76)	17-230(89)	18-230(109)	18-176(110)	16-180(72)
Asp	2-12 (6)	2-16(7)	3-12(6)	3-10(5)	2-8(5)	2-8(5)	1-9(5)	1-10(4)	2-7(4)
Hyp	20-320 (146)								
Thr	20-138 (53)	17-92(44)	14-56(33)	15-62(29)	10-48(20)	9-36(20)	9-27(17)	8-28(17)	7-29(13)
Ser	80-282 (149)	42-194(124)	50-137(98)	45-124(79)	32-94(61)	38-93(54)	23-69(48)	23-67(41)	21-50(30)
Gln	52-205 (119)	63-229(137)	74-197(128)	62-165(107)	45-236(86)	52-133(81)	13-97(61)	20-112(59)	20-76(36)
Pro	21-213 (70)								
Gly	283-1 097 (609)	210-743(397)	114-415(259)	110-356(207)	111-326(194)	91-246(155)	84-236(129)	64-165(114)	43-173(107)
Ala	75-244 (149)	72-206(133)	36-162(92)	41-130(83)	33-115(62)	27-92(50)	17-65(36)	21-62(38)	16-68(30)
Val	3-26 (11)	4-19(11)	6-19(13)	7-21(13)	6-20(12)	3-15(9)	3-15(7)	3-17(7)	3-13(5)
Cys	24-78 (49)	13-48(27)	12-29(19)	10-26(17)	8-30(15)	8-22(13)	8-21(13)	7-23(12)	6-34(13)
Met	7-27 (13)	6-22(11)	8-29(15)	7-29(15)	5-21(12)	5-20(10)	3-17(8)	3-10(6)	2-16(6)
Leu	3-25 (10)	4-12(8)	4-16(10)	3-17(10)	4-18(8)	3-13(6)	3-16(7)	3-14(6)	2-11(5)
Tyr	6-55 (22)	12-52(28)	11-54(30)	13-48(27)	10-30(17)	9-35(18)	7-26(14)	6-25(14)	2-23(10)
Phe	4-32 (13)	7-28(16)	11-28(19)	10-31(18)	7-21(14)	6-26(13)	5-20(11)	5-17(10)	2-19(7)
Lys	22-171 (81)	15-199(52)	13-79(36)	16-69(27)	10-46(24)	10-68(24)	10-44(23)	10-56(23)	7-58(17)
His	80-295 (152)	72-342(185)	92-278(167)	87-287(167)	68-255(132)	61-216(120)	45-184(109)	43-159(98)	26-153(79)
His (tMe)	20-39 (30)	19-40(29)	20-47(32)	22-57(38)	20-59(36)	21-61(37)	18-59(33)	20-56(36)	19-47(32)
Total	**956-2 917**	**828-2 236**	**637-1 457**	**700-1 465**	**576-1 231**	**513-1 247**	**408-975**	**418-975**	**252-950**
	(1 845)	**(1 446)**	**(1 106)**	**(1 043)**	**(830)**	**(763)**	**(693)**	**(637)**	**(514)**

Table III. Amino acid changes in the main aminoacidopathies

	Aminoacidopathy	Enzyme deficiency	Amino acid profile	
			Plasma	Urine
Urea cycle defects	Hyperargininaemia	Arginase	Arg ↗	
	Argininosuccinic aciduria	Argininosuccinate lyase	Presence of Asa Cit, Glu+Gln, Ala, Lys ↗ ; Arg ↘	Presence of Asa Cit, Glu+Gln, Ala, Lys ↗
	Citrullinaemia	Argininosuccinate synthetase	Cit ↗ Glu-Gln, Ala and Lys ± ↗ Arg and Orn ↘	Cit ↗ Glu-Gln, Ala and Lys ± ↗
	Triple H syndrome: hyperammonaemia, hyperornithinaemia, homocitrullinuria	Transport of Orn across the mitochondrial membrane	Orn ↗ ; Glu-Gln, Ala and Lys ± ↗	Orn ↗ ; Glu-Gln, Ala and Lys ± ↗ Presence of HCi (Cys)2, Arg sometimes ↗
Defects in the metabolism of branched amino acids	Leucinosis	Branched ketoacid dehydrogenase	Val, Ile, Leu : ↗ ; presence of alle	
Defects in the metabolism of sulphur-containing amino acids	Homocystinuria	Cystathionine b-synthase	(HCy)2, Met : ↗ ; presence of (Cys-Hcy) ; (Cys)2 : ↘	
	Homocystinuria	N5-methylene tetrahydrofolate reductase, N5-methylene tetrahydrofolate methyltransferase	(HCy)2 : ↗ ; (Cys)2 and Met ↘ ; presence of (Cys-Hcy)	
	Sulphite oxidase deficiency	Sulphite oxidase	Presence of sulphocysteine (Cys)2 ↘	Presence of sulphocysteine (Cys)2 ↘ ; Tau ↗
Other enzyme deficiencies	Hyperglycinaemia without ketosis	Cleavage of glycine	Gly ↗ (same in CSF)	
	Hyperphenylalaninaemia	Phénylalanine hydroxylase, dihydrobioptérine synthetase, dihydrobioptérine reductase	Phe ↗	
	Type II tyrosinaemia	Tyrosine aminotransferase	Tyr ↗	
	Aspartylglucosaminuria	aspartylglucosaminidase	± presence of 2-acetamido-1-L-aspartamido-1,2-dideoxy-D-glucose	
	Hyperornithinaemia	Ornithine aminotransferase	Orn ↗	Orn ↗ ; (Cys)2, Lys and Arg ± ↗
	Type I hyperprolinaemia	Proline oxydase		
	Type II hyperprolinaemia	Pyrroline 5 carboxylate deshydrogenase	Pro ↗	
Transport defects	Familial protein intolerance with lysinuria		Lys, Orn, Arg : ↘ Glu +Gln, Ala, Cit, Gly ± ↗	Lys ↗ Orn, Arg, (Cys)2, Cit, Hci, Glu + Gln ± ↗
	Cystinuria : homozygous form		None or (Cys)2, Orn, Lys, Arg	Cys)2, Orn, Lys, Arg ↗
	Cystinuria : heterozygous form (Type II or III)		None	Cys)2, Lys,↗
	Cystinosis	Impaired lysosomal transport of cysteine	Kidney failure – tubule disease profile	

FOR FURTHER INFORMATION

■ Ph. Parvy, D. Rabier, P. Kamoun, *Analyse des acides aminés dans les liquides biologiques*.
http://bioch.ap-hop-paris.fr/analyses/Bioforma/ACIDES.htm

AMIODARONE

DEFINITION

Amiodarone is a Class 3 anti-arrhythmic drug as defined in the Vaughan-Williams classification system.

It is available as hydrochloride in 200 mg tablets and 3 ml vials for injection (150 mg). It is also available in generic form.

It is a potent compound prescribed to prevent the recurrence of serious arrhythmia (ventricular and supraventricular tachycardia, ventricular fibrillation) and treat supraventricular tachycardia. Its anti-arrhythmic activity results from prolongation of phase 3, of the action potential in heart cells (among other effects); through a slowdown of the potassium flux without any effect on sodium or calcium transport and it also has a bradycardiac effect.

When administered orally, the usual dosage is 3-5 tablets a day for 8-10 days, followed by a daily maintenance dosage of ½-2 tablets (the lowest effective dose).

In severe cases, when oral administration is not appropriate, parenteral administration is indicated but this is only possible in a hospital setting and requires close monitoring. The average dosage is 5 ml/kg which is always administered by infusion over 20-120 minutes, two or three times in the first 24 hours, followed by 10-20 ml/kg/day for a few days until a switch can be made to oral administration.

Amiodarone has a long half-life and can interfere with thyroid function·

PHARMACOKINETICS

Bioavailability (oral administration)	Absorption is slow and variable (mean: 50%)
Plasma peak (Tmax)	After a single oral dose: 3-7 hours
Steady state	Therapeutic effects seen after one week (on average). Steady state in long-term treatment; > 5 months
Protein binding	96% (including 62% to albumin)
Metabolism	In the first passage through the liver: about 25%. One metabolite identified: mono-N-desethylamiodarone (the activity of which is comparable to that of the parent compound).
Plasma half-life	20-100 days (major variability between different patients)
Elimination	The compound loses a fraction of its iodine which is cleared in the urine as iodide; the rest (the great majority) is excreted in the faeces after passage through the liver.

INDICATION FOR MEASUREMENT

Given the seriousness of the situations in which this drug is indicated, coupled with its potential toxicity, a patient on amiodarone should be closely monitored to avoid overdosing and to check efficacy. It is believed that its plasma concentration correlates with therapeutic effects.

INFORMATION

SAMPLE

Serum or plasma drawn into EDTA or heparin. Do not use tubes containing a separating gel.

Draw blood just before the next dose (trough concentration); alternatively, 4-6 hours after a dose to measure the peak concentration.

Long-term treatment: A steady state is not reached before five months of treatment.

QUESTIONS FOR THE PATIENT

Any request for a drug assay should always include.

- The reason the drug was prescribed (for efficacy or toxicity evaluations).
- The time at which the test sample was taken.
- The date of the beginning of the course of treatment and/or dates on which the dosage was changed.
- Dosing details (administered dose, frequency, route of administration).
- The age, height and weight of the patient.

Are you taking any other drugs?

As a rule, only one anti-arrhythmic drug should be taken at a time (unless a combination has been prescribed by a specialist). Amiodarone is contraindicated in combination with other drugs that can induce "torsades de pointes", namely Class 1 (quinidine, hydroquinidine, disopyramide) and Class 3 antiarrhythmic drugs (ibutilide, sotalol) and certain other products (cisapride, bepridil, sultopride, IV erythromycin and IV vincamine).

However, amiodarone can affect the metabolism of many drugs—oral anticoagulants, cyclosporin, phenytoin and digitalis derivatives—thereby entailing a risk of overdose. Close clinical and laboratory monitoring are necessary.

SAMPLE STORAGE AND TRANSPORT

Centrifuge and decant within one hour of blood drawing.

Freeze at –20°C if the test is to be postponed. Transport the sample frozen at –20°C.

ASSAY METHODS

High performance liquid chromatography.

NORMAL EXPECTED VALUES

Trough concentrations (given for reference purposes):

Amiodarone: 0.5-2.5 mg/l

Desethylamiodarone: 0.5-2.5 mg/l.

Plasma or serum amiodarone concentrations of >2.5 mg/l are considered as toxic. There is little information about overdoses with oral amiodarone. However, a few cases have been reported of sinus bradycardia, ventricular arrhythmia and impaired liver function. Given its long clearance half-life, monitoring has to be prolonged. Therapeutic adjustment is pointless in patients with renal failure, and the drug's

pharmacokinetics has not been adequately studied in patients with liver failure.

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AMMONIA

DEFINITION

At physiological pH, 98% of ammonia in the body is in the form of the ammonium ion (NH4+), with just 2% as ammonia itself (NH3). Ammonia is generated in the course of the breakdown of protein and is cleared via the liver. Ammonaemia is the concentration of ammonia in the blood and it is measured to detect hyperammonaemia which can result from hereditary conditions (metabolic abnormality) or can be acquired (acidosis, liver disease).

INTRODUCTION

Almost all this ammonia is produced in the gut, kidney or muscles. In the digestive tract, large quantities are generated by the gut flora from dietary protein, and then pass into the general circulation via the portal vein. In muscle tissue, ammonium is released when amino acids, notably glutamic acid, are oxidatively deaminated and from the purine nucleotide cycle. In the body, ammonium is toxic and it is eliminated via the liver after conversion to urea in the urea cycle.

INDICATIONS FOR MEASUREMENT

Ammonium is assayed in the blood in the investigation of hereditary metabolic disease, notably disorders involving enzymes in the urea cycle. In addition, hyperammonaemia can be acquired secondary to certain conditions such as severe liver failure and metabolic acidosis, and also in response to valproic acid treatment.

INFORMATION

SAMPLE

Plasma collected into EDTA (ethylenediaminetetraacetic acid) or heparin, i.e. lithium or sodium heparinate (depending on the assay method) but not ammonium heparinate.

Ammonium can also be measured in arterial or capillary blood (in which reference values are higher than those in plasma), red blood cells, urine, saliva, sweat, cerebrospinal fluid and faeces.

The patient should have been fasting for at least six hours.

The patient should not clench his/her fist during blood drawing, since muscle contraction can stimulate NH3 release.

Smoking should be forbidden in the six hours before the sample is taken because tobacco smoke contains a large amount of NH3.

Avoid contamination with sweat (which is rich in ammonium) or atmospheric pollutants, notably cigarette smoke and some detergents which give off ammonia in vapour form.

QUESTIONS FOR THE PATIENT

Are you taking valproic acid? This drug can induce hyperammonaemia.

SAMPLE STORAGE AND TRANSPORT

The following instructions must be strictly followed to preclude the release of ammonia from amino acids in the plasma.

Immediately cool the sample down to +4°C.

Centrifuge at +4°C, decant-off the plasma and run the test within 30 minutes; any delay in separating plasma and cells can misleadingly raise the apparent ammonia concentration *in vitro*, as a result of the deamination of glutamine.

If the test is postponed, the plasma should be frozen within 30 minutes of blood drawing.

Reject haemolysed samples (red blood cells contain two-to-three times more ammonia than plasma) and cloudy or hyperlipaemic samples (enzyme-based methods are lipid-sensitive).

Transport conditions: Centrifuge and freeze the sample within 30 minutes of blood drawing.

ASSAY METHODS

Before every test, the distilled water has to be checked for the absence of NH4+. Smoking is prohibited during testing.

Enzyme-based methods are the most common (the glutamate dehydrogenase method); others have been reported, such as: Ion-selective electrodes, microdiffusion in alkaline medium, or ion exchange followed by a colourimetric reaction, but these are seldom used.

NORMAL EXPECTED VALUES

Can fluctuate somewhat, between different methods.

For reference:

	Venous blood (µmol/l)	Arterial or capillary blood (µmol/l)
New-born babies (up to 3 days)	34-102	50-128
Babies (3-28 days)	37-63	
Children and adults	14-38	16-50

Conversion factor: 1 µg/l = 0.059 µmol/l

1 µmol/l = 17 µg/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Age-dependent *(see above)*: Values are higher in young babies.

PATHOLOGICAL VARIATIONS

Hyperammonaemia points to either a hereditary metabolic disorder or an acquired condition.

Hereditary disorders

Deficiencies in enzymes of the urea cycle

Ornithine carbamyl-transferase (OCT) deficiency is the most common. Its transmission is partly dominant, being X-linked. The severity of the clinical symptoms depends on the degree of the deficiency. Distinction is made between neonatal forms, which are characterised by severe neurological involvement, respiratory disorders, coma, hepatic cytolysis and low levels of clotting factors, and forms that onset later with neuropsychiatric and/or gastrointestinal symptoms, resulting in retarded growth and development.

Other deficiencies that can underlie hyperammonaemia (carbamyl phosphate synthetase, arginosuccinate synthetase, arginosuccinate lyase and arginase deficiencies) are transmitted as autosomal recessive traits.

Hereditary metabolic disorders:

Deficiencies in enzymes involved in the catabolism of branched amino acids, and deficiencies in pyruvate decarboxylase, pyruvate dehydrogenase and mitochondrial ornithine transporters (or triple H syndrome; hyperammonaemia, homocitrullinaemia, hyperornithinaemia) as well as disorders affecting the oxidation of fatty acids and transient hyperammonaemia in premature new-borns, are all often associated with acute foetal distress.

- **Acquired abnormalities**

– Severe liver failure, either acute (fulminant viral hepatitis, poisoning, Reye's syndrome) or chronic (cirrhosis).

Since it is in the liver that ammonia is detoxified, severe hepatocyte damage will compromise this function.

– A marked increase in ammonia levels in an adult confirms a diagnosis of hepatic encephalopathy, e.g. in a patient with a portacaval shunt, severe hepatitis or cirrhosis.

– Metabolic acidosis: The levels of NH4+ rise at the expense of the NH3 form which is the initial substrate in the urea cycle; in consequence, the rate of ammonia clearance in the urine drops, entailing hyperammonaemia.

– Ingestion of exogenous organic acids, accidental or as medicinal products, e.g. valproic acid.

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AMOEBIASIS

DEFINITION

Amoebiasis is a strictly human parasitic infection caused by the protozoa called *"Entamoeba histolytica"*. It is usually confined to the intestine but may spread to other organs, notably the liver and lungs. The parasite exists in three forms:

– A ***cyst*** which is the resistant form of the amoeba that is disseminated.

– ***Two vegetative forms or trophozoites:*** The intestinal saprophytic *minuta* form, and the pathogenic *histolytica* amoeba form.

Synonyms: *Entamoeba histolytica or Entamoeba dysenteriae*.

INTRODUCTION

EPIDEMIOLOGY

Amoebiasis is fairly ubiquitous but is endemic in the tropics and sub-tropics where hygiene conditions are poor and faecal contamination is common. It is also seen in temperate zones in tourists who have visited countries in which the infection is endemic. The parasite's reservoir is man. Transmission from one human host to another can be direct, via dirty cyst-laden hands, or indirect, via contaminated water, drinks or food.

LIFE CYCLE

Cysts passed into the external environment are highly resistant. Humans become infected when they ingest cysts, at which point, two cycles are possible:

A non-pathogenic cycle

in which the ingested cyst divides to generate amoebules, when in the colon, transform into *minuta* trophozoites to give rise to new cysts for excretion in the faeces. This cycle does not cause disease in the immediate host but asymptomatic carriers ensure dissemination of the infection.

A pathogenic cycle

in which *minuta* forms in the colon transform into pathogenic *histolytica* forms, which cause ulceration of the colic mucosa and abscesses in the sub-mucosa. *Histolytica* forms can also spread in the blood to reach secondary foci, notably in the liver and lungs.

SYMPTOMS

Amoebiasis infection, the asymptomatic form, corresponding to the non-pathogenic cycle.

Amoebiasis disease is the symptomatic form due to parasites in the intestine or elsewhere, corresponding to the pathogenic cycle. Whether or not the parasite goes into the pathogenic or non-pathogenic cycle depends on factors related to both host (such as weakness or intercurrent disease) and parasite (the strain's virulence).

Intestinal amoebiasis

Acute dysenteric form: The classic symptoms are massive diarrhoea with blood and mucus in the stool (rectal sputum) together with stomach ache and tenesmus, but no fever.

Sub-acute diarrhoeal form: The more common form with watery and viscous or paste-like stools together with colic pain but no fever.

Malign colic amoebiasis

This rare form can strike weakened individuals and has a poor prognosis. It combines a dysenteric syndrome with major infectious toxicity.

Amoeboma

An inflammatory mass in the colon which is very rare (some cases in Latin America and South Africa).

Hepatic amoebiasis (or abscess)

The most common extra-intestinal focus, due to dissemination of *histolytica* forms to the liver. It accompanies or succeeds colic amoebiasis and manifests as pain in the right hypochondriac region with sustained high fever and hepatomegaly.

Pulmonary amoebiasis

This is most commonly due to amoebal dissemination out of a neighbouring hepatic abscess, resulting in acute pneumonia in the right basal lung or pulmonary abscesses.

Other possible foci

The brain (amoebal abscess), skin, pericardium and spleen, among others

SEARCH INDICATIONS

Diagnosis of intestinal amoebiasis in a patient returning from an endemic region.

Diagnosis of hepatic amoebiasis concomitant with or secondary to intestinal amoebiasis.

Diagnosis of extra-hepatic tissular amoebiasis.

Differential diagnosis vis-à-vis other pathogenic amoebae (e.g. *Entamoeba coli, Entamoeba dispar, Entamoeba hartmanni, Endolimax nana, and Blastocystis hominis*) or another dysentery-causing pathogen, parasite, virus or bacterium.

Differential diagnosis vis-à-vis a pyogenic abscess or hepatic carcinoma.

INFORMATION

SAMPLE

Faeces: Collected into a clean, dry recipient for a parasitological examination, directly on passage because the mobile, vegetative form is fragile and soon dies. Repeat the collection and examination procedure several times over several days as long as the result is negative.

Blood: Drawn into a dry tube for serological tests.

Pus: Sterile aspiration biopsy.

QUESTIONS FOR THE PATIENT

Symptoms and date of onset?

Have you visited an endemic region?

Have you had amoebiasis in the past?

Are you taking drugs to treat a parasitic infection?

SAMPLE STORAGE AND TRANSPORT

Faeces: Preferably passed at the laboratory; otherwise, dispatch immediately after defecation.

Serum: Keep at +4°C.

Pus: Dispatch as soon as possible.

DIAGNOSTIC METHODS

PARASITOLOGICAL DIAGNOSIS

- **Direct examination of freshly passed faecal material to detect vegetative forms and cysts.**

  – *The E. histolytica trophozoite,* form *histolytica,* measures between 20 and 40 µm, has a small, off-centre nucleus, can contain intravacuolar erythrocytes, and is motile at 37°C (by virtue of pseudopods).

  – *The E. histolytica trophozoite* form *minuta*, measures between 10 and 15 µm, has a similar morphology to that of the *histolytica* form, and is motile by virtue of long pseudopods, with peak motility at temperatures below 37 °C.

  – *The cyst* is spherical and refringent, has a diameter of 10-14 µm with, when mature, four nuclei; less mature forms have one or two larger nuclei and a vacuole.

- **Staining:** With lugol or merthiolate-iodine-formol (MIF).
- **Concentration:** Modified Ritchie or Bailenger methods.
- **Culture:** Not often undertaken.
- **Antigen assays:** Distinction can be made between *E. histolytica* and *E. dispar* (a non-pathogenic species with very similar morphology) by the direct detection of amoebal antigens in faeces using species-specific monoclonal antibodies.
- **Molecular biology:** The vegetative forms and cysts of *E. histolytica* in faecal material can also be differentiated from those of *E. dispar* using PCR.

IMMUNOLOGICAL DIAGNOSIS

– Passive haemagglutination is based on treating sheep red blood cells with a soluble *E. histolytica* antigen.

– Latex bead agglutination is a simple, rapid test.

– Indirect immunofluorescence is based on whole parasite antigen, derived from *E. histolytica* trophozoites grown in axenic conditions.

– ELISA methods based on soluble antigens.

– Precipitation methods: Immunoelectrophoresis and co-electrosyneresis are more specific and such tests can be ordered to confirm a positive screen result, but they are not commercially available.

INTERPRETATION

– A parasitological stool examination examination is indispensable in the diagnosis of intestinal forms of the disease but the test must be performed on freshly passed material. Detection of *E. histolytica histolytica* in the faeces points to acute amoebiasis, whereas the presence of minuta forms or *cysts*, suggests that the patient is either an asymptomatic carrier or has had amoebiasis in the past without sterilising treatment.

– Serological tests are essential for the diagnosis of extra-intestinal forms and are less relevant to intestinal amoebiasis because, in this form of the disease, antibodies are not always elicited.

TREATMENT

– Extra-intestinal amoebicides: 5-nitro-imidazolates, administered orally or IV, diffuse through the tissues and destroy histolytica amoebae: metronidazole, tinidazole, secnidazole and ornidazole.

– Contact amoebicides: Administered orally, kill intraluminal *minuta* forms: tiliquinol and tilbroquinol.

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AMPHETAMINES

DEFINITION

Amphetamine and its derivatives are synthetic products which used to be prescribed to treat narcolepsy, paediatric hyperactivity and to suppress appetite. These days, amphetamines are almost exclusively misused for their stimulating or entactogen properties, by drug addicts, as recreational drugs or by athletes as doping agents. The most important of these narcotic substances are:

– Amphetamine (alpha-methylphenethylamine) also referred to as Benzedrine, Dexedrine or speed.

– Methamphetamine (N-alphadimethylphenethylamine or N-methylamphetamine), also referred to as ice, speed, crystal meth or crank.

– MDMA (methylenedioxymethamphetamine): Ecstasy, Adam, XTC or "E".

– MDEA (N-ethyl-3-4-methylenedioxyamphetamine): Eve or MDE.

– MDA (Alpha-methyl-1, 3-benzodioxole-5-ethanamine): Love drug, Love pill or tenamphetamine.

– MBDB (N-methyl-1-(1, 3-benzodioxazol-5-yl)-2 butanamine): Eden.

INTRODUCTION

■ WHEN POISONING CAN OCCUR

Poisoning generally occurs when an amphetamine has been consumed for its psychostimulatory properties in a recreational context. Often the exact dose consumed is unknown and the amphetamine was taken together with other products such as cannabis, alcohol or psychotropic drugs. Amphetamine and its derivatives are usually taken orally but sometimes by inhalation, or in the case of amphetamine itself, by intravenous injection.

■ MODE OF ACTION

Amphetamine induces the release of dopamine into the synapses of central dopaminergic neurons, especially in the caudate nucleus. This has a number of consequences, first and foremost, the extracellular concentration of noradrenaline in the hippocampus and frontal cortex is raised, and at high doses, the concentration of serotonin in the caudate nucleus is also elevated.

MDMA binds with high affinity to serotoninergic receptors (5HT2), adrenergic muscarinic M1 receptors and H1 histamine receptors. Its affinity for the active serotonin transporter is forty times higher than that of dopamine. The mechanism underlying the predominant indirect serotoninergic effect of MDMA is similar to that of amphetamine on dopaminergic neurons.

The mechanism of action of MDEA is identical to that of MDMA. In rats, neurotoxic effects have been observed in the central serotoninergic system.

■ MAIN EFFECTS AND TOXICITY

Substance	Main effects (actively sought)	Toxicity
Amphetamine	Enhanced mental and physical capacity and euphoria.	Long-term: Agitation, anxiety, weight loss, insomnia, hallucinations, paranoia and dependence. Overdose: Agitation, convulsions, violence, suicidal or homicidal ideas, hyperthermia, cardiac problems, cerebral haemorrhage, loss of consciousness, respiratory failure and death.
Methamphetamine	Feeling of well-being, enhanced concentration, suppression of appetite and increased libido.	Low-dose (10-25 mg): Irritability, insomnia, headache and akinesia. High-dose (25-60 mg): Confusion, sweating, hyperreflexia, tremor, hallucinations, hyperthermia, mydriasis and paranoia. Acute poisoning: Extreme anxiety, breathing difficulties, severe cardiac and neurological problems, psychological dependence and tolerance.
MDMA (Ecstasy) MDEA, MDA, MBDB	Suppression of appetite, used as a "truth serum", used to enhance communication in psychotherapy. Empathy, altered perceptions of space and time.	Toxic effects can occur after "normal", as opposed to excessive consumption and effects are highly variable from one person to another: • Tachycardia, dry mouth, jaw percussion, anorexia, sweating and nausea. • Hyperthermia, convulsions, stiff muscles, mydriasis, neuropsychiatric problems (psychosis, panic attacks, etc.) cardiovascular problems, DIC, rhabdomyolysis, acute kidney failure, psychological dependence and tolerance.

Withdrawal symptoms include lassitude, mood disorders, insomnia, psychomotor insensibility and even depression.

■ PHARMACOKINETICS

	Amphetamine	MDMA, MDEA
Oral ingestion	rapid	Absorbed in 20-60 min
Plasma peak	2.5 hours after ingestion (plasma peak after the administration of a dose of 0.5 mg prolonged-release amphetamine to a child: 70 ng/ml after 4 hours, 64 ng/ml after 8 hours). In a regular user, the peak is reached one hour after the intravenous injection of 160 mg: 590 ng/ml	2 h and MDA (a metabolite) peak after 4 h
Metabolism	A fraction is deaminated to generate phenylacetone which is oxidised to benzoic acid which is in turn, conjugated with glycine to form hippuric acid. Then formation of active hydroxyamphetamine which is subsequently conjugated.	2 metabolic pathways: Demethylenation and N-dealkylation + formation of potentially neurotoxic derivatives
Clearance half-life	7-34 hours (usually 8-13 hours)	
Clearance	Amphetamine begins to appear in the urine 20 minutes after administration. The rate of clearance depends on the pH of the urine. In normal conditions, 20-30% is excreted as the parent molecule within 24 hours. When the pH of the urine is between 5.5 and 6, up to 74% is eliminated in unchanged form in the urine. When the pH of the urine is between 7.5 and 8, 1% is excreted in unchanged form, the majority being eliminated as deaminated metabolites.	8 metabolites of MDMA are found in the urine: The 3 main ones are MDA (active), HMMA and HHMA. The urinary metabolites of MDEA are conjugates (HME, DHE and MDA).

NB: The metabolism of diverse compounds yields amphetamine, namely methamphetamine, amphetaminil, clobenzorex, dimethamphetamine, fencamine, fenethylline, fenproporex, meneforex, mesocarb and prenylamine. Other substances can get converted to methamphetamine and then amphetamine: Selegiline, benzphetamine, fenfenorex and famprofazone.

INDICATIONS FOR MEASUREMENT

Screening/confirmation of amphetamine poisoning or consumption, either in a medical context (convulsions, coma), or for forensic purposes (e.g. in a driver involved in a fatal accident or an athlete suspected of doping).

INFORMATION

SAMPLE

For non-forensic purposes, collect 40 ml of urine into a special recipient at the laboratory. Take precautions against deception (substitution, dilution with water or addition of adulterating agents) by immediately measuring the sample's pH and density, as well as its temperature (temperature >30°C). Close the bottle well and mark it with exact identifying details as well as the time, date and place of sampling. If the test has been ordered for a judicial investigation, the recipient should be officially sealed before dispatch to a qualified laboratory.

QUESTIONS FOR THE PATIENT

If possible, about the circumstances and time of poisoning, any ill effects experienced, and details of any medicinal products or psychoactive substances taken concomitantly (possibly from witnesses).

SAMPLE STORAGE AND TRANSPORT

Keep at +4°C if the test is to be run within 48 hours. If testing is to be postponed, freeze at –20°C (at which temperature, it is stable for months).

ASSAY METHODS

Urine screening by immunoassay: RIA, EIA, FPIA

Specific confirmation methods: Gas-phase chromatography coupled with mass spectrometry, HPLC coupled with mass spectrometry and capillary electrophoresis.

NORMAL EXPECTED VALUES

Zero unless the subject has taken an amphetamine.

INTERPRETATION

Results must be interpreted in the light of the compound's complex pharmacokinetic and metabolic properties. Many urine screening tests are available. The usual thresholds for positive results are 300 ng/ml for amphetamine and 1000 ng/ml for methamphetamine. In France, the legislation on screening for narcotic products for people involved in a fatal road accident (in force as of 1 October 2001) sets the threshold for a positive result at 1000 ng/ml.

Amphetamine can be detected in urine for three days after administration and methamphetamine remains detectable for seven days.

MDMA can be detected in urine for 72 hours after ingestion and conjugated metabolites can be found eight days after ingestion.

Screening immunoassays performed on urine can give falsely positive results in patients on sympathomimetic amines and medicinal products such as labetalol, tranylcypromine, ranitidine, cafedrine, nasal decongestants (ephedrine, phenylephrine, etc.) and appetite suppressants (fenfluramine, clobenzorex, etc.). Attention should also be paid to substances that are used to adulterate urine in vitro (hydrogen peroxide, detergents, liquid soap, table salt, benzalkonium chloride, glutaraldehyde, etc.).

If a urine screening test gives a positive result, confirmation is essential using an appropriate method to identify the specific products taken, and make an accurate quantitative determination. In general, persons who regularly consume amphetamines tolerate very high levels in the blood and there is not necessarily any correlation between the measured blood concentration and symptoms. Accidents are not therefore always dose-dependent.

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AMYLASE

DEFINITION

Amylases (or alpha-amylases) are enzymes that catalyse the hydrolysis of polymers of alpha-D-glucose by cleaving C1-C4 bonds. There are two genetically distinct groups of isoenzymes, defined according to their source (pancreatic P or salivary S). Five pancreatic fractions and six salivary ones have been distinguished by electrophoresis, isoelectric focusing or ion exchange chromatography. Both P and S isoforms are found in the blood, each accounting for half of all amylase activity in the circulation. Amylase is also found in tears, breast milk, perspiration and various cells and organs, including leukocytes, platelets, intestinal epithelial cells, the lungs, the prostate and the female genital systems (these isoenzymes resemble the salivary form). The enzyme is filtered in glomeruli and excreted in the urine.

INTRODUCTION

The main function of amylase is to hydrolyse dietary starch to release glucose, maltose and dextrin.

INDICATIONS FOR MEASUREMENT

– Investigation of pancreatic disease.

– Investigation of disorders affecting the saliva.

INFORMATION

■ SAMPLE

Assaying amylase (or the salivary and pancreatic isoenzymes) in blood: The ideal sample-type is serum which should be centrifuged and separated rapidly after drawing. Depending on the methods, heparin or EDTA may be used but not other anticoagulants, i.e. oxalate, citrate or fluoride.

Preferably, the patient should be fasting (unless the test is being conducted in an emergency situation).

Urine assay: 24-hour urine collection or a freshly passed urine sample (amylase is unstable in urine).

Aspiration biopsies and drain or endoscopic samples: Amylase is labile so the test should be run as soon as possible.

Take precautions to avoid contamination of either sample or reagent with saliva or sweat, both of which contain a large amount of amylase.

■ QUESTIONS FOR THE PATIENT

Are you taking any of the following medications? Valproic acid, opiates, cholinergic agonists, thiazide diuretics, aspirin, corticosteroids, oral contraceptives or indomethacin? All of these can raise the amount of amylase in the blood.

NB: In HIV-positive subjects, didanosine can induce severe or even fatal acute pancreatitis associated with a marked rise in the concentration of pancreatic amylase and regular monitoring is recommended.

SAMPLE STORAGE AND TRANSPORT

Blood: Centrifuge and decant-off the sample as soon as possible. Haemolysed and fatty samples should be discarded.

Storage of serum: Freeze at -20°C within four hours of blood drawing.

Urines: As long as the pH is close to 7 (amylase activity is destroyed at low pH), urine samples can be kept for 48 hours at +4°C.

ASSAY METHODS

Total α-amylase activity: Dynamic measurement of the enzyme's activity on a chromogenic substrate. Various substrates can be used:

– The reference method (IFCC 1998) is based on NPG7ethylidene (4-nitrophenyl-1, 4-alpha-D-maltoheptaoside) with an alpha-glucosidase.

– Another IFCC-validated substrate is CNPG3 (2-chloro-4nitrophenyl-alpha-D-maltotriose).

European Union-approved reference enzyme material (CRM 476) has been developed to standardise this test.

α-amylase isoenzymes:

– Separation by electrophoresis, isoelectric focusing or chromatography (specialist laboratories).

– Specific assay for the pancreatic isoenzyme after inhibition of the salivary isoenzyme (S) using specific monoclonal antibodies (dynamic test with a chromogenic substrate).

NORMAL EXPECTED VALUES

Total alpha-amylase activity will vary depending on assay method and temperature. For reference purposes:

		30 °C (IU/l)	37 °C (IU/l)
Serum	New-born babies	2- 22	2 - 32
	Adults	10 – 45	12 – 62
Urine		10 - 150	12 – 210

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

– Total alpha-amylase activity is lower in new-born babies (due to pancreatic immaturity) and young children; adult levels are reached by about five years of age (and those of the pancreatic isoenzyme at about 15).

– Activity is sometimes slightly higher during pregnancy, in the elderly (probably due to impaired kidney function) and in people of African and Asian descent.

■ PATHOLOGICAL VARIATIONS

There are many possible causes of elevated amylase activity in the blood and/or urine. Interpretation is helped if the result of a blood lipase assay is available.

NB: The first aetiology to consider in a subject with elevated blood amylase activity is acute pancreatitis (an emergency diagnosis). Hyperamylasaemia persisting after an acute episode points to complications.

■ **Concomitant rises in amylasaemia, amylasuria and lipasaemia**

– This points to pancreatic disease, most likely acute

pancreatitis. Blood amylase peaks 12-72 hours after the onset of pain (reaching 4-6 times the normal reading). The extent of the rise does not correlate with the severity of the condition. Amylasuria rises in parallel over time but the change is greater and it is staggered by about eight hours. Care is warranted because amylase levels in the blood can be normal in a patient with genuine acute pancreatitis although this is rare if the test is performed early enough. In practice, because the clearance half-life of amylase is short (a matter of hours), a urinary amylase test should be combined with the blood test because amylasuria may remain high after amylasaemia has returned to normal.

– Other causes of concomitant rises in amylasaemia and amylasuria, usually associated with elevated lipasaemia: Chronic pancreatitis, cancer of the pancreas, a perforated ulcer, upper intestinal obstruction, mesenteric infarction, viral hepatitis, acute cholecystitis, and lithiasis with obstruction of the hepatic ducts.

- **Elevated amylasaemia and amylasuria – lipasaemia normal**

  This pattern is usually due to elevated levels of salivary amylase. The most common cause is alcoholism and is due to direct stimulation of the salivary glands by alcohol. The rise in amylasaemia is usually moderate (2-3 times the upper normal limit). It is seen in about one-third of all long-term heavy drinkers.

  Other causes are diseases affecting the salivary glands, such as infection, parotid lithiasis, tumour, maxillofacial surgery, irradiation of the parotid glands but also mumps, female genitourinary problems (salpingitis, ectopic pregnancy, rupture of an ovarian cyst) and some forms of cancer (lung, prostate, ovarian). In these situations, the rise in amylase activity can be dramatic.

- **Elevated amylasaemia with normal amylasuria and lipasaemia**

  This pattern points to either kidney failure (with the hyperamylasaemia remaining moderate), or macroamylasaemia due to the binding of a fraction of blood amylase by a macromolecule; an immunoglobulin (most commonly IgA or sometimes IgG), a glycoprotein or a polysaccharide, thereby inhibiting its renal filtration. The increase in amylasaemia is usually moderate but levels ten times higher than normal are sometimes seen. The elevation is stable over time. This common abnormality (accounting for 0.5% of cases of hyperamylasaemia and 20% of "unexplained elevations") is not considered as pathological.

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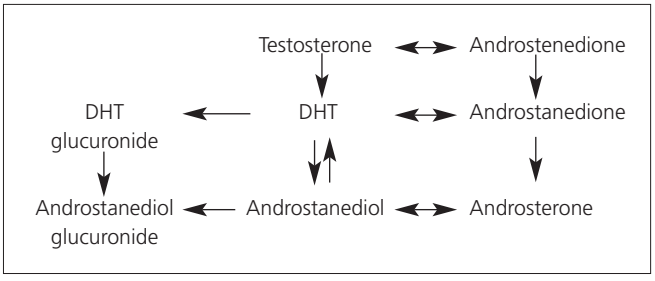
ANDROSTANEDIOL
(GLUCURONIDE)

DEFINITION

Androstanediol (5α-androstane-3 α, 17-diol, ADIOL) is generated from dihydrotestosterone (DHT) by 3-hydroxysteroid dehydrogenase. Although both the α and β derivatives can be formed in this reduction reaction, far more of the former is produced. Androgens are metabolised in the liver and the target tissues. Androstanediol represents the end-product of testosterone metabolism commonly referred to as the 17 β-hydroxy pathway as opposed to the 17-oxo pathway which passes via androstenedione. From a metabolic point of view, the 17 β-hydroxy pathway is a minor one in the metabolism of testosterone (most of which is metabolised in the 17-oxo pathway to generate androsterone and etiocholanolone).

Equal quantities of androstanediol are derived from androstenedione and DHT (see the figure). It is then conjugated with glucuronate at either the 3α or the 17β position, with the latter markedly predominating in humans. However, only a minority of the androstanediol glucuronide (GADIOL) in the circulation comes from androstanediol, and DHT is a minor precursor. In fact, it is DHA and androstenedione that are the most significant precursors. Most GADIOL is synthesised from precursors in a pathway that does not involve DHT or androstanediol but rather the glucuronide of DHT (see the figure).

Moreover, as there is a relationship between the level of GADIOL and that of bioavailable testosterone, it is the latter rather than total testosterone that can enter cells and get metabolised by 5α-reductase. GADIOL is then mainly excreted in the urine.



The high levels of adrenal GADIOL precursors in women explain why its concentration in the blood drops after long-term corticosteroid therapy and rises after stimulation of the adrenal cortex.

Since GADIOL is mainly synthesised in target tissues; notably in the skin and pilosebaceous follicles, its peripheral concentration depends on the activity of 5 α-reductase in the skin. This is why this measurement is generally considered as reflecting this enzyme's activity. In contrast, the concentration in the urine is unrelated to cutaneous 5 α–reductase activity. In addition, significant positive correlation between the concentrations in serum and urine has only been demonstrated in young males. In consequence, it is generally accepted that most serum GADIOL has an extrasplanchnic origin whereas that urine GADIOL has both splanchnic and extrasplanchnic origin.

INDICATIONS FOR MEASUREMENT

GADIOL is usually assayed as part of an investigation of hirsutism in women and children. It is also recommended in the investigation of masculine hermaphroditism for the detection of 5α-reductase deficiency.

INFORMATION

■ SAMPLE

The assay is conducted on serum, plasma or an aliquot of a 24-hour urine collection. Blood can be drawn at any time of day and, in women, at any phase of the menstrual cycle. Anticoagulants do not interfere with the assay, and neither high lipid content in the sample or haemolysis pose any problem because the method used includes extraction and chromatography steps.

■ QUESTIONS FOR THE PATIENT

Since GADIOL is derived from androstenedione and DHA sulphate, find out about corticosteroid use.

■ SAMPLE STORAGE AND TRANSPORT

Decanted plasma and serum as well as 24-hour urine should be kept at +4°C and sent to the laboratory at that temperature.

They can be stored for 6-12 months at –30°C.

ASSAY METHODS

Androstanediol is rarely assayed in routine clinical practice and a serum or urinary GADIOL test is more commonly ordered. For the test, a volume of serum or urine is specifically hydrolysed using glucuronidase. This releases free androstanediol which is then purified (by extraction followed by chromatography) and measured using a radioimmunologic assay. Specificity will depend on efficient purification.

A kit available on the market allows the direct radioimmunologic assay of serum GADIOL without hydrolysis, extraction or chromatography steps but this is only suitable for assaying urine.

UNITS

Serum GADIOL results are expressed in either ng/ml or nmol/l (the ng→nmol conversion factor is 2.134). In urine, the result is expressed in terms of "androstanediol" equivalents as either µg/24 hours or µmol/24 hours (the mg→µmol conversion factor is 3.419).

USUAL VALUES

Plasma or serum GADIOL concentrations range:

– In adult men, between 2.2 and 26 ng/ml or between 4.70 and 55.48 nmol/l

– In adult women, between 0.6 et 4.8 ng/ml or between 1.28 and 10.24 nmol/l.

In urine, the rate of GADIOL excretion ranges:

– In adult men, between 55 and 170 µg/24 hours or between 188 and 581 nmol/24 hours

– In adult women, between 12 and 80 µg/24 hours or between 41 and 274 nmol/24 hours.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

The serum concentration of androstanediol glucuronide is very low in children of both genders, beginning to rise at about 8 in girls, and at about 11 in boys. Concentrations rise further at puberty in both girls and boys. As of Tanner Stage III, levels in boys rise above those in girls.

In adults, there are neither diurnal nor, in women, menstrual variations. GADIOL concentrations are substantially lower in women. In men, they steadily drop off with age but this waning has not been significant in all studies. Similarly, in post-menopausal women, GADIOL concentrations are lower than those in women of child-bearing age. In urine, GADIOL concentrations follow the same patterns in both men and women.

PATHOLOGICAL VARIATIONS

Serum androstanediol glucuronide

Elevated concentration

Disorders of puberty

GADIOL levels rise in early puberty and premature adrenal maturation (adrenarche).

Congenital adrenal hyperplasia

GADIOL concentrations are elevated in 21-hydroxylase deficiency with or without salt loss. They correlate with those of androstenedione, 17-hydroxyprogesterone and testosterone, in both boys and girls. They are also elevated in 11β-hydroxylase deficiency. In both cases, this parameter can be used to monitor the efficacy of treatment.

Hirsutism

Increased cutaneous 5α-reductase activity can be accompanied by rises in GADIOL concentration although this is not observed in all females with this form of the condition. Similarly, GADIOL is not systematically elevated in women with another form of hirsutism (due to impaired ovarian or adrenal function). Nevertheless, some experts have observed that such increases are more common in women in whom hirsutism is accompanied by menstrual problems than in those with a normal menstrual cycle.

Since the treatment of hirsutism with prednisone or spironolactone lowers GADIOL whereas treatment with leuprolide (a LHRH agonist) does not, it has been concluded that the GADIOL concentration seems to reflect the rate of adrenal androgen production rather than that of androgen metabolism in the skin.

Decreased concentrations

5α–reductase deficiency (see DHT)

This deficiency involves Type 2 5α–reductase and results in masculine pseudo-hermaphroditism. The syndrome is hereditary and transmitted as an autosomal recessive trait. The gender of the new-born baby is ambiguous and he is often believed to be female. At puberty, these boys with a female phenotype undergo a greater or lesser extent of somatic and behavioural virilisation, where muscles develop, the voice breaks and the external genitals develop; there is never gynaecomastia. Overall, post-pubertal morphology is typically masculine apart from the distribution of pubic hair which tends to follow a feminine pattern.

Laboratory diagnosis is relatively straightforward after puberty, characterised by a high ratio of testosterone to DHT (see DHT). The GADIOL concentration is very low which makes diagnosis possible without the need for a HCG stimulation test.

Androstanediol glucuronide in the urine

A urine GADIOL measurement is usually carried out before the serum result is available. Most studies have been in the context of the investigation of hirsutism and levels have shown to be increased in only a fraction of patients, with disparate results from one study to another.

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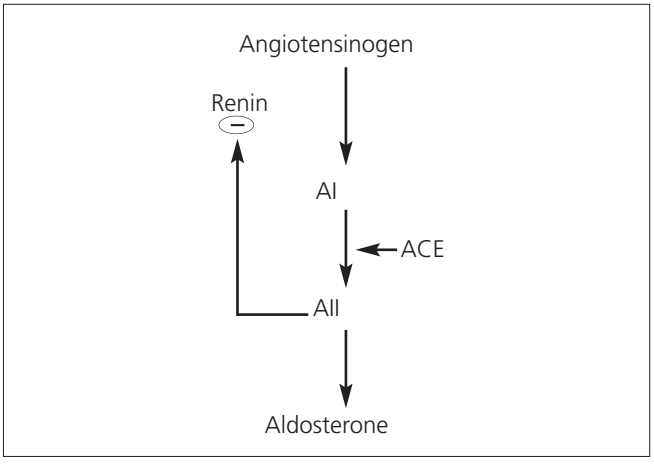
ANGIOTENSIN II

DEFINITION

Angiotensin II (AII) is an 8-amino acid peptide (1045 Da) and is a potent vasoconstrictor produced in the plasma of the blood, following activation of the renin-angiotensin system.

INTRODUCTION

Angiotensin II is a key link in the renin-angiotensin system.



Regulation of the renin-angiotensin system (RAS)

To understand the role of AII, the main stimulant of aldosterone production, the successions of reactions that lead to its formation have to be analysed. Since the RAS is of particular therapeutic importance in patients with arterial hypertension, it is important to outline the impact of certain circumstances and medicinal products on this system.

Angiotensinogen synthesised in the liver is cleaved by renin to generate a decaptide, which is angiotensin I.

Renin is synthesised by the juxtaglomerular myoepithelial cells of the afferent arterioles. Production is:

– Stimulated by a drop in arteriolar pressure, hypovolaemia, low sodium concentration and the presence of prostaglandin or an adrenergic substance, as well as by standing up and physical activity.

– Inhibited by AII.

AI is converted to AII (an octapeptide) by:

– Angiotensin-converting enzyme (ACE) to a great extent;

– To a lesser extent, by vascular carboxypeptidases.

AII has autocrine and paracrine activities in the regulation of many organs, including the heart, lungs and kidneys. All of AII activities depend on the binding of specific receptors:

– AT1 receptors: Which stimulate the production of aldosterone in the adrenal cortex (among other effects);

– AT2 receptors: Opposing effects.

Aldosterone synthesis and secretion is mainly RAS-dependent but is also affected by ACTH and kalaemia; atrial natriuretic peptide, vasopressin, dopamine are inhibitors.

NB: The primary class of anti-hypertensive drugs are ACE inhibitors and compounds that block the binding of AII to AT1 receptors.

In investigations of the RAS and all factors that can affect volaemia need to be assessed, including the sodium ion concentration.

INDICATIONS FOR MEASUREMENT

A work-up to investigate hypertension and hyperaldosteronism usually includes assays for active renin and aldosterone although plasma and urinary electrolyte levels remain the primary guiding parameters.

INFORMATION

■ SAMPLE

AII has to be measured in plasma from blood collected into EDTA in a pre-cooled tube.

Ideally, the blood should be drawn in the morning after the patient has been calm and lying down for one hour and then again after stimulation by one hour of walking.

It should be checked that the patient's sodium intake was normal over the three days prior to blood drawing.

■ SAMPLE STORAGE AND TRANSPORT

As soon as the blood has been drawn, the tube should be placed on ice. Centrifuge as soon as possible at low temperature and, if the test is to be postponed, freeze the plasma within one hour of blood drawing. Store the plasma frozen at –20°C until testing.

AII is stable at –20°C for at least a month; do not repeatedly thaw and refreeze an aliquot.

■ QUESTIONS FOR THE PATIENT

Are you taking any drugs (anti-hypertensives, NSAIDs)? Are you on a low-sodium diet?

ASSAY METHODS

Competitive immunoassay

Based on specific monoclonal antibodies, after ethanol extraction.

High performance liquid chromatography (HPLC) ± mass spectrometry (MS)

Useful to establish the ratio of AII to AI.

NB: Dynamic tests can be performed (RAS suppression by salt loading or using captopril).

NORMAL EXPECTED VALUES

For reference: 20-100 pg/ml.

PHYSIOLOGICAL VARIATIONS

– Values tend to be higher in babies and young children.

– Values are lower in the elderly.

– The following are associated with elevated values:

- Pregnancy.
- Low sodium diet.
- Oral contraception.

PATHOLOGICAL VARIATIONS

Assaying AII is less common than assaying for renin, aldosterone and electrolytes. As an aetiological diagnosis is undertaken before instigating a course of treatment for hypertension, interpretation of the results aims to differentiate between primary hyperaldosteronism (Conn's syndrome or bilateral hyperplasia of the zona glomerulosa of the adrenal cortex) and secondary hyperaldosteronism (due to RAS hyperactivity).

What does assaying the AII concentration contribute?

– Although the AII concentration does not correlate with blood pressure, a high value points to activation of the RAS cascade.

– A drop in AII level in a captopril test (suppression due to ACE inhibition) suggests that anti-hypertensive treatment based on an ACE inhibitor or an AT1 blocker could be beneficial.

If the patient is being treated, the test should be performed within a therapeutic window with regulated sodium intake.

Drug-induced perturbation of the RAS

– β-blockers: Renin down, aldosterone down

– Diuretics: Renin up, aldosterone up

– Prostaglandin inhibitors (non-steroid anti-inflammatory drugs and salicylate): Inhibition of the RAS

– Aldosterone antagonists (spironolactone): AII up

– Antidopaminergic drugs: Aldosterone up

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ANGIOTENSIN CONVERTING ENZYME

DEFINITION

Angiotensin converting enzyme (ACE) catalyses the hydrolysis of angiotensin I into angiotensin II and degrades bradykinin, producing vasoconstrictor effects. ACE is synthesised by the vascular endothelial cells.

Synonym: Angioconvertase.

INTRODUCTION

ACE plays an important role in vascular tone and permeability of the vasoconstrictor angiotensin II by degrading kinins, particularly bradykinin which is a vasodilator. It is a marker of endothelial damage. ACE is also believed to be involved in the degradation of the neuronal peptides, such as substance P, encephalins and LH-RH.

ACE is synthesised by vascular endothelial cells. In disease states, it can be produced by circulating macrophages and lymphocytes and be found in large amounts in the circulation.

ACE is also present in the renal tubular epithelial cell brush border, bowel and choroid plexus.

INDICATIONS FOR MEASUREMENT

The main indication for ACE measurement is in the diagnosis and monitoring of the treatment of sarcoidosis. It can also be used to monitor the effectiveness of treatment with a converting enzyme inhibitor.

INFORMATION

SAMPLE

Serum: 0.5 to 1 ml. Plasma, particularly EDTA plasma must not be used (EDTA inhibits the enzyme's activity).

CSF or BAL.

QUESTIONS FOR THE PATIENT

Are you being treated with angiotensin converting enzyme inhibitors? These drugs reduce ACE.

SAMPLE STORAGE AND TRANSPORT

Serum storage: 8 days at +4°C; then three months at –20°C

Transport at + 4°C (if less than < 8 days).

CSF and BAL: Centrifuge immediately, freeze the supernatant within an hour of sampling and transport at –20°C.

ASSAY METHODS

ACE activity is measured using artificial substrates including hippuryl-histidylleucine (HHL) and furylacryoloyl-phenylalanyl-glycyl-glycine (FAPGG). With the latter, the fall in absorbance at 340 nm is proportional to the amount of substrate hydrolysed. This method can be automated. Activity is expressed in ACE units (ACEU) which represents the hydrolysis of one micromole of substrate per minute.

NORMAL EXPECTED VALUES

FAPGG method.

Adults: 12- 68 ACEU.

Children: 27-113 ACEU.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Activity is higher at birth and in childhood until adolescence.

PATHOLOGICAL VARIATIONS

Serum concentrations are raised in 50 to 75% of cases of sarcoidosis although the rise is not specific and is seen in various disorders: non-sarcoid respiratory disease (silicosis, asbestosis, berrylliosis, etc.) and non-respiratory diseases (leprosy, Gaucher's disease, schistosomiasis, etc.). Serum Ace can also be raised in cholestasis, renal insufficiency and hypothyroidism.

A large rise associated with symptoms of multi-organ involvement (particularly lung but also skin, eyes, etc.), however, suggests sarcoidosis. Although a normal ACE concentration does not exclude the diagnosis particularly in non-pulmonary muco-cutaneous and joint forms of the disease.

A modest rise in sarcoidosis is a good prognostic indicator. Corticosteroids reduce the serum enzyme activity and recovery without complications is seen in approximately 2 years in 50% of cases. ACE measurement is useful to adjust the corticosteroid dosage; a further rise in ACE indicates recurrence of the disease.

In sarcoidosis, ACE may also be increased in BAL fluid and CSF if granulomas are present in the central nervous system. ACE measurement in CSF is used in the differential diagnosis from other neurological diseases (multiple sclerosis, malignant tumours, etc.).

FOR FURTHER INFORMATION

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ANISAKIASIS

DEFINITION

Anisakiasis is a gastro-intestinal helminthozoonosis due to infestation by anisakidae larvae in human beings. These are marine mammal parasites. It is a visceral form of *larva migrans* caused by several species of nematodes, primarily *Anisakis simplex*.

Synonyms: anisakiasis, anisakiosis, anisakidosis.

INTRODUCTION

EPIDEMIOLOGY

Human infection occurs through consumption of raw, poorly cooked, smoked or traditionally prepared marinated seafood. Most commonly eaten fish are infected (herring, sardine, mackerel, whiting, tuna, salmon, etc).

Anisakiasis is ubiquitous and common in countries in which raw or poorly cooked fish are commonly eaten. It is endemic in Japan, present in North America and in Europe (in France and Spain particularly but also in countries bordering the North Sea). Human-to-human transmission in not possible.

LIFE CYCLE

The cycle is indirect and human beings are an accidental host and a block to the parasite. The definitive host is a marine mammal and the intermediary host being sea fish and molluscs.

The adults live in marine mammal stomachs (whales and pinnipeds). The eggs are excreted in the animals' faecal matter and release stage-1 larvae into seawater. The larvae are ingested by plankton where they become stage-2 larvae. Small crustaceans and then sea fish act in turn as intermediary hosts for the larvae which reach stage-3 and only become adults if the infested fish are eaten by a marine mammal. Human beings are infested by ingesting the larvae (approximately 3 cm long at stage-3) contained in the flesh of parasite-infested fish. The larvae penetrate the gastro-intestinal mucosa causing inflammatory and allergic reactions.

SYMPTOMS

These are characterised by an acute abdominal syndrome occurring a few hours after eating infested fish.

Clinical symptoms are atypical, dominated by stomach and intestinal disorders but also by various allergic reactions (urticaria, bronchospasm, anaphylactic shock etc).

The disease progresses in 2 stages:

- An initial painful early stage which lasts for between 12 and 72 hours (acute gastritis or ulcer syndrome).

- Established disease phase in which the clinical symptoms are polymorphic (abdominal pain, nausea, vomiting, bowel transit disorders). In view of its lack of specificity it is often discovered late at the stage of surgical gastro-intestinal complications.

SEARCH INDICATIONS

Diagnosis of anisakiasis in a patient with painful acute abdominal syndrome or allergic reactions developing after recently eating raw fish.

Differential diagnosis with another helminthiasis.

INFORMATION

SAMPLE

Serum (Dry tube): For the serological diagnosis and measurement of total and specific IgE.

Whole blood: Taken into EDTA for a full blood count and differential cell count.

Other samples: Biopsies.

QUESTIONS FOR THE PATIENT

Clinical symptoms.

Raw or poorly cooked fish eaten recently?

Current antihelminth treatment.

SAMPLE STORAGE AND TRANSPORT

Serum: Store at 4°C for 1 week, then freeze at–30°C for 1 year.

BIOLOGICAL DIAGNOSTIC METHODS

GUIDING DIAGNOSIS

– ***Raised eosinophil count:*** Common but mild.

– ***Hyperleukocytosis:*** Particularly intestinal forms of the disease.

– ***Total and specific IgE:*** Raised.

DIRECT DIAGNOSIS

Parasitological examination of stools is always negative. An unequivocal diagnosis can only be made when the larva is found directly on endoscopic examination, in an excision section or in vomit.

INDIRECT DIAGNOSIS

Serum antibody testing is possible although is often negative at the start of the infestation, in addition to which cross-reactions with other helminthiases are very common. The commercially available RAST (radioallergosorbent test) technique used to measure IgE is positive early and highly specific.

RESULTS

The diagnosis of anisakiasis is difficult as the clinical symptoms are not particularly suggestive and the direct biological diagnosis is limited. It is usually made from serology or from a histopathological finding where a macroscopic examination reveals a nodular tumour measuring a few cm in diameter. Histological examination reveals a granuloma rich in eosinophils, lymphoplasmacytes and giant cells with the larva found in the centre.

If a gastric form of the disease is suspected, gastroscopy can visualise the parasite bound to the gastric mucosa and remove it.

Imaging is decisive in atypical extra-gastric forms of the disease as it can identify the affected segment and avoid complications.

In summary, the arguments supporting the diagnosis of anisakiasis (raised eosinophilia count, gastro-intestinal pain and history of eating raw or poorly cooked fish) can be confirmed using endoscopic, histological and/or serological techniques.

TREATMENT

– ***Medical:*** The disease resolves spontaneously and symptomatic therapy is generally sufficient. Benzimidazole derivatives (albendazole), occasionally combined with corticosteroid therapy are useful in diffuse and allergic forms of the disease.

– ***Surgical:*** Indicated for gastro-intestinal complications.

– ***Prophylaxis:***

– Avoidance of raw or poorly cooked fish from the diet.

– Eating adequately cooked fish (at least 10 minutes at 60°C).

– Freezing fish intended to be eaten raw at– 20°C for at least 24 hours.

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ANTI-STREPTOCOCCAL ENZYME ANTIBODIES

DEFINITION

Anti-Streptococcal enzyme antibodies are antibodies against the enzymes of groups A, C and G β-haemolytic Streptococci from the Lancefield classification. There are 4 of these: ***anti-streptolysin O antibodies (ASLO), anti streptodornase B antibodies (ASD), antistreptokinase (ASK)*** and ***anti-streptohyaluronidase (ASH)***.

INTRODUCTION

The Streptococcal enzymes are haemolysins or other extracellular substances secreted by Streptococcus pyogenes (group A), responsible for the majority of bacterial sore throats. They are also secreted by groups C and G Streptococci.

- **Streptolysin O** is a cytolytic toxin or haemolysin which is labile in the presence of oxygen and is secreted by many strains of group A Streptococci. It is highly immunogenic, therefore allowing the antibodies which it induces to be measured: ASLO. It can destroy red cell membranes as well as those of polynuclear cells and platelets.
- **Streptodornase or deoxyribonuclease:** there are 4 types: A, B, C and D, which can depolymerise DNA. They are antigenic and induce the formation of antistreptodornase or deoxyribonuclease (DNA ase) antibodies.
- **Streptokinase or antigenic fibrinolysin:** is produced by groups A, C and G Streptococci and also by a few strains of groups B and F. It has fibrinolytic activity, i.e. it catalyses the conversation of plasminogen into plasmin, which lyses fibrin.
- **Hyaluronidase** is produced by some types of group A and also by a few strains of groups B, C and G. It is immunogenic and induces the formation of antistreptohyaluronidase or ASH antibodies. It hydrolyses hyaluronic acid and increases spread of the infection by degrading the base material for connective tissue.

INDICATIONS FOR MEASUREMENT

Diagnosis of non-suppurative, post-Streptococcal disorders due to group A Streptococcus, particularly:

– Acute Articular Rheumatism or AAR.

– Acute Glomerulonephritis or AGN.

– Erythema nodosum.

These disorders are due to an immunological process due to antigenic commonality between body tissues and some constituents of the Group A Streptococcus. The bacterium is never found in the inflammatory lesions, which occur distant to the Streptococcal infection. The clinical symptoms have the common feature of presenting one to several weeks after the initial infection.

■ ACUTE ARTICULAR RHEUMATISM (ARR) OR ACUTE RHEUMATIC FEVER:

This is a rare complication which occurs two to three weeks after a Streptococcal sore throat due to Group A Streptococcus, which has been either untreated or inadequately treated. The risk of AAR following sore throat is in the region of 1 to 3%: it mostly affects children between 4 and 15 years old from disadvantaged backgrounds and people who have already suffered AAR. It is still common in North African countries and in the French overseas *departements* and territories.

- **Rheumatic fever** or the classical form of AAR is a combination of joint features (large joints) and cardiac features (particularly common in younger people). It appears that cell mediated (cytotoxic T lymphocyte) immunity has a major role in the pathogenesis of the cardiac disorders. The arthritis appears to be due to the formation of circulating immune complex deposition on the synovaie.
- **Acute Sydenham's chorea** is another isolated clinical form of AAR which occurs late, 1 to 6 months after Streptococcal pharyngitis and particularly in girls between 5 and 15 years old. It has a progressive insidious onset with changes in mood and reduced muscle tone followed by ataxia and severe frequent abnormal choreiform movements. It resolves spontaneously with possible long term relapses.
- **Nowadays, post-Streptococcal reactions** are often incomplete and may involve isolated monoarticular arthritis or highly inflamed painful oligo-arthritis or alternatively isolated cardiac dysrhythmias.

■ ACUTE GLOMERULONEPHRITIS (AGN):

This is a complication of sore throat or skin infection due to Group A Streptococcus which occurs in 10 to 15% of cases. Only some so-called "nephritogenic" serotypes are involved in AGN. Clinical features develop 10 days after a sore throat and 21 days after a skin infection, and is characterised by a combination of fever, abdominal pain, oedema, hypertension and oligo-anuria.

■ POST-STREPTOCOCCAL ERYTHEMA NODOSUM:

This is an acute dermo-hypodermitis characterised by sudden onset of painful nodules located mostly on the legs. It occurs mostly in women between 25 and 40 years old. The condition has many causes although previous Streptococcus A infection is very often implicated.

INFORMATION

■ SAMPLE

Serum sample in an empty tube. Serum should not be haemolysed, or icteric.

■ QUESTIONS FOR THE PATIENT

Has the patient previously had a Streptococcal infection? If yes, when?

Clinical features?

Age?

At risk person?

■ SAMPLE STORAGE AND TRANSPORT

Serum stored at + 4°C or frozen at – 30°C for 1 year.

ASSAY METHODS

These are based on measurement of the serum antibodies ASLO, ASD and ASK. The most widely used serological reaction is for ASLO, based on the principle of antibody inhibition of the haemolytic activity of streptolysin O on rabbit red blood cells.

– Rapid screening tests are available which can detect several anti-streptococcal antibodies simultaneously by haemagglutination: this is the Streptozyme® method.

– Each anti-enzyme antibody is measured by neutralisation.

NORMAL EXPECTED VALUES

As an indication:

ASLO: < 200 IU/ml in adults and < 100 IU/ml in children

ASD: ≤ 400 U/ml

ASK: < 180 U/ml.

PATHOLOGICAL VARIATIONS

ASLO develops approximately 10 days after the onset of an acute infection, rising to a peak between the 3rd and 4th week and then falling, returning to normal after 3 to 6 months. Re-infection results in a new rise in ASLO with accelerated kinetics. Measurement is therefore very useful to diagnose post-infectious complications of pharyngeal Streptococcal infections. On the other hand, they increase little if at all after a skin infection as streptolysin O is inactivated by cholesterol in the skin.

The pathological thresholds for the antibodies vary depending on the kits, manufacturers and units proposed: a titre > 200 IU/ml in an adult is considered positive. False positives occur because of cross-reactions with groups C and G Streptococci and also in a number of pathological circumstances: myeloma, SLE, inflammatory rheumatic disease such as rheumatoid arthritis, some liver disorders with cholestasis, nephrotic syndrome and some forms of hyperlipoproteinaemia. Various factors such as age and season influence antibody titres in healthy people. In addition, 20 to 30% of people who have had a Streptococcus A infection do not produce ASLO.

ASD is a good indicator of skin Streptococcus A infection (raised in 89% of cases). Their kinetics of development and disappearance are slower than those of the ASLO.

ASK and **ASH** are not specific to Streptococcus A and because of this have ceased to be useful. Increases in these antibodies are small and often erratic.

Summary: the most widely used serological markers are ASLO and ASD: a combination of the 2 markers increases diagnostic efficiency with a sensitivity approaching 100% for the diagnosis of AAR and AGN. Isolation of an isolated titre is difficult and the best approach is to demonstrate a clear rise in antibodies over a 15 day interval in 2 samples.

FOR FURTHER INFORMATION

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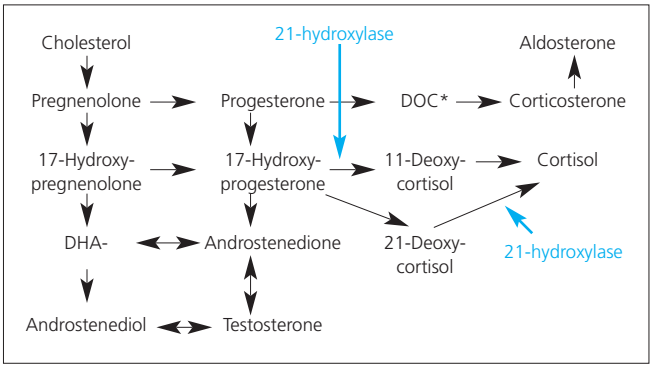
ANTI-21-HYDROXYLASE ANTIBODIES

DEFINITION

21-hydroxylase is a cytochrome P-450 microsomal enzyme complex which plays a key role in steroid biogenesis in the adrenal cortex *(see diagram on the biosynthesis of steroids in the adrenal cortex)*. It is responsible for converting progesterone and 17-hydroxyprogesterone into deoxycorticosterone and 11-deoxycortisol.



** DOC = deoxycorticosterone*

The protein found in the microsomal fraction after precipitation with serum from patients suffering from Addison's disease who are positive for anti-adrenocortical antibodies on immunofluorescence, was found to be 21-hydroxylase (21-OH). Antibodies isolated from the serum of patients suffering from Addison's Disease inhibit the activity of the enzyme in vitro, with the result that progesterone is no longer converted into deoxycorticosterone.

INTRODUCTION

These antibodies are able to cause adrenal failure because of their inhibition of 21-hydroxylase.

INDICATION FOR MEASUREMENT

Measurement is used in the investigation of Addison's patients to demonstrate an autoimmune origin for the disease.

INFORMATION

SAMPLE

Anti-21-hydroxylase antibodies are measured from serum.

SAMPLE STORAGE AND TRANSPORT

Following separation the serum should be frozen as soon as possible at –20°C. Transport the sample in its frozen state.

ASSAY METHOD

Anti-21-OH antibodies are currently measured radioimmunoassay. After incubating the samples with labelled 21-OH, anti-21- OH autoantibodies present bind to the labelled enzyme. In a second incubation the complexes formed are precipitated with protein A. The radioactivity of the precipitates separated by centrifugation is proportional to the amount of anti-21-OH antibodies initially present in the samples.

REFERENCE VALUES

The result is expressed in arbitrary units. Concentrations of 1 U/ml or more are considered to be positive for anti-21-OH antibodies.

PATHOLOGICAL VARIATIONS

ADDISON'S DISEASE

Anti-21-OH antibodies have not been found in Addison's disease secondary to tuberculosis or leukodystrophy. They have however been found in most patients with anti-adrenal antibodies.

There is a good correlation between the presence of anti-adrenal antibodies and anti-21-OH antibodies, suggesting that 21-hydroxylase is the major auto-antigen for these antibodies.

In Addison's disease, anti-21-OH antibodies tend to fall over time. It is likely that they disappear completely in some patients after the adrenal cortex has been totally destroyed.

MULTIPLE ORGAN AUTOIMMUNE SYNDROMES

Anti-21-OH antibodies have been found in most patients with both, type I and type II autoimmune polyglandular syndromes.

OTHER AUTOIMMUNE DISEASES

Anti-21-OH antibodies have been found in a small proportion (1.3 – 4.%) of patients with:

– Chronic idiopathic hypoparathyroidism,

– Insulin-dependent diabetes,

– Hashimoto's thyroiditis,

– Graves' disease.

In these patients the adrenal failure (Addison's disease) only developed after a latent period which was shorter in children than in adults. These antibodies could therefore be considered to be an early specific marker of latent adrenocortical involvement, which may become overt in future months or years.

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ANTI-ACETYLCHOLINE RECEPTOR ANTIBODIES

DEFINITION

Anti-acetylcholine receptor antibodies are found in the serum of patients with autoimmune myasthenia gravis or Lambert-Eaton myasthenic syndrome (LEMS).

Myasthenia is an autoimmune disease caused by circulating antibodies directed against the acetylcholine receptors in the post-synaptic membrane of striated muscles. The symptoms include predominant muscle fatigability in proximal muscles, particularly in the shoulder girdle, which is aggravated by exercise and at the end of the day. There is also ocular impairment (ptosis and diplopia), and damage to pharyngeal and laryngeal muscles, producing a characteristic hoarse voice.

LEMS is a paralysing autoimmune disorder characterised by predominant neuromuscular fatigability in the lower limbs, sometimes associated with dysphagia, ptosis, dysautonomia and/or hyporeflexia. In this disease, anti-acetylcholine receptor antibodies react with P/Q and N voltage-dependent calcium channels, which regulate the release of acetylcholine and are situated in neuromuscular junctions and in neurons.

Other antibodies directed against various components of striated muscle (actin, connectin, myosin and ryanodine receptors) have been described in myasthenic patients and they may also be induced by D-penicillamine and are often associated with a thymoma.

Synonyms: anti-cholinergic receptors, anti-AChR.

INTRODUCTION

The acetylcholine receptor is a protein complex composed of subunits known as alpha, beta, gamma and delta. Approximately 80% of myasthenia patients have antibodies against the acetylcholine receptor, reacting essentially with the alpha subunit and sometimes against the other subunits (beta, delta and gamma). The transplacental passage of anti-gamma antibodies from a myasthenic mother to her baby causes multiple congenital arthrogryposis, a foetal malformation characterised by deforming joint stiffness accompanied by paralysis. Certain forms of the illness are genetic, while others are autoimmune consequences of the transplacental passage of maternal anti-AChR antibodies.

In autoimmune myasthenia, the binding of autoantibodies to nicotine receptors in the post-synaptic membrane (which are calcium channels) causes either a modulation of the complexes by endocytosis (modulatory antibodies), or an inhibition of the receptors (inhibitory antibodies), which can no longer fix acetylcholine. The presence of modulatory antibodies suggests the (frequent) presence of a thymoma.

Among the 20% of myasthenia patients who are seronegative for anti-AChR antibodies, a certain number have antibodies against Muscle Specific Kinase (MuSK), a protein kinase, causing aggregation of anti-acetylcholine receptors.

In the case of LEMS, the fixing of autoantibodies to P/Q and N calcium channels prevents the release of acetylcholine. In more than 50% of cases, the syndrome is associated with a small-cell cancer of the lung; in this context, tumour cells carrying the receptors recognised by the autoantibodies are believed to be the reason for their production.

INDICATIONS FOR MEASUREMENT

Helps in diagnosing myasthenia or LEMS.

Monitoring the evolution of myasthenia in patients.

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not required.

Please ensure the sample is not haemolysed.

QUESTIONS FOR THE PATIENT

Are you taking any of the following drugs?

– D-penicillamine: This can be the reason for the presence of antibodies against striated muscle components (anti-connectin, anti-myosin, etc.).

– Myorelaxants: They may result in false positives for modulatory anti-AChR antibodies.

SAMPLE STORAGE AND TRANSPORT

Storage of serum: Work on fresh serum if at all possible, or freeze to -20° C immediately after centrifugation if analysis is to be delayed.

Transport frozen at -20° C.

ASSAY METHODS

Radioimmunological assay (RIA): Global anti-AChR measurement, specific RIA measurement of modulatory anti-AChR antibodies (on muscle cells in culture); specific RIA measurement of inhibitory anti-AChR antibodies; specific RIA measurement of antibodies against P/Q or N calcium channels.

Indirect immunofluorescence: Measurement of antibodies against other striated muscle components.

NORMAL EXPECTED VALUES

These autoantibodies are normally undetectable in the blood. Positive result thresholds vary according to the laboratory. Indicative values: anti-AChR antibodies < 0.5 nmol/l.

INTERPRETATION

IN MYASTHENIA

Diagnostic value of autoantibodies:

The presence of anti-acetylcholine receptor antibodies is a consideration in diagnosing the disease and measurement sensitivity in this condition ranges between 70% (in the absence of ocular impairment) and 90% (generalised myasthenia); specificity is slightly lower. The antibodies are not in fact pathognomonic for the disease and they may be

absent, or they may appear late, months or even years after the onset of clinical symptoms. They may also be found in other pathological circumstances (see below). Their presence is therefore not sufficient to make the diagnosis, but forms part of an overall clinical approach.

In cases of strong clinical suspicion, a negative result requires specific measurements of inhibitory and/or modulatory anti-acetylcholine receptor antibodies. Inhibitory anti-acetylcholine receptor antibodies are positive in approximately 50% of patients with generalised myasthenia and in 30% of those with an ocular form; specificity is better than that of anti-AChR antibodies (above 80%). Modulatory anti-AChR antibodies are at least as sensitive as anti-AChR's and their specificity is equivalent; they are more frequently positive in children or when the disease has only recently appeared, and very strongly positive in cases with associated thymoma. They are never found in the absence of anti-AChRs and inhibitory anti-AChRs.

Specific antibodies against other components of striated muscles are found in 30 to 60% of patients with generalised myasthenia and in 80% of cases with associated thymoma (and in 25% of cases with isolated thymoma); their specificity is similar to that of anti-AChR antibodies. Anti-connectin antibodies are most often detected in patients who develop the disease late or have an associated thymoma. The presence of anti-myosin antibodies has been correlated with the severity of the disease.

- **Value during monitoring:**

  The serum concentration of anti-AChR antibodies is generally correlated with the evolution of the disease.

IN LAMBERT-EATON SYNDROME

Anti-acetylcholine receptor antibodies are detected in 13% of cases of LEMS. Antibodies against P/Q and N calcium channels are more sensitive and more specific than anti-AChR antibodies in this context, being positive in approximately 50% of cases and in 73% of cases when the syndrome is associated with small-cell lung cancer; their specificity is greater than 90%. Their presence therefore suggests the diagnosis, but must be interpreted according to the clinical context.

POSITIVE ANTI-ACHR RESULTS DURING OTHER ILLNESSES

Apart from myasthenia and LEMS, anti-AChR antibodies are detected in autoimmune hepatitis and in 5 to 10% of small-cell lung cancer cases.

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ANTI-ACTIN ANTIBODIES

DEFINITION

Anti-actin antibodies are a diagnostic test for type-1 autoimmune hepatitis. The assay has a sensitivity and specificity of 85%. (0.7% of cases of hepatitis C, occasional drugs and some autoimmune diseases can interfere).

Only high titre (>160), anti-smooth muscle antibodies with anti-actin specificity are associated with type-1 autoimmune hepatitis.

INDICATIONS FOR MEASUREMENT

Type-1 autoimmune hepatitis is a rare disease (10 to 20 cases/million) mostly seen in women (80%), which effects people either between the ages of 10 and 20 years old or between 45 to 70 years old. There is commonly a dysimmune background with thyroiditis, diabetes, connective tissue disease etc. It occasionally presents with severe hepatitic changes and jaundice, although it is usually insidious with a slow progression towards cirrhosis. Laboratory findings include raised transaminases (x5), cholestasis (not invariably), raised polyclonal IgG and anti-smooth muscle antibodies, with anti-actin specificity. Homogeneous anti-nuclear antibodies without anti-native DNA are found in 40% of cases and led to this form of the disease being called lupoid hepatitis. Anti-SLA (soluble liver antigen) antibodies are reported in 5 to 10% of cases and are highly specific. Treatment is long and involves corticosteroids and azathioprine.

INTRODUCTION

■ SAMPLE

Serum: A fasting sample is not required.

■ DESIRABLE INFORMATION

Information about any increase in liver enzymes and the serological status for viral hepatitis B and C.

■ SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling.

Transport at +4°C.

ASSAY METHODS

Immunofluorescence, using 2 substrates:

– Hep-2 cells: Actin "cables" are looked for: the usual Hep-2 cell preparations are not optimised for this test and can produce false negative results.

– Triple substrate (rat kidney/liver/stomach): This is examined for the concomitant presence of smooth muscle appearances on the muscularis mucosae and blood vessels, the presence of radial filaments in the stomach, a reticulated appearance in the liver and above all, the presence of fine needles in the renal tubules.

ELISA and immunoblot: The two second line methods use purified rabbit F-actin; whilst the ELISA produces a quantitative result, immunoblot has the advantage of being able to use for single tests.

UNITS AND REFERENCE VALUES

Immunofluorescence: the result is expressed as the reciprocal of the dilution.

The threshold is 80 on HEp-2 cells and 40 on the triple substrate.

The upper titration limit is 1280 on Hep2 cells and 640 on the triple substrate.

ELISA: the result is expressed in arbitrary units. There is no international standard.

Immunoblot: the result is expressed as negative/positive against an internal control. « + » type quantification is possible.

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ANTI-ADRENAL ANTIBODIES

DEFINITION

Anti-adrenal antibodies are autoantibodies against different structures of the adrenal cortex. They are pathogenic, blocking ACTH receptors or destroying the adrenal glands and are responsible for autoimmune adrenal failure.

Synonyms: anti-adrenal gland antibodies, anti-adrenocortical antibodies.

INTRODUCTION

Organ-specific autoimmune disease commonly affects the endocrine glands and 5 to 7% of the general population may develop autoimmune endocrine disease. The immunopathological mechanism is usually direct damage of the hormone-producing cell (as is the case with anti-adrenal antibodies) although more rarely involves neutralisation of the hormone by an anti-hormone antibody.

The adrenal glands are formed from two parts:

The adrenal medulla and;

– The external layer or zona glomerulosa secrets mineral corticoid hormones, mostly aldosterone;

– The zona fasciculata secretes glucocorticoid hormones, mostly cortisol and small amounts of androgens;

– The zona reticulosa produces small amounts of glucocorticoids and androgens.

In autoimmune adrenal failure, the anti-adrenal antibodies may be against various antigen targets:

– The main target is 21-hydroxylase (see anti-21 hydroxylase antibodies page) which is present in all three layers of the adrenal cortex but mostly in the zona glomerulosa (and is absent from other organs such as the ovaries, testes and placenta). This enzyme, which is found in cytochrome P450 (it is also called P450 C21), is involved in the biosynthesis of glucocorticoids (conversion of 17-OH-progesterone into 11-deoxycortisol) and in the mineralocorticoid pathway (conversion of progesterone into 11-deoxycortisol).

– 17-hydroxylase and 20-22 desmolase (P450 C17 and P450 SCC) are steroid biosynthesis enzymes and also form part of cytochrome P450. They are located in the zona fasiculata and zona reticulosa layers of the adrenal cortex respectively and P450 C17 is also found in the in gonads (ovaries and testes). P450 SCC is found in the three layers of the adrenal cortex and in the gonads and placenta.

– Other targets: 3-beta-hydroxysteroid dehydrogenase (3-HSD), a 51 kDa antigen also present in the islets of Langerhans, the ovarian granulosa cells and in the placenta and 18-hydroxylase (a target described in hypoaldosteronism with antibodies binding only to the adrenal cortex zona glomerulosa).

Anti-adrenal antibodies are responsible for autoimmune adrenal failure. This condition may be isolated or associated with other autoimmune diseases in a context of polyendocrinopathy. Anti-adrenocortical antibodies have been described in the type I and II polyendocrinopathy.

Type I polyendocrinopathy also called Whittaker or Blizzard's Disease or APECED *(Autoimmune Polyendocrinopathy, Candidiasis Ectodermal Dystrophy)*; it is an autosomal recessive condition developing before the age of 15 years old. Its major clinical signs are adrenal failure, hypoparathyroidism and muco-cutaneous candidiasis, sometimes associated with pernicious anaemia, vitiligo, chronic hepatitis, type 1 diabetes or thyroiditis. The diagnosis is made from at least two of these disorders being present and is confirmed by the finding of AIRE *(autoimmune regulator)* gene mutations. The antibodies found in this disease bind to the adrenal cortex and steroid - producing cells in the gonads.

Type II polyendocrinopathy or Schmidt's Disease is characterised by autoimmune dysthyroidism, adrenal failure and/or type I diabetes.

SEARCH INDICATIONS

Diagnostic investigation of adrenal failure (aetiological diagnosis) whether or not in a context of type I or II polyendocrinopathy.

INFORMATION

■ SAMPLE

Serum (Empty tube): A fasting sample is not required.

■ SAMPLE STORAGE AND TRANSPORT

Conservation of serum: one week at +4°C; then freeze at –20°C.

Transport at +4°C, unless sample is already frozen, in which case transport the sample in its frozen state.

ASSAY METHODS

Indirect immunofluorescence (IIF) on human or primate adrenal sections: The sensitivity and specificity of anti-adrenal antibodies detected by IIF for the diagnosis of primary adrenal failure are 40%, and 90% respectively.

Radioimmunoassay of anti-21-hydroxylase or 17-hydroxylase antibodies: the sensitivity and specificity of anti-21-hydroxylase antibodies by RIA for the diagnosis of primary adrenal failure are 85%, and 90% respectively.

NORMAL EXPECTED VALUES

These autoantibodies are normally undetectable in blood.

Results are reported either as serum dilutions or as titres and even low dilutions (from 1/10) are generally considered to be positive.

INTERPRETATION

The presence of anti-adrenal antibodies suggests that the adrenal failure is autoimmune in origin, whether or not it forms part of a polyendocrinopathy (depending on clinical features).

Anti-adrenal antibodies are found in approximately 80% of cases of primary isolated adrenal failure (Addison's Disease), previously called idiopathic. Anti-21-hydroxylase and anti-17-

hydroxylase antibodies are found in 64 to 91% and 5 to 9% of isolated Addison's disease cases respectively.

The sensitivity of anti-steroid cell autoantibodies for diagnosing the types I and II autoimmune poly-endocrinopathies are shown in the table below:

Autoantibody	Type I Polyendocrinopathy	Type II Polyendocrinopathy
Anti-21 hydroxylase	10-92%	85-96%
Anti-17 hydroxylase	55%	33%
Anti 20-22 desmolase	45%	36%

In addition, the finding of anti-21 hydroxylase antibodies in the absence of clinical signs of adrenal failure appears to predict the development of adrenal failure in adults, and even more so in children. Ovarian failure develops in approximately 40% of women with autoimmune Addison's disease who have positive adrenocortical antibodies, 10 to 15 years after the appearance of the autoantibodies in the circulation.

An alternative cause of the adrenal failure should be considered if these antibodies are negative such as infection (tuberculosis, fungal or other), tumour, haemorrhage or genetic disease (adrenoleukodystrophy, congenital lipoid hyperplasia, congenital hypoplasia).

Anti-adrenal antibodies are also seen however:

– In less than 2% of normal people;

– In less than 10% of people suffering from tuberculous adrenal failure;

– In less than 10% of people suffering from adrenoleukodystrophy;

– In autoimmune diseases without adrenal involvement: autoimmune thyroiditis or type 1 diabetes (2% of cases), chronic idiopathic hypoparathyroidism (10%), atrophic gastritis (3%). On the other hand they have not been reported in rheumatoid arthritis, systemic lupus erythematosus or myasthenia.

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ANTI-BETA 2 GLYCOPROTEIN 1 ANTIBODIES

DEFINITION

Anti- β_2 glycoprotein 1 antibodies or anti- β_2GPI form part of the heterogeneous family of anti-phospholipid (APL) antibodies. The anti-phospholipid antibodies recognise either isolated anionic (phosphatidylinositol, phosphatidylserine) or neutral (phosphatidylethanolamine) phospholipids, phospholipid/protein cofactor complexes (β_2 glycoprotein I, prothrombin, etc.) or the cofactors alone. Anti-β_2GPI has been identified as the main anti-cardiolipin cofactor.

Anti- β_2GPI (or apolipoprotein H) is a 326 amino acid protein synthesised by the liver. It is organised into 5 domains of 60 amino acids, the binding site for anti- β_2GPI and anionic phospholipids being located on the fifth domain.

INTRODUCTION AND CLINICAL INDICATIONS

The anti-cardiolipin antibodies (aCL) can be found in autoimmune diseases such as systemic lupus erythematosus (SLE), and also other situations such as infection, neoplasia, iatrogenic and physiological situations (Table I). The aCL associated with infectious diseases are usually transient and do not have thrombotic effects. On the other hand when persistently present at significant titres and associated with venous and/or arterial thromboses and repeated miscarriages, they define the anti-phospholipid syndrome (APLS) (Table II). The preliminary criteria for classifying the APLS defined in 1999 in Sapporo were re-examined in the XIIth International Symposium on antiphospholipid antibodies (2006). Anti-cardiolipin antibodies and anti-β_2GPI antibodies (IgG and IgM) were added to the laboratory criteria. The time between 2 samples to confirm persistent laboratory criteria was increased from 6 weeks to 12 weeks and the APLS was defined as the association of at least one clinical with one laboratory criterion.

It should be noted that thrombocytopaenia was an integral part of the definition of the APLS a few years ago and is a relatively common finding in the secondary form of APLS associated with lupus. APLS is said to be "primary" if it is not associated with any other autoimmune disease and "secondary" if associated with another disease (SLE). Primary APLS may develop over time into a secondary APLS.

One rare very serious form is the catastrophic anti-phospholipid syndrome which is a medical emergency and may be associated with laboratory findings of DIC. The development of ELISA tests has shown that these antibodies are often present in lupus patients with severe thrombotic reactions. Considerable clinical heterogeneity however rapidly became apparent, some people having anti-cardiolipin antibodies but no clinical features whatsoever. This finding led to two types of autoantibodies being distinguished in 1990: antibodies against anionic phospholipids (cardiolipin) alone and antibodies against a cardiolipin/protein cofactor complex. The support for this cofactor activity was found to be β2 glycoprotein, initially introduced by the calf or beef sera contained in the ELISA saturation buffers. Depending on the reagent used therefore and particularly on the dilution and/or saturation buffer compositions (animal sera, gelatine), either cardiolipin β_2 GPI-dependent anti-cardiolipin antibodies or β_2 GPI- independent antibodies (found with tests without cofactors) were found. For this reason, ELISA kits specifically detecting anti-β_2 GPI antibodies were developed. In view of their clinical involvement, it is now recommended that reagents which identify β_2GPI-dependent anti-cardiolipin antibodies be used. Antibodies which recognise the cardiolipin /β_2GPI complex or even the cofactor alone appear to be more associated with the APLS and with autoimmune diseases than those which recognise cardiolipin alone, which appear to be associated more with infection. Most of the APLS associated antibodies are of the IgG type.

The anti-β_2 GPI antibodies are believed to be more specific for the APLS than circulating lupus anticoagulants or anti-cardiolipin Ab alone. There is however a large amount of, occasionally contradictory, data in the literature.

Drugs associated with anti-phospholipid antibodies	*Infectious diseases associated with anti-phospholipid antibodies*
β-blockers	Syphilis
Chlorpromazine	Kala-Azar
Cocaine	Leptospirosis
Hydralazine	Lyme's Disease
Interferon α	Mycoplasmosis
Phenothiazine	Tuberculosis
Phenytoin	Malaria
Procainamide	Pneumocystis
Pyrimethamine/sulfadoxine	Viral infections
Quinidine	HIV
Quinine	Epstein-Barr virus
	Parvovirus B19
	Hepatitis

Table I: Main drugs and infectious diseases potentially associated with the presence of anti-phospholipid antibodies.

Clinical criteria	***1 .Vascular thrombosis*** At least one clinical episode of arterial, venous or small vessel thrombosis in any territory. The thrombosis must be confirmed by imaging, Doppler ultra sound or histology except for superficial venous thromboses. For histological confirmation the thrombosis must be present in the absence of significant signs of vessel wall inflammation.
	2 .Obstetric Disease: **(a)** At least one unexplained death of a morphologically normal foetus from the 10th week of pregnancy onwards (normal foetal morphology must be confirmed on ultrasound or by direct examination of the foetus) or; **(b)** At least one premature birth of a morphologically normal newborn baby before 34 weeks of pregnancy because of severe pre-eclampsia, eclampsia or severe placental failure or; **(c)** At least three spontaneous miscarriages before the 10th week of pregnancy with no anatomical or maternal hormonal abnormality and no maternal or paternal chromosomal abnormality.

Laboratory criteria	**1.** Presence of anti-cardiolipin antibodies or anti-β_2 glycoprotein 1 antibodies, Isotype IgG and/or IgM at moderate or high titres in at least two samples taken at least 12 weeks apart measured by a standardised ELISA technique identifying anti-cardiolipin β_2-GPI-dependent antibodies.
	2. Presence of a circulating lupus anticoagulant found in at least two samples taken at least 12 weeks apart under conditions defined by the International Society for Haemostasis and Thrombosis.

Table II: International Consensus classification for APLS in 1999 *from Wilson et al. revised in 2006 by Miyakis. APLS is defined by the presence of at least one clinical and one laboratory criterion.*

Mechanism of action

Because it binds to anionic phospholipids, *in vivo,* β_2GPI may have an inhibitory effect on platelet aggregation and on the different coagulation stages.

INFORMATION

■ SAMPLE

Serum or plasma. A fasting sample is not required.

■ QUESTIONS FOR THE PATIENT

Report any autoimmune or other known disease.

■ SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling.

Transport at +4 °C (avoid repeated freeze-thaw cycles).

ASSAY METHODS

Commercial immunoenzymatic ELISA kits are available to test specifically for anti-β_2GPI antibodies. Results are not well standardised and vary depending on the kit used, according to the type of antigen, the way of calculating the threshold value, the calibrators and type of microtitre plate. In order to optimise standardisation of the ELISA methods to detect anti-β_2GPI antibodies, it is recommended that the monoclonal antibodies HCAL and EY2C9, distributed by the Atlanta Centres for disease control and prevention and by some diagnostics companies be used as standards. Irradiation of the microtitre plate polystyrene support enables a higher concentration of β_2GPI to be obtained in the base of the plate wells and should enable improvement recognition of the antigen because of conformational changes. The main isotype tested for is IgG.

REFERENCE VALUES

These vary greatly between kits.

INTERPRETATION

Diagnostic testing for an anti-phospholipid syndrome involves a combination of coagulation and immunological tests. The first line immunological tests include anti-cardiolipin and anti-β_2GPI IgG antibodies. APLS is diagnosed from high levels of antibodies persisting in 2 samples taken 12 weeks apart. If the initial tests are negative despite a high index of clinical suspicion it may be useful to test for rarer specificities (anti-phosphatidylethanolamine).

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ANTI-CARDIOLIPIN ANTIBODIES

DEFINITION

Cardiolipin, or diphosphatidylglycerol, is a phospholipid present in plasma (bound to lipoproteins) and in the internal mitochondrial membrane.

Anti-cardiolipin antibodies (aCL) belong to the extremely heterogeneous family of anti-phospholipid antibodies (the term "cardiolipin" and "cardiolipid" are used interchangeably).

Two types of antibodies are distinguished; those against cardiolipin alone and those against a cardiolipin/protein cofactor complex, the support for the cofactor activity usually being β_2 glycoprotein I (β_2 GPI), prothrombin, annexin V, etc. *(cf. corresponding page)*.

INTRODUCTION

The aCL can be found in autoimmune diseases such as systemic lupus erythematosus (SLE), and also other situations such as infection, neoplasia, iatrogenic and physiological situations (Table I). The aCL associated with infectious diseases are usually transient and do not produce thrombotic effects. On the other hand when persistently present at significant titres and associated with venous and/or arterial thrombosis and repeated miscarriages they define the anti-phospholipid syndrome (APLS) (Table II). The preliminary criteria for classifying the APLS defined in 1999 in Sapporo were re-examined in the XIIth International Symposium on antiphospholipid antibodies (2006), and anti-cardiolipin antibodies and anti-β_2GPI antibodies (IgG and IgM) were added to the laboratory criteria. The time between 2 samples to confirm persistent laboratory criteria was increased from 6 weeks to 12 weeks and the APLS was defined as the association of at least one clinical with one laboratory criterion.

It should be noted that thrombocytopaenia was an integral part of the definition of the APLS a few years ago. It is a relatively common finding in the secondary form of APLS associated with lupus. APLS is said to be "primary" if it is not associated with any other autoimmune disease and as "secondary" if associated with another disease (SLE). Primary APLS may develop over time into secondary APLS.

One rare very serious form is the catastrophic anti-phospholipid syndrome which is a medical emergency and may be associated with laboratory findings of DIC. The development of ELISA tests has shown that these antibodies are often present in lupus patients with severe thrombotic reactions. Considerable clinical heterogeneity however rapidly became apparent, some people having anti-cardiolipin antibodies but no clinical features whatsoever. This finding led to two types of autoantibodies being distinguished in 1990:

- Antibodies against anionic phospholipids (cardiolipin) alone and;

- Antibodies against a cardiolipin/protein cofactor complex.

The support for this cofactor activity was found to be β_2 glycoprotein, initially introduced by the calf or beef sera contained in the ELISA saturation buffers. Depending on the reagent used therefore and particularly on the dilution and/or saturation buffer composition (animal sera, gelatine) either cardiolipin β_2 GPI-dependent anti-cardiolipin antibodies or β_2 GPI- independent antibodies (found with tests without cofactors) were found. For this reason, ELISA kits specifically detecting anti-β_2 GPI antibodies were developed. In view of their clinical involvement, it is now recommended that reagents which identify β_2GPI-dependent anti-cardiolipin antibodies be used. Antibodies which recognise the cardiolipin /β_2GPI complex or even the cofactor alone appear to be associated more strongly with the APLS and with autoimmune diseases than those which recognise cardiolipin alone, which appear to be associated more with infection. Most of the APLS-associated antibodies are of the IgG type.

Drugs associated with anti-phospholipid antibodies	***Infectious diseases associated with anti-phospholipid antibodies***
β-blockers	Syphilis
Chlorpromazine	Kala-Azar
Cocaine	Leptospirosis
Hydralazine	Lyme's Disease
Interferon α	Mycoplasmosis
Phenothiazine	Tuberculosis
Phenytoin	Malaria
Procainamide	Pneumocystis
Pyrimethamine/sulfadoxine	Viral infections
Quinidine	HIV
Quinine	Epstein-Barr virus
	Parvovirus B19
	Hepatitis

Table I: Main drugs and infectious diseases potentially associated with the presence of anti-phospholipid antibodies.

Clinical criteria	**1.** Vascular thrombosis: At least one clinical episode of arterial, venous or small vessel thrombosis in any territory. The thrombosis must be confirmed by imaging, Doppler ultra sound or histology, except for superficial venous thromboses. For histological confirmation the thrombosis must be present in the absence of significant signs of vessel wall inflammation.
	2. Obstetric Disease: **(a)** At least one unexplained death of a morphologically normal foetus from the 10th week of pregnancy onwards (normal foetal morphology must be confirmed on ultrasound or by direct examination of the foetus) or **(b)** At least one premature birth of a morphologically normal newborn baby before 34 weeks of pregnancy because of severe pre-eclampsia, eclampsia or severe placental failure or **(c)** At least three spontaneous miscarriages before the 10th week of pregnancy with no anatomical or maternal hormonal abnormality and no maternal or paternal chromosomal abnormality.
Laboratory	**1.** Presence of anti-cardiolipin antibodies or anti-β_2 glycoprotein 1 antibodies, Isotype IgG and/or IgM at moderate or high titres in at least two samples taken at least 12 weeks apart measured by a standardised ELISA technique identifying anti-cardiolipin β2-GPI-dependent antibodies. **2.** Presence of a circulating lupus anticoagulant found in at least two samples taken at least 12 weeks apart under conditions defined by the International Society for Haemostasis and Thrombosis.

Table II: International Consensus classification for APLS in 1999 *from Wilson et al. revised in 2006 by Miyakis. APLS is defined by the presence of at least one clinical and one laboratory criterion.*

Several hypothetical mechanisms of action are proposed to explain APLS, raising the possibility of interactions between phospholipids and platelets or phospholipids and endothelial cells followed by activations triggering a thrombotic reaction.

INDICATIONS FOR MEASUREMENT

There are many indications, although it is essential to combine measurement of aCL with testing for circulating lupus anticoagulant, as anti-phospholipid antibodies are only found simultaneously by both methods in 60% of cases:

– Raised ACT suggesting circulating lupus anticoagulant.

– Initial assessment and monitoring of autoimmune diseases (SLE).

– Etiological assessment of unexplained venous and/or arterial thrombosis.

– Obstetric complications including repeated foetal loss following the definition shown in table II.

Kits detecting aCL- β_2 glycoprotein I-dependent IgG should be used in preference. IgM antibodies are less specific. IgA antibodies are tested for in Anglo-Saxon countries but not in France. Testing for persistent aCL must be performed 12 weeks apart.

INFORMATION

SAMPLE

Serum sample (Dry Tube).

Frozen Citrated plasma can be used.

QUESTIONS FOR THE PATIENT

Past history of venous or arterial thrombosis, miscarriages, known autoimmune disease and medical drug history.

SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling.

Transport at +4 °C (avoid repeated freeze-thaw cycles). If frozen plasma is being sent then please send in its frozen state.

ASSAY METHOD

The aCL are identified by immunological ELISA tests. It is important to have a good understanding of the reagent used (anti-cardiolipin dependent or independent of protein cofactors) and for the diagnosis of APLS it is recommended that kits which detect aCL-β_2 GPI dependent IgG be used.

REFERENCE VALUES

These vary greatly from one kit to another. Despite the existence of so-called "standards" which vary from one batch to the next, the expression of results in GPL, MPL or APL units (for IgG, IgM, IgA types respectively) in no way reflects effective standardisation. The results vary greatly between manufacturers and an PL European standardisation initiative is ongoing.

FOR FURTHER INFORMATION

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ANTI-CITRULLINATED PEPTIDE ANTIBODIES

DEFINITION

Several auto-antibodies are now considered to belong to this family. The first of them to be described, in 1964, were the antiperinuclear antibodies. The "cottage industry" technique, cumbersome and non-standardisable, was used by very few laboratories.

In 1979, antibodies marking the stratum corneum of rat oesophagus were described and wrongly named ***anti-keratins***: Testing for these has now become a routine procedure (see corresponding document).

Immunohistochemical studies carried out in the 1990's identified the major antigenic target as filaggrin, whose function is to aggregate filaments of keratin.

Recent work has shown that synovial plasmocytes synthesise antibodies directed against citrullinated peptides. Under the action of an enzyme, peptidyl arginine deiminase (PAD), which is present in synoviocytes, the inflammatory cells, the arginine of the α and β chains of fibrin and fibrinogen is oxidised to citrulline. The reaction is heterogenic and depends on a number of individual parameters, including membership of certain HLA groups and the PAD haplotype (antibody production will be greater with DRB1*0401/*0101 and PAD4).

These *citrullinated peptides* or *deiminated fibrin* are recognised as the true antigenic targets of the immunological reaction and sustain the local inflammation.

Reagents currently available use a cyclic citrullinated peptide (CCP), which offers excellent results. In rheumatoid arthritis (RA), anti-citrullinated peptide antibodies constitute the best biological marker, together with rheumatoid factors (RF). Work is in progress using human deiminated fibrinogen and may allow further improvement in diagnostic sensitivity.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

Anti-CCP antibodies appear very early, sometimes preceding the clinical onset of the disease. They are independent of other markers such as RF's (presence in 1/3 of RA cases without RF), predictive of greater aggressiveness of the disease, and easily detectable.

Their specificity for RA is close to 98%. Sensitivity is approximately 65% in RA cases of less than 6 months' evolution, and more than 80% in full-blown RA.

Biological investigations now provide valuable help in the early diagnosis of RA, thus permitting optimal treatment from the early months and a more favourable long-term functional prognosis.

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not necessary.

SAMPLE STORAGE AND TRANSPORT

Decant the serum within half a day of sample collection.

Store and transport at +4° C.

ASSAY METHODS

The immune reaction is directed against multiple peptides and there is no single, perfect antigenic target. Various citrullinated peptides have been tested and developed; these include 2nd and 3rd generation cyclic citrullinated peptide (CCP2 and CCP3 respectively), citrullinated vimentin, citrullinated fibrinogen, etc. Tests using these different antigenic sources show broadly similar performance.

UNITS AND REFERENCE VALUES

There are international units – each kit has its own units and reference values.

FOR FURTHER INFORMATION

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ANTI-D ALLOIMMUNISATION ANTIBODY

DEFINITIONS

Alloimmunisation: Immunisation of an individual against an antigen which they do not possess but which is common to a group of individuals.

Incompatible pregnancy: A pregnancy is said to be incompatible for the RH1 antigen if the mother has the RH negative (D negative) phenotype and is carrying a Rhesus positive (D positive) child.

Anti-Rh1 alloantibody: Immunisation antibody appearing in RH1 subjects or, more rarely, in subjects carrying a partial D antigen or certain weakly-expressed variants of a partial D antigen after contact with the RH1 antigen following a transfusion or a pregnancy.

Passive antibodies: Found after injection of specific anti-D immunoglobulins (anti-D Ig) aimed at preventing Alloimmunisation ("passive" anti-D antibodies or residual anti-D).

INTRODUCTION ET INDICATIONS

MATERNAL IMMUNISATION

Immunisation occurs after a transplacental foetomaternal haemorrhage (FMH), which is possible from the 6th week of pregnancy. Immunisation is thought to be less frequent if there is ABO incompatibility. It is unpredictable, but sometimes results from a micro-FMH and, in 50% of cases, from a bleed of moderate volume (< 30 ml). Alloimmunisation is also observed after ectopic pregnancies.

FOETAL HAEMOLYSIS

Maternal alloantibodies are IgG's which cross the placental barrier by an active transport mechanism. When in the foetal bloodstream, they can cause haemolysis, of which the severity depends on the quantity of antibodies produced, their immunological characteristics (heavy-chain isotype, affinity, etc) and the duration of exposure. Haemolysis produces regenerative anaemia (foetal erythroblastosis) and an excess of bilirubin, which can be difficult for the foetus to resorb (bilirubin elimination by glucuronosyltransferase not having reached maturity). It is now rare to encounter the classical death in-utero caused by foetoplacental anasarca *(hydrops foetalis*, which combines generalised oedema, hepatomegaly and anaemia). Retarded intrauterine development, premature birth and neurological effects linked to deposits of free bilirubin in the central nervous system are sometimes observed. The excess free bilirubin is eliminated by the maternal bloodstream, but is toxic for the newborn child.

MODE OF ACTION OF ANTI-D IG'S

Efficacious prevention of anti-Rh1 immunisation is obtained by injecting specific human IgG's, at least after every situation known to be capable of stimulating FMH, as well as systematically. Anti-D Ig's are believed to have the effect of covering the epitopes of foetal erythrocytes, which are thus very quickly eliminated by the mononucleated phagocyte system (in 6 to 8 hours for a moderate FMH). This prophylaxis remains effective in the days, even weeks, following an FMH and therefore relies on the recognition of each occurrence and the greatest possible accuracy in estimating its volume (Kleihauer test).

EPIDEMIOLOGY OF MATERNOFOETAL RH1 INCOMPATIBILITY

In Europe, fewer than 10% of pregnancies are affected by this incompatibility. Without prophylaxis, alloimmunisation would occur in 17% of these pregnancies. At present, with the generalisation of systematic injections of anti-D Ig's at 28 and 32 weeks of gestation and immediately post partum, there is less than 1 per thousand alloimmunisations after incompatible pregnancies. This residual level is attributed, excluding unmonitored pregnancies, to prophylaxis which is under-dosed, absent or instigated too late.

MODES OF PREVENTION

Screening and monitoring: Screening in France is defined in regulations published in the Official Journal on 4 May 2002. The principles are as follows:

– In early pregnancy: Full Rh phenotyping and screening for abnormal agglutinins must be carried out. Any positive irregular antibody (IA) test must be followed up by identification. The programme of mandatory IA tests for every pregnant woman, is testing in the 1st quarter and the 6th, 8th and 9th months of pregnancy.

– In every Rh negative woman, a determination is required of whether or not a pregnancy is incompatible. If the father is rhesus negative, then so will the child, and the pregnancy will be compatible. In the opposite situation, in the event of a positive IA test with anti-D, the indication of antenatal genotyping must be discussed. This genotyping is performed by searching, with amplification, for rhesus D DNA of foetal origin circulating in the maternal plasma, which avoids trophoblastic biopsies and umbilical cord blood collection. IA testing with titration monitoring and quantification is necessary every month, or even every 15 to 10 days, depending on the severity of immunisation. Following the prophylactic administration of antiglobulins, the same procedure must be followed if the IA screening test is positive.

– When prophylaxis is initiated, it is desirable to carry out an IA test prior to injection.

Indications for prophylaxis:

– Any Rhesus negative woman who has given birth to an RH1 child and produces a positive IA test

– Any Rhesus negative woman who is not anti-RH1 immunised, whenever there is a suspicion of repeated or accidental transfer of foetal erythrocytes to the mother after an elected termination of pregnancy, a spontaneous miscarriage, an ectopic pregnancy, a therapeutic abortion or foetal death in utero (if embryonic erythrocyte phenotyping requires it or has not been carried out). In the antenatal period, after any situation which might result in an FMH: amniocentesis, foetal blood collection, chorionic villi biopsy, embryo reduction, cervical cerclage, external cephalic version, abdominal trauma, metrorragia or threatened premature birth.

- **Quantity of anti-D Ig:** A minimum dose of 100 µg/ml for an FMH < 5 ml must be injected within a maximum of 72 hours after the incident. The standard dose is from 100 to 300 µg (100 µg = 500 IU) administered by intravenous or intramuscular injection, but is increased if the volume of the FMH is substantial (Kleihauer test > 5 foetal erythrocytes/10000 maternal erythrocytes).

  A Kleihauer test must be performed within 12 to 48 hours of injection to confirm that foetal erythrocytes have disappeared from the maternal blood. If the reduction in foetal erythrocytes is insufficient, a further injection is required.

  Despite immunoglobulins prophylaxis, an allo immunisation can occur, that is why a regular follow up of anti D titre is necessary all along the pregnancy.

INFORMATION

SAMPLE

The sample must be collected in a dry tube and in an EDTA tube. When looking for passive anti-D's, the sample must be collected in the 24 to 48 hours the injection of anti-D immunoglobulin.

QUESTIONS FOR THE PATIENT

Stage of pregnancy? Previous pregnancies? Known immunisation?

Anti-D immunoglobulin injections? If so: date(s)? Dose?

Circumstances of the request (monitoring pregnancy, miscarriage, abortion, obstetric manipulation, etc)?

STORAGE

Frozen serum must be stored throughout the pregnancy, to allow titre comparisons for monitoring the evolution of any alloimmunisation.

DIAGNOSTIC METHODS

Testing for residual agglutinins must be carried out with respect to an identification range of 10 test erythrocytes. If an anti-RH1 antibody is found, it must be titred, since the antibody titre and its evolution are the basis for verifying that there is no active immunisation. If diagnostic uncertainty remains, the doubt can be lifted by quantification, combined if appropriate with a sensitive method of comparative micro-titration with a standard anti-D.

INTERPRETATION

Distinguishing between a passive anti-D and an immunisation anti-D can be difficult in the early stages of immunisation. The clinical context (haemolytic disease in previous children, dose and lapse of time since administration), the titre and its evolution are factors in diagnosis. In the majority of cases, the level of agglutinins allows a distinction to be made between these two possibilities:

– The residual level for administered antibodies (graphs are available showing the titer expected depending on the dose injected and the lapse of time since injection),

– Level elevated, stable or even rising in cases of active alloimmunisation.

FOR FURTHER INFORMATION

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- *Suivi de l'allo-immunisation fœto-maternelle* par L. MANNESSIER, Transfusion clinique et biologique 2003; 10: 258-262.
- *Prévention de l'allo-immunisation fœtomaternelle* par L. MANESSIER. Transfusion clinique et biologique 2000; 7: 525-526
- Dictionnaire Vidal®: *immunoglobuline humaine anti D.*

ANTI-DNA ANTIBODIES

DEFINITION

Anti-DNA (deoxyribonucleic acid) antibodies are a subgroup of anti-nuclear antibodies which recognise 2 forms of DNA: native or double-stranded DNA (nDNA or dsDNA) and denatured or single-stranded DNA (dDNA or ssDNA). They are homogeneous with indirect immunofluorescence (IIF) on HEp-2 cells.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

Anti-dsDNA are commonly found in systemic lupus erythematosus (SLE). Their diagnostic sensitivity and specificity are such that they form part of the criteria recommended by *the American Rheumatism Association* (ARA) to diagnose the disease. Anti-dsDNA antibodies are highly specific for SLE (although not absolutely specific, particularly depending on the method used).

They are also prognostic indicators. Relapse of lupus is preceded by an increase in anti-double-stranded DNA antibody titres, sometimes even several months before the clinical relapse occurs.

Anti-ssDNA antibodies are not specific for SLE and can be seen in many diseases including the connective tissue diseases, drug-induced lupus, viral infections, etc. They have low diagnostic value.

INFORMATION

SAMPLE

Serum. A fasting sample is not necessary.

CLINICAL DETAILS

Report any autoimmune or other known disease and where available, the results of anti-nuclear antibody testing.

SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling.

Store and transport at 4°C.

ASSAY METHOD

Three groups of techniques are available.

FARR TEST

This test is considered to be the reference method and is based on direct binding of serum antibodies to purified DNA radio-labelled with iodine 125. The complexes formed are separated by precipitation with ammonium sulphate. It is a sensitive and specific test which identifies high avidity anti-DNA antibodies and therefore those which are potentially more aggressive to the kidney. It is tending, however, to be abandoned because of restrictions on the use of radioactive elements.

INDIRECT IMMUNOFLUORESCENCE (IIF)

This is a technique using smears of flagellated protozoa *(Crithidia luciliae)* as substrates, the kinetoplast of which is rich in circular double-stranded DNA. It is a simple method to use but has low sensitivity. The reading of fluorescence is subjective and may be difficult. The test is not suitable for large volume testing.

ELISA IMMUNOENZYMATIC ASSAYS

Many ELISA tests have been developed in which the wells are coated with different double-stranded DNA preparations. These methods have the advantage of measuring large batches of sera with objective spectrophotometric reading.

They are the most sensitive methods, sometimes at the expense of specificity (well contamination by fragments of single-stranded DNA).

Dot blot tests exist although these are single sample qualitative tests, reducing their merits.

Anti-single-stranded DNA antibodies are tests using the ELISA technique.

The wide range of methods available also produces in a wide range of results, and method differences must be taken into account in interpretation. Patients should preferably be monitored using the same method.

The main features of each method are summarised in the table below:

	Farr Test	IIF on C. luciliae	ELISA
Antigen	Native DNA* in solution	Intact native DNA	Purified native DNA bound to microtitre plate
Use	Quantitative reference test	Specific simple	Automatable Quantitative
Limitations	Contamination by denatured DNA	Subjective reading Semi-quantitative	Contamination by denatured DNA
Sensitivity	+++	+	++++
Specificity	+++	++	+/- to ++

RECENT TECHNOLOGIES

Luminex® methods including Bioplex®: flow immunofluorimetry.

UNITS AND REFERENCE VALUES

Immunofluorescence: The result is expressed as the reciprocal of the dilution

The screening dilution is 1/10 on *C. luciliae*.

The reference values for radioimmunological or immunoenzymatic methods depend on the reagent used.

FOR FURTHER INFORMATION

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biomnis
SPECIALISED MEDICAL PATHOLOGY

ANTI-ENA ANTIBODIES OR ANTI-TCE ANTIBODIES OR ANTI-SOLUBLE NUCLEAR ANTIGENS

DEFINITION

Anti-ENA *(Extractable Nuclear Antigen)* antibodies, formerly called anti-TCE (thymic cell extract) antibodies bind to soluble cell nuclear and cytoplasmic components. Many antibodies have been described although only around ten are of known clinical significance (SSA, SSB, Sm, RNP, JO1, SCL70).

These antibodies are more or less specifically associated with certain connective tissue diseases and are a valuable aid in their diagnosis.

The fluorescence appearance on HEp-2 cells may suggest certain ENA although precise determination requires appropriate methods. In France, these methods are used systematically by laboratories when the anti-nuclear antibody (ANA) titres are > 80 on indirect immunofluorescence (IIF).

SEARCH INDICATIONS

Anti-ENA antibodies must be tested following the detection of any positive anti-nuclear antibody. They are more or less specifically associated with the connective tissue diseases and are an important diagnostic and occasionally prognostic marker. A positive test requires confirmation and titration. The following antibodies are tested for routinely:

■ ANTI-SSA/RO Ab

This antigen consists of low molecular weight ribonucleoproteins described for the first time in the serum of a patient suffering from Gougerot-Sjögren's syndrome. The antibodies are revealed by IIF on HEp-2 cells by granular nuclear fluorescence. Not all HEp-2 cells detect anti-SSA unequivocally and for this reason they may be tested directly using a specific method even if ANA are negative. HEp-2 cells transfected with SSA (which overexpress the antigen) are available to increase the sensitivity of screening for anti-SSA antibodies by IIF (HEp2000®).

Anti-SSA antibodies are found in:

– 30 to 50% of cases of systemic lupus erythematosus (SLE)

– 40 to 80% of Sjögren Sicca syndromes

– 60 to 90% of cases of subacute cutaneous lupus

– 3 to 5% of cases of rheumatoid polyarthritis

– Neonatal lupus: anti-SSA antibodies cross the placenta and can cause neonatal lupus with atrio-ventricular block.

■ ANTI-SSB/LA Ab

These very often co-exist with the anti-SSA and are found in 40% of cases of Sjögren's syndrome and 10% of SLE.

■ ANTI-RNP Ab

The antigen consists of a ribonucleoprotein, one of the *"small nuclear ribonucleoproteins"*. These antibodies produce speckled fluorescence with average sized irregular granules on HEp-2 cells and at high titres are markers of mixed connective tissue disease (SHARP syndrome) and also SLE.

ANTI-ENA ANTIBODIES OR ANTI-TCE ANTIBODIES OR ANTI-SOLUBLE NUCLEAR ANTIGENS

■ ANTI-SM Ab

The Sm antigen belongs to the "small ribonucleoproteins". It consists of several polypeptides. Antibodies are seen on IIF in the nuclei of HEp-2 cells with medium sized granular speckled fluorescence.

These antibodies are highly specific for SLE although are rarely found (10% of Caucasians although 30% of black Americans).

■ ANTI-SCL70 Ab

These antibodies are directed against topo-isomerase 1 and produce a speckled nuclear fluorescence with very fine, very dense granules which may appear homogeneous. The antibodies are a marker of systemic scleroderma associated with a more severe prognosis, often with pulmonary fibrosis.

■ ANTI-JO1 Ab

These are anti-cytoplasmic antibodies, the target antigen of which is histidyl RNt synthetase. They produce a finely granular fluorescence appearance in the cytoplasm of Hep-2 cells on IIF.

They are present at variable rates in polymyositis and dermatomyositis, and more specifically in forms with pulmonary fibrosis.

■ ANTI-PCNA OR PROLIFERATING CELL NUCLEAR ANTIGEN Ab

These antibodies do not bind to quiescent cell nuclei but recognise the nuclei of dividing cells. A positive reaction produces a speckled appearance which is heterogeneous in intensity or pleiomorphic in the nuclei. They are also identified by the Ouchterlony and Dot Blot methods.

They are very rare antibodies but are highly specific for lupus.

■ ANTI-PM1 AND PM/SCL Ab

These antibodies produce homogeneous fluorescence in the nucleoli of HEp-2 cell nuclei. They can also be tested for using the Ouchterlony method or Dot Blot methods. These antibodies are associated with scleroderma and the polymyositis and scleroderma overlap syndromes.

The main clinical associations of the anti-ENA are shown on the table below

Anti-RNP	Sharp (100%) SLE (25%)
Anti-Sm	SLE (10%)
Anti-SSA	Sjögren (50%), SLE (30%)
Anti-SSB	Sjögren (80%), SLE (5%)
Anti-PCNA	SLE (3%) specificity+++
Anti-Scl70	Diffuse scleroderma
Anti-JO1	Polymyositis-dermatomyositis
Anti-Pm/Scl or PM1	Scleroderma, polymyositis

INFORMATION

■ SAMPLE

Serum: A fasting sample is not required.

■ CLINICAL DETAILS

Report any other autoimmune or known disease and the results of anti-nuclear antibody testing.

■ SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling.

Transport at 4°C.

DETECTION METHOD

■ DOUBLE IMMUNODIFFUSION (OUCHTERLONY)

This system identifies anti-ENA antigen antibody systems using reference sera.

The antigen and antiserum are placed on an agar gel. Migration towards each other results in a precipitation line at the equilibrium point which is visible either to the naked eye or through a magnifying glass.

Electrosyneresis using migration in an electrical field is faster. Screening is generally performed initially with an antigen consisting of a crude extract of ground lymphoid organs (thymus or spleen). If a positive reaction is found the specific antibodies are identified using reference anti-sera of known specificity.

Immunodiffusion is a simple technique which is specific but relatively insensitive. In general it requires early (5 to 6 hours) followed by late reading (24 hours). It does however detect some autoantibodies for which no commercial kit is available.

■ IMMUNOENZYMATIC TECHNIQUES

The ELISA methods are currently widely used to screen for and characterise anti-ENA. The kits available generally screen with a combined total antigen in each reagent well. Positive screens can subsequently be identified using strips in which each well contains a purified antigen.

The ELISA method is more sensitive than the Ouchterlony although differences in results and specificity may be seen between kits depending on the nature and method of preparation of the antigen.

■ IMMUNOBLOTTING TECHNIQUES

Anti-ENA can be tested for using Western Blot techniques (separation of nuclear antigens by electrophoresis) or by Dot Blot (loading native antigens onto membranes), allowing simultaneous detection of one or more autoantibodies.

■ RECENT METHODS

The Bioplex® flow immunofluorimetry methods (used particularly in the USA) and Luminex® technology.

REFERENCE VALUES

These depend on the method used.

All of the immunodiffusion and Dot Blot or Western Blot methods are qualitative: the result is either positive or negative.

For ELISA methods the positivity threshold used depends on the reagent.

FOR FURTHER INFORMATION

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ANTI-ENDOMYSIAL ANTIBODIES

DEFINITION

Anti-endomysial (AEA) antibodies are autoantibodies against antigens present in the connective tissue which surrounds the smooth muscle fibres in most species of animal, including human beings. They are highly specific antibodies for coeliac disease (gluten intolerance).

INTRODUCTION

Coeliac disease is an intolerance to gluten causing an enteropathy resulting in malabsorption in genetically predisposed people (95% of patients express HLA class II DQ2).

There are two different clinical forms:

Childhood coeliac disease: This has a fairly typical clinical presentation and it usually presents before the age of 2 years old and has a prevalence of approximately 1/2500 in France. Classically it involves a combination of chronic diarrhoea and relatively severe signs of malnutrition. It is associated with constant anorexia and vomiting in 50% of cases. Deterioration in general health is a constant finding. This leads quickly to growth retardation and a falling off of the weight curve.

In adults: The classical form is only seen in 20% of cases. In all of the other cases the clinical features may only reflect malabsorption and present with low grade non-specific symptoms (bone demineralisation, arthralgia, neurological problems, subfertility) which may make it difficult to diagnose. Laboratory findings in this situation may include those of malabsorption (anaemia with iron, folate and vitamin B12 deficiency, deficiency of vitamin K dependent factors, etc).

The treatment of coeliac disease involves a strict lifelong gluten-free diet. All foods containing wheat, barley or rye must be excluded from the diet. Laboratory and clinical findings improve often spectacularly over a few months on a gluten-free diet. This is the only means of preventing the long term complications of the disease (notably small bowel lymphoma).

Coeliac disease is diagnosed from serological screening, followed by confirmation of the disease by histological examination of a small bowel biopsy to confirm the presence and extent of villous atrophy.

INDICATIONS FOR MEASUREMENT

Coeliac disease is associated with antibodies in response to eating gluten. The antibodies tested for are anti-endomysial IgA, anti-gliadin IgG and IgA and anti-transglutaminase antibodies. The anti-reticulin antibodies should no longer be tested for because of their low sensitivity. Similarly, anti-gliadin antibodies have poorer sensitivity and specificity than anti-endomysial Ab and/or anti-transglutaminase Ab, particularly in adults. Testing for these antibodies is no longer recommended in France.

Anti-endomysial Ab (together with anti-transglutaminase Ab) are currently considered to be the most specific and sensitive indicators of coeliac disease. They have a sensitivity of 88% to 100% and a specificity of 100%. Disappearance of these antibodies over a few months confirms good compliance with a gluten-free diet.

INFORMATION

SAMPLE

Serum: A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Check that the patient is not already following a gluten-free diet.

SAMPLE STORAGE AND TRANSPORT

Separate the serum within half a day of sampling.

Transport at +4°C.

ASSAY METHOD

Anti-endomysial antibodies are tested for by indirect immunofluorescence on monkey oesophagus sections. A positive result is given by smooth muscle fluorescence producing a bee's nest appearance.

It is important that anti-endomysial IgA are tested using monospecific anti-α chain immune serum. In exceptional cases patients may be tested for anti-endomysial IgG when severe congenital IgA deficiency is present.

REFERENCE VALUES

Immunofluorescence:

The result is expressed as the reciprocal of the dilution.

The accepted positivity threshold is 10.

FOR FURTHER INFORMATION

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ANTI-EXOCRINE PANCREAS ANTIBODIES

DEFINITION

Anti-exocrine pancreas antibodies are autoantibodies specific for the exocrine ducts of the pancreas (they are therefore different from the endocrine anti-pancreas antibodies present in type 1 diabetes: anti-GAD 65, anti-insulin, anti-IA2 or anti-islet cells).

These antibodies, which are found by indirect immunofluorescence on human or monkey pancreas sections, may be directed against various antigenic targets: a macromolecule belonging to the exocrine pancreatic cell secretion products (involved in the pathogenesis of Crohn's Disease), a p60 protein located on the secretory ducts of the human exocrine pancreas and salivary glands or human type II carbonic anhydrase located on the secretory ducts of many exocrine organs: pancreas, salivary and lachrymal glands, liver, oesophagus, distal renal tubules (in pancreatic or autoimmune diseases).

Anti-exocrine pancreas antibodies are sometimes found in the serum of patients suffering from pancreatic or chronic inflammatory bowel diseases (particularly Crohn's disease).

INTRODUCTION

According to Stoker's theory, the immune reaction which develops in Crohn's disease is started by pancreatic secretions passing into the lumen of the bowel. Antigens in these secretions are then believed to bind to anti-exocrine pancreas autoantibodies forming immune complexes which lodge in the mucosa and submucosa causing an Arthus reaction. The antigens may also spread into the blood and lodge on enterocyte cells. This hypothesis is supported by the fact that patients suffering from Crohn's disease eat large amounts of carbohydrates. Glucose decreases intestinal pancreatic secretions and by increasing their carbohydrate intake, patients suffering from Crohn's disease would reduce their pancreatic secretions and therefore the amount of antigens in their bowel.

INDICATIONS FOR MEASUREMENT

These autoantibodies are tested for in the diagnostic investigation of some pancreatic disorders (chronic idiopathic pancreatitis) or Crohn's disease (an aid to the differential diagnosis from ulcerative colitis).

In this latter situation the anti Saccharomyces cerevisiae or ASCA antibodies are currently preferred.

INFORMATION

SAMPLE

Serum (Empty tube): A fasting sample is not required.

SAMPLE STORAGE AND TRANSPORT

Storage of serum: 1 week at +4°C; then freeze at– 20°C.

Transport at +4°C or frozen if the sample is already in the frozen state.

ASSAY METHODS

Indirect immunofluorescence on human group O or monkey pancreas sections using an anti-IgG conjugate.

INTERPRETATION

Anti-exocrine pancreas antibodies are found in approximately 40% of patients suffering from Crohn's disease (generally at high titre > 100) and in a few of their relatives whereas they are only rarely found in people suffering from ulcerative colitis. The differential diagnosis often uses testing for anti-Saccharomyces cerevisiae antibodies (which are also common in Crohn's disease and rare in ulcerative colitis).

FOR FURTHER INFORMATION

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■ Monier JC, Auger C, Fabien N. *Les auto-anticorps 2003-2004*, CD-Rom Pharmacia Diagnostics.

ANTI-FACTOR ANTIBODIES

DEFINITION

Anti-factor antibodies are acquired pathological coagulation inhibitors. They are directed specifically against a haemostasis factor and predispose to bleeding. They need to be distinguished from the non-specific Circulating anticoagulant (CAC) against a coagulation phase (the most common being the lupus anticoagulants), which can artificially reduce the activity of coagulation factors and predispose to thrombotic effects *(see circulating anticoagulant–lupus anticoagulant section)*.

Anti-factor antibodies are immunoglobulins without anti-phospholipid activity. Their presence in plasma produces an increase in one or more coagulation test(s) (the activated partial thromboplastin time (APTT), Prothrombin time (PT) or thrombin time (TT)), which are not corrected by adding control plasma or by adding phospholipids (unlike the lupus anticoagulants), associated with deficiency of a coagulation factor against which the antibody is specifically directed.

They are mostly neutralising antibodies, i.e. antibodies against the active site of the haemostasis factor and therefore can neutralise the same factor in control plasma in-vitro. Less commonly (some anti-II or anti-von Willebrand factors), the antibody is not neutralising and after binding to the "factor" protein it forms a complex, which is rapidly removed by the reticulo-endothelial system leading secondarily to deficiency of the haemostasis factor.

Synonyms: anti-haemostasis or coagulation factor antibodies, anti-VIII, anti-IX, anti-XIII, anti-XII, anti-XI, anti-X, anti-VII, anti-V, anti-II, anti-fibrinogen, anti-von Willebrand factor.

INTRODUCTION

In general, the anti-factor antibodies can develop:

– In people with a constitutional haemostasis factor deficiency treated with repeated replacement transfusions. These are then alloantibodies specifically directed against the deficient factor, which after they have developed in the patient make the usual replacement treatment ineffective. The most common are the anti-VIII (or anti-IX) which develop in treated haemophilia A (or B) patients. The diagnosis is then generally made from, occasionally severe, bleeding.

– Spontaneously, in the absence of any haemostatic abnormality in the elderly, associated with underlying disease (malignancy, auto-immune disease, monoclonal gammopathies), post-partum, or in association with some drugs. In these situations they are autoantibodies.

INDICATIONS FOR MEASUREMENT

Investigation for anti-factor antibodies is indicated in unexplained bleeding or bleeding occurring in a person deficient of a haemostasis factor treated with repeated replacement transfusions.

The test is also used when a very low coagulation factor is found fortuitously, in the absence of a known past history.

INFORMATION

These have been defined precisely and the investigation relies on them being followed strictly.

SAMPLE

Take the sample into citrate, concentration 3.2% (0.109 M) 1/10 (0.5 ml per 4.5 ml of blood). 3.8% (0.129 M) citrated tubes are acceptable. CTAD tubes (citrate, theophylline, adenine, dipyridamole) are recommended in patients receiving heparin. No other anticoagulant may be used.

The sample should preferably be taken in the morning between 0700 and 1100 hours with the patient resting, seated, for at least 5 minutes. A light low fat snack is permitted but caffeine, tobacco and physical activity must be avoided during the hour before the sample is taken. The samples must be centrifuged promptly after the sample is taken and the test performed within 2 to 4 hours of sampling. Check that no micro-clots are present and discard haemolysed or lipaemic samples.

For more information refer to the *"General pre-analytical conditions in haemostasis"* section.

QUESTIONS FOR THE PATIENT

Are you known to have a haemostasis factor deficiency?

Are you taking any of the following drugs?

– Anticoagulation:

Unfractionated heparin, low molecular weight heparin, hirudin and derivatives, dabigatran, rivaroxaban: these treatments prolong (or can prolong) the APTT.

Anti-vitamin K agents, dabigatran, and rivaroxaban: these treatments prolong the PT.

– Anti-factor antibodies have also been described in people taking the following treatments: penicillin, ampicillin, gentamicin, isoniazid and phenylbutazone.

SAMPLE STORAGE AND TRANSPORT

The test is performed on platelet poor plasma obtained by double-centrifugation (2000 g, 15 minutes between 10°C and 20°C separating the plasma obtained from the first centrifugation.

Can be stored for 2 to 4 hours, at laboratory temperature (never store the sample at + 4°C); separated plasma can be stored for 2 weeks at – 20°C or for 6 months at – 70°C. It is recommended that samples are thawed promptly in a water bath at 37°C.

Transport the platelet poor plasma frozen at – 20°C within 2 hours of sampling.

ASSAY METHODS

Assays of coagulation factors (cf corresponding sections) carried out for prolonged coagulation time, corrected by adding control plasma.

The relevant finding is deficiency of the factor against which the antibody is specifically directed.

After identifying an anti-factor antibody by assaying factors at different dilutions, the antibody must be titred (in a specialist laboratory) using a functional chronometric method.

Antibody titration

Method: example of anti-factor VIII

The inhibitor can be quantified approximately by testing for the patient plasma dilution, which inhibits 50% of the control plasma.

– The test plasma should be heated to 56°C for 30 minutes in order to destroy residual factor VIII and release the antibody from the VIII-anti VIII complex.

– One volume of undiluted test plasma or test plasma diluted in physiological saline (1/2, 1/4, 1/8, 1/16…) is mixed with one volume of control plasma.

– One volume of control plasma is mixed with one volume of physiological saline.

– The different mixtures are incubated at 37°C for 2 hours.

– Factor VIII is assayed in the different mixtures of the test plasma (at different dilutions) + control plasma. The factor VIII titre, R2 is recorded.

– Factor VIII is assayed in the control + physiological saline mixture after incubating under the same conditions as the test plasma, the factor VIII titre, R1 is recorded.

– Residual factor VIII activity is defined by the ratio R2/R1 x 100.

To titre, the test plasma dilution, which produces a residual factor VIII titre of approximately 50%, should be used. The equivalence between Bethesda units / ml should then be read from a curve of the percentage residual factor VIII activity (logarithmic scale) on the Y axis and Bethesda units /ml (linear scale) on the X axis: 50% residual factor VIII is equivalent to one Bethesda unit / ml.

INTERPRETATION

Assay of coagulation factors by the prolonged coagulation test corrected by adding control plasma

This assay must be performed on several successive plasma dilutions (1/10, 1/20, 1/40, 1/80). When an anti-factor antibody is present the activity of the factor against which the antibody is directed is reduced. This reduction persists with dilution. The activity of other factors can also be reduced at low dilutions (interference from the anti-factor antibody), although this fall does not persist as the dilutions are increased (titres of other factors are normal).

Antibody titring: expressing and interpreting results

Results are expressed in Bethesda units, one unit being defined as the amount of antibodies contained in 1 ml of plasma, which neutralises 50% of the activity of normal plasma.

< 5 Bethesda units (BU): low titre anti-factor antibody.

5 – 10 BU: moderate titre anti-factor antibody.

> 10 BU: high titre anti-factor antibody.

A patient deficient in a coagulation factor who has a low or moderate titre of anti-factor antibody can be treated with concentrates of the factor, producing immune tolerance. Beyond 10 BU, the antibody cannot be diluted and the patient must be given alternative replacement therapy (concentrates of animal factors or activated coagulation factors).

Specific features of anti-factor antibodies

	Development circumstances	Clinical features
Anti-VIII	– In haemophilia A: 10 to 30% of patients treated – Post-partum (transient), The elderly (often long-lasting), Auto-immune disease, Treatment with Ampicillin and Phenylbutazone	*Severe bleeding, fatal in 20 to 25% of cases*
Anti-IX	– In treated haemophilia B – Post-partum (transient), Auto-immune disease infection – Disappears spontaneously within 6 months	*Bleeding*
Anti-XI	– Rare, described in lupus erythematosus (progression of antibodies in parallel to the disease)	*Bleeding*
Anti-X or anti-XII	– Very rare	*Non-haemorrhagic*
Anti-II	– Isolated or associated with Lupus anticoagulant, often in childhood viral infection – Transient and non-neutralising	*Variable bleeding depending on factor II titre*
Anti-V	– In antibiotic treatments (Gentamicin) or with haemostatic glues – Usually transient; and disappearing spontaneously	*Variable bleeding*
Anti-VII	– Very rare, in malignancy	*Severe bleeding*
Anti-XIII	– Rare, in treatment with Isoniazid and Penicillin	*Severe bleeding*
Anti-fibrinogène	– Very rare	
Anti-von - Willebrand factor	– In various disorders (immunological and haematological diseases); Antibodies progress in parallel with the underlying disease	*Muco-cutaneous bleeding*

FOR FURTHER INFORMATION

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■ Samama M.M., Elalamy I., Conard J., Achkar A., Horellou M.H., *Hémorragies et thromboses: du diagnostic au traitement,* Collection «Les abrégés», Edition Masson, Paris, 2004.

ANTI-GAD 65 ANTIBODIES

DEFINITION

Anti-GAD 65 antibodies are antibodies against islet of Langerhans βcell glutamate decarboxylase. This enzyme catalyses the conversion of glutamate into gamma-aminobutyric acid (GABA). GAD 65 is one of the two main isoforms of this enzyme and is expressed in the human brain as well as the pancreas where it is mostly found in the islet of Langerhans βcells.

Synonyms: anti-glutamic acid decarboxylase antibodies; anti-glutamate decarboxylase antibodies.

INTRODUCTION

Type 1 "autoimmune" diabetes (DM1) begins with progressive destruction of the pancreatic islets where insulin is produced (Langerhans β cells). The factors responsible for this "autoimmune" destruction include innate predisposition (HLA) and probable viral pancreatitis (Coxsakie virus). This form of DM1 is associated with anti-pancreatic cell autoantibodies or antibodies against the cell secretion products. Amongst these, anti-GAD autoantibodies can occasionally be detected 8 years before symptomatic DM1. Since the role of anti-GAD was demonstrated in initiating mouse DM1, many research teams have tried to demonstrate an equivalent role in human DM1.

Anti-GAD autoantibodies are directed against conformational epitopes of GAD 65 in the central and C-terminal domains which share structural homology with a sequence of the Coxsackie B virus P2-C protein. They are present at the time of diagnosis in 50 to 70% of new cases of DM1 and in 2% of the general population. They have a higher prevalence in older age groups and in HLA-DR3 individuals. Anti-GAD autoantibodies are also found in 80% of cases of a spastic neurological disease, *stiff man syndrome* or *stiff person syndrome* (this name has been proposed secondarily as the disease affects as many women as men) which is characterised by muscle rigidity, painful spasms and cramps. In this case antibodies are against the linear epitopes common to the two GAD 65 and 67 isoforms, explaining cross-reactivity in tests.

INDICATIONS FOR MEASUREMENT

Detection of autoantibodies associated with DM1 allows the risk of DM1 developing in a population of at risk people, mostly those first generation relatives of a person suffering from DM1 and in patients suffering from autoimmune polyendocrinopathy, to be estimated. Screening involves a combination of the measurement of immunological markers (anti-GAD, anti-islet cells, anti-IA2, anti-insulin), testing for genetic markers (HLA DR Q α and β homology level test with affected family members) and measurement of insulin-secreting capacity of the cells after intravenous (IV) induced hyperglycaemia.

- **In people at high risk of DM1 (first generation relatives):**
  Anti-GAD autoantibodies are predictive markers of type 1 diabetes, allowing treatment to be started in the pre-diabetes phase. Together with anti-IA2, they are the two most useful markers in this situation and are used first line before anti-insulin antibodies in children and before anti-islet cell antibodies in adults (second line).
- **When signs of DM1 develop in a person without risk factors for DM1, particularly in young people:**
  The presence of anti-pancreatic autoantibodies (GAD etc.) confirms the autoimmune origin of the disease and allows other associated autoimmune endocrinopathies to be tested for. Their absence suggests an alternative type of diabetes (MODY, secondary diabetes, diabetes of mitochondrial origin).
- **In people with hyperglycaemia not initially suggesting DM1: The role of testing for autoantibodies as markers of an autoimmune cause:**
  In type 2 diabetics: 10% of these patients have detectable autoantibodies guiding the diagnosis towards slow progression type 1 diabetes (LADA).
  In women at risk of gestational diabetes: Screening should be combined with testing for anti-GAD autoantibodies. Their presence in gestational diabetes (particularly at delivery), appears to correlate closely with subsequent development of DM1 in the mother but not in the child.
  In the assessment of autoimmune polyendocrinopathy.
  Pre-pancreatic or islet of Langerhans transplant: Prognostic use.
  In stiff man syndrome: Testing for anti-GAD allows the autoimmune origin of the disease to be identified.
  General population screening: 90% of new cases of DM1 occur in the absence of known risk factors and the role of general population screening is starting to be examined.

INFORMATION

SAMPLE

Serum (Empty tube): A fasting sample is not required. Please ensure the sample is not haemolysed.

QUESTIONS FOR THE PATIENTS

Are you diabetic or have you a close relative suffering from diabetes? Are you being treated with insulin?

SAMPLE STORAGE AND TRANSPORT

Storage of serum: Room temperature for 8 h; 2 days at +4 °C.

Transport frozen.

ASSAY METHODS

Radioimmunological or ELISA methods.

NORMAL EXPECTED VALUES

Positivity thresholds vary between laboratories.

As an indication: anti-GAD 65 Ab < 1 kU/l.

INTERPRETATION

The prevalence of anti-GAD in the general population is approximately 3%.

The prevalence at the time of diagnosis of DM1 is 70-90%.

In screening for autoimmune diabetes in at risk people, the presence of these antibodies correlates closely with the presence of anti-islet cell antibodies (ICA). They can be detected up to 8 years before development of clinical symptoms and persist after the disease has started. The prevalence of anti-GAD in people with a first generation relative suffering from type 1 diabetes is 5 to 13% and is increased considerably (66 to 89%) in relatives of patients with positive ICA (> 20 JFD units). 75% of this population develops type 1 diabetes. The finding of anti-GAD therefore increases the positive predicted value of ICA by 66 to 75%, confirming the use of early joint testing in this population *(cf. table)*.

Pre-pancreatic or islet of Langerhans transplant: the presence of anti-GAD antibodies indicates a poor prognosis (compared to absence) in terms of transplant success.

In type 2 diabetes, 10 to 15% of patients have antibodies against one of the islet of Langerhans βcell components (usually anti-GAD).

At least one of the autoantibodies (anti-GAD, ICA or anti-IA2) are present in approximately 18% of cases of gestational diabetes and the risk of developing type 1 diabetes within two years after delivery is 30% if the antibodies are positive compared to 2% if they are negative.

In stiff *man syndrome*: anti-GAD testing confirms the autoimmune origin of the disease (80% of cases) and excludes a paraneoplastic cause associated with amphiphysin I autoantibodies. Antibody titres are far higher in this disease than are found in diabetes.

Sensitivity and positive predictive value of immunological markers for diabetes (percentage values) from Verge et al. Diabetes 1996; 45 (7): 926-33.

	Sensitivity	Positive predictive value at 5 years
Anti-GAD	90	52
ICA (> 20 JDF units)	74	51
Anti-insulin	76	59
Anti-IA2 (ICA 512 bdc)	64	81
Anti-GAD + Anti-insulin	68	68
Anti-GAD + ICA 512 bdc	52	86
Anti-insulin + ICA 512 bdc	54	100
1 antibodies	18	15
2 antibodies	28	44
3 antibodies	52	100

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ANTI-GANGLIOSIDE ANTIBODIES

DEFINITION

Gangliosides are glycosphingolipids present in the mammal cell membrane lipid bilayer. They consist of a hydrophobic membrane ceramide and one or more sugars and sialic acids forming the extracellular part.

Around twenty gangliosides are described. Their name always begins with G (for ganglioside), the 2nd letter indicates the number of sialic acids (**M**ono, **D**i, **T**ri, **Q**uad) and the figure indicates that the molecule contains (5n) sugars. This figure is occasionally followed by a small letter representing the migration order in thin layer chromatography.

GM1 therefore consist of one sialic acid and 4 sugars, GQ1b of 4 sialic acids and 4 sugars.

The gangliosides are involved in many functions: Growth and cell differentiation, apoptosis, adhesion, immune system modulation, etc. They are also receptors for a large range of bacteria, viruses and toxins.

GM1, GD1a, GD1b and GT1b are present in large amounts in peripheral nerves.

GQ1b is only present in the oculomotor nerves.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

Although the pathogenic potential of the anti-ganglioside antibodies is disputed, their identification in various peripheral neuropathies provides an important diagnostic aid.

– ***In motor neuropathies with conduction block:*** anti-GM1 and anti-GD1b class IgM antibodies are found in almost 75% of cases.

– ***In motor neuropathies without conduction block,*** particularly amyotrophic lateral sclerosis (ALS).

– ***In chronic peripheral neuropathy:*** Associated with a monoclonal IgM, 5 types of antibodies are found anti-GM1, GM2, GD1a, GD1b or GT1b defining 5 separate clinical entities.

– ***In axonal forms of Guillain-Barré syndrome:*** Following Campylobacter jejunii infection, anti-GM1 or GD1b class IgG antibodies are found in 95% of cases in the acute phase and then disappear over 6 months if the disease resolves. Conversely, in "classical" Guillain-Barré syndrome, anti-GM1 are found in 20 to 30% of cases and their titre is of limited significance.

– ***In Miller-Fischer syndrome*** (ophtalmoplegia associated with polyradiculoneuritis): High titres of anti-GQ1b class IgG antibodies are found in 90% of cases.

INFORMATION

SAMPLE

Serum or CSF: A fasting sample is essential.

ESSENTIAL INFORMATION

State the antibodies to be tested or disease suspected.

SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling.

Store and transport at +4° C.

ASSAY METHODS

In their native state, gangliosides are found in a three-dimensional configuration, any change in which alters their recognition by antibodies. Operating conditions must therefore be followed particularly carefully and incubation performed at +4°C.

Immunodetection on thin layer chromatography: Semi-purified glycolipids from nerve tissue are loaded onto high resolution aluminium-silica thin layer chromatography plates and migrated. Highly diluted serum is added and revealed with an anti-human conjugate. If positive, the antibody specificity IgG, IgM, κ or λ may be described.

ELISA: Different tests are commercially available with a single antigen (usually GM1), or different antigen in each well (GM1, GM2, GD1a, GD1b, GQ1b).

They are revealed with an anti-IgG + M or anti-IgG or anti-IgM conjugate.

Immunodot: Different commercial tests are available, one with GM1, GM2, GM3, GD1a, GD1b, GD3, GT1a, GT1b, GQ1b, sulfatides, and another with GM1, GM2, GM3, GD1a, GD1b, GT1b, GQ1b. They are revealed with an anti-IgG + M or anti-IgG or anti-IgM conjugate.

INTERPRETATION

Regardless of methods, only markedly high levels are significant. Low titres of natural polyvalent IgM autoantibodies of low affinity are found in all people.

Cross-reactions are seen between different antigens (for example GM1 and GD1b) and the dominant antigen in the profile found is reported.

Immunodetection on thin layer chromatography: The result is expressed as negative/positive against an internal standard. "+" type quantification is possible.

ELISA: Results are expressed in arbitrary units after calculating a ratio to a control serum.

Immunodot: The result is expressed as negative/positive against an internal standard. "+" type quantification is possible.

TREATMENT

Different treatments have been tested either alone or in association in ***motor neuropathies with conduction block***; corticosteroids and plasmaphereses do not appear to be effective and may even be harmful. Azathioprine and above all cyclophosphamide can be used although infusions of high dose immunoglobulins are the most effective.

Motor neuropathies without conduction block are far less responsive to treatments.

Corticosteroid therapy should be avoided in ***acute polyradiculoneuritis***; plasmaphereses and high dose immunoglobulins produce good results.

FOR FURTHER INFORMATION

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ANTI-GLIADIN ANTIBODIES

DEFINITION

Anti-gliadin antibodies are autoantibodies against different fractions of gliadin, which is the ethanol-soluble fraction of gluten. Their pathological role is unclear although they are serological markers of coeliac disease (gluten intolerance).

INTRODUCTION

Coeliac disease is an intolerance to gluten characterised by enteropathy causing malabsorption in genetically predisposed people (95% of patients express HLA class II, DQ2).

Two clinical forms are distinguished:

Childhood coeliac disease, which has a fairly typical presentation:

This generally presents before the age of 2 years old and has a prevalence of approximately 1/2500 in France. Classically it is an association of chronic diarrhoea and relatively severe signs of malnutrition. It is accompanied by a constant history anorexia, vomiting in 50% of cases and always with deterioration in general health. This leads quickly to growth retardation and a falling off of the weight curve.

In adults:

The classical form is only seen in 20% of cases.

In all of the other cases the clinical features may only reflect malabsorption and present with low grade non-specific symptoms (bone demineralisation, arthralgia, neurological problems, fertility disorders) which may make it difficult to diagnose. Laboratory findings in this situation may include those of malabsorption (anaemia with iron, folate and vitamin B12 deficiency, deficiency of vitamin K dependent factors, etc).

The treatment of coeliac disease involves a strict lifelong gluten-free diet. All foods containing wheat, barley or rye must be excluded from the diet. Laboratory and clinical findings improve often spectacularly over a few months on a gluten-free diet. This is the only means of preventing the long term complications of the disease (notably small bowel lymphoma).

The disease is diagnosed by initial serological testing followed by confirmation from histological examination of a small bowel biopsy to confirm the presence and extent of villous atrophy.

ROLE OF MEASUREMENT

Coeliac disease is characterised by the presence of antibodies in response to eating gluten. The antibodies tested are anti-endomysial IgA, anti-gliadin IgG and IgA and anti-transglutaminase antibodies *(cf. corresponding pages)*. Anti-reticulin antibodies should no longer be tested because of their low sensitivity.

Similarly, anti-gliadin antibodies have lower sensitivity and specificity to anti-endomysial and/or anti-transglutaminase Ab particularly in adults, and it is no longer recommended that they be tested in France. New tests however have recently emerged using modified gliadin peptides (deaminated peptides or peptides bound to transglutaminase). These new tests show far better performance than the first generation tests using native gliadin.

INFORMATION

■ SAMPLE

Serum: A fasting sample is not necessary.

■ QUESTIONS FOR THE PATIENT

Are you following a gluten-free diet?

■ SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling.

Transport at 4 °C.

ASSAY METHODS

Currently, anti-gliadin IgG and IgA are tested for using ELISA or immunoblot methods. There is a lack of standardisation between kits due particularly to the antigen preparations.

REFERENCE VALUES

ELISA values are expressed in arbitrary units which vary according to the kit.

By immunoblot, the result is expressed as negative/positive against an internal standard. "+" type quantification is possible.

FOR FURTHER INFORMATION

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ANTI-GLOMERULAR BASEMENT MEMBRANE ANTIBODIES

DEFINITION

The glomerular basement membrane comprises several components. Type IV collagen is the main one and others include macromolecules such as laminin, fibronectin, entactin and proteoglycans. Anti-glomerular basement membrane antibodies (anti-GBM) are, in most cases, auto-antibodies directed against the molecule of type IV collagen and they are detected mostly in the serum of patients with Goodpasture's syndrome. The collagen IV molecule is composed of several alpha chains arranged in three domains: a non-collagenous (NC1) globular C-terminal domain; a triple helix domain formed by two alpha1 chains (IV) and one alpha2 chain (IV), which determines the mechanical properties of the molecule; and domain 75 at the N-terminal extremity. The Goodpasture antigen is the alpha3 chain (IV) and the antibodies associated with this disease are mainly directed against the NC1 domain (also designated M2). These are usually IgG, but sometimes IgA. Other anti-GBM antibodies, which do not correspond with Goodpasture's syndrome, are detected in the serum of patients with a variety of kidney diseases. In post-streptococcal glomerulonephritis, the target antigen is the 7S domain of type IV collagen. In auto-immune tubulo-interstitial nephritis, the TIN (tubulo-interstitial nephritis antigen) component of the basement membrane is the target. Other components of the basement membrane can also be involved in this way: fibronectin (IgA-fibronectin complexes) in Berger's disease, laminin in eclampsia and Chagas disease, entactin in systemic lupus erythematosus and heparan sulphate in post-streptococcal glomerulonephritis.

Synonym: anti-GBM.

INTRODUCTION

Goodpasture's syndrome is very rare in France. It usually occurs in young men and presents with lung involvement (haemoptysis) and kidney problems, as the anti-GBM antibodies cause rapidly progressive glomerulopathy. Typically there are anti-GBM antibodies in the serum and immunofluorescence shows linear binding of IgG along the membranes of the glomerular capillaries. This suggests auto-immunisation against the basement membrane.

INDICATIONS FOR MEASUREMENT

When Goodpasture's syndrome is suspected.

Investigation of glomerulonephritis or tubulo-interstitial nephritis.

Differential diagnosis of Wegener's disease. ANCA (anti-neutrophil cytoplasmic antibody) should be looked for in this situation.

Assessment of the prognosis and for follow-up of progress in patients with Goodpasture's syndrome.

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not necessary.

SAMPLE STORAGE AND TRANSPORT

Store serum for one week at 4° C, and then freeze.

Transport at + 4° C or in its frozen state if already frozen.

ASSAY METHODS

Indirect immunofluorescence on sections of monkey kidney pre-treated with 6M urea at pH 3.5 for 30 minutes to expose the antigen.

ELISA or immunoblot using collagen IV. ELISA allows for quantification and, therefore, has a role in follow-up of patients, but it is not useful in emergency diagnosis, whereas immunoblot can be used for this purpose.

NORMAL EXPECTED VALUES

With immunofluorescence the positive threshold is usually 5. Titration must be exact because the progress of the titre is used to follow the effectiveness of therapy.

ELISA: The result is expressed in arbitrary units as there is no international standard.

Immunoblot: The result is expressed as negative or positive with reference to an internal standard; "+" quantification is possible.

INTERPRETATION

Anti-GBM antibodies are found in the serum of patients with a variety of nephropathies, in particular Goodpasture's syndrome. In this condition, a high antibody titre is a poor prognostic feature. Following plasmaphoresis or immunosuppressant treatment, these antibodies disappear after a mean period of 3 months. They can, however, persist for up to 18 months, even though clinical symptoms have disappeared. Renal transplant can be considered once the antibodies have disappeared.

FOR FURTHER INFORMATION

■ Noël L.H., Lesavre P., *Anti-glomerular basement membrane antibodies*. In : Meyer O., Rouquette A.M., Youinou P., Autoanticorps marqueurs des maladies auto-immunes (Auto-antibody markers of auto-immune disease), Bmd Ed, Paris, 1999: 381-91.

■ Humbel R.L., *Immune nephropathies*. In : Humbel R.L., Auto-anticorps et maladies auto-immunes (Auto-antibodies and auto-immune disease), 2nd Ed. Collection Option/Bio, Ed Elsevier, Paris, 1997 : 166-79.

ANTI-HEPATIC CYTOSOL ANTI-LC1

DEFINITION

Anti-LC1 *(liver cytosol)* antibodies, together with anti-LKM1 antibodies, are the markers of type 2 autoimmune hepatitis (AIH 2).

They are present in 30% of AIH 2 in association with anti-LKM1, in 10% of AIH 2 in isolation and in 0.5% of cases of hepatitis C.

The antibodies may be negative in the acute phase and a 2 month follow up test is essential in this situation. They are usually greatly raised in the cirrhotic phase. They become negative over 1 to 3 years with effective treatment, their persistence suggesting relapse.

INDICATIONS FOR MEASUREMENT

Type 2 autoimmune hepatitis is a very rare disease (1 to 2 cases/million) mostly affecting girls (90% of cases) between 2 and 15 years old. It is often associated with other autoimmune diseases including diabetes and thyroiditis etc. It often presents with severe acute hepatitis, in 80% of cases progressing to cirrhosis within 3 years. The diagnosis relies on the finding of anti-LKM1 antibodies often associated with anti-LC1 antibodies. Anti-LC1 antibodies may also be found in isolation.

In addition to a large rise in transaminases, the IgG is commonly raised.

Conventional treatment is with prednisolone and azathioprine. Cyclosporin is used in fulminant hepatitis and if this fails, the last resort is a liver transplant.

INFORMATION

■ SAMPLE

Serum: A fasting sample is not necessary.

■ DESIRABLE INFORMATION

Information about any rise in liver enzymes and serological status for viral hepatitis B and C.

■ SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling.

Transport at +4°C.

ASSAY METHODS

Triple substrate immunofluorescence (rat kidney/liver/stomach):

Kidney: Negative.

Liver: Homogeneous hepatocyte fluorescence sparing hepatocytes around the centro-lobular veins.

Stomach: Negative.

Anti-LC1 Ab may not be recognised by IFI if high titres of anti-LKM1 are present.

Immunodiffusion: Confirmatory method. Based on the findings of a precipitation arc with an extract containing cytosol, which should coalesce with a known positive serum.

Immunoblot: Confirmatory method using the antigen formiminotransferase cyclodesaminase. Tests are also available to type for other antibodies implicated in autoimmune liver disease (M2, LKM1, SLA, etc.).

REFERENCE VALUES

Immunofluorescence: The result is expressed as the reciprocal of the dilution, with a threshold of 40. The upper limit of the method is 640.

Immunodiffusion: The result is expressed as negative/positive.

Immunoblot: The result is expressed as negative/positive against an internal standard. «+» type quantification is possible

FOR FURTHER INFORMATION

■ *Les Autoanticorps 2003-2004,* CD-Rom produced by Monier J.C., Auger C., Fabien N.

■ Humbel R.L., *Autoanticorps et maladies autoimmunes,* collection Option/Bio, Ed Elsevier, 2e Ed, Paris, 1997.

■ Revue Française des Laboratoires (supplement 361 bis) 3e greffe hépatique. GEAI Congress, March 2004.

ANTI-HISTONE ANTIBODIES

DEFINITION

In the nucleus of eukaryocytic cells all the DNA is tightly incorporated within a protein complex formed by an association of 5 types of histone: H1, H2a/H2b, H3 and H4. This grouping forms a structure known as the nucleosome. The core of the nucleosome consists of an octamer of histones (2 H2a/H2b dimers and an H3, H4 tetramer).

Various histone antigenic patterns can be present in the chromatin complex. Histones seem to fulfil major regulatory functions, especially in the transcription of certain genes.

INTRODUCTION

Anti-histone antibodies have been observed since 1957. They were initially described in systemic lupus erythematosus (SLE) and, in particular, in drug-induced lupus (hydralazine, procainamide, etc.). They are also found, however, in many other conditions such as rheumatoid arthritis, scleroderma, primary biliary cirrhosis and some infectious diseases. Thus, their importance is limited, because their specificity in relation to any particular auto-immune disease is low.

INDICATIONS FOR MEASUREMENT

Positive anti-nuclear antibodies, in the presence of negative anti-native DNA, and anti-ENA.

INFORMATION

■ SAMPLE

Serum (Dry tube): A fasting sample is not required.

■ CLINICAL INFORMATION

Note any auto-immune disease as well as other conditions and the result of tests for antinuclear antibodies.

■ SAMPLE STORAGE AND TRANSPORT

Remove serum within 12 hours of sampling and store at +4° C

Transport at +4° C.

ASSAY METHODS

In current practice anti-histone antibodies are measured by ELISA techniques.

The variety of antigenic preparations in use (total histone, histone sub-units, nucleosome etc) means that the results obtained can be very different depending on the reagent used.

UNITS AND REFERENCE VALUES

These vary, depending on the kit that is used.

FOR FURTHER INFORMATION

■ Decker P., Muller S., *Anti-histone antibodies*. In: Meyer O., Rouquette A.M., Youinou P., Auto-anticorps marqueurs de maladies auto-immunes (Autoantibody markers of auto-immune disease), Paris: BMD, 1999; pp. 103-112

ANTI-HLA ANTIBODIES

DEFINITION

Anti-HLA antibodies are involved in acute rejection of transplanted organs. They can be directed against the HLA antigens of the graft and it is mandatory that they should be looked for in the recipient's serum before any organ transplant procedure.

Synonym: antibodies against major histocompatibility complex (MHC) class I or class II.

INTRODUCTION

Anti-HLA antibodies may appear after blood transfusion, pregnancy or organ allograft. These antibodies are detectable from 10 days following transfusion and usually disappear within a month. After pregnancy or transplant, they more often persist over the long term.

These antibodies are directed against class I HLA antigens (A, B or C), which are expressed on the surface of all cells, other than red cells, or against class II HLA antigens (DR, DQ, DP), expressed only on the surface of B lymphocytes, monocytes/macrophages and dendritic cells.

Antibodies against class I HLA antigens lyse B and T lymphocytes, whereas antibodies to class II HLA antigens lyse B but not T lymphocytes. Their damaging role is well established for IgG antibodies against class I HLA antigens (lysing donor T lymphocytes) but is less certain for antibodies to class II antigens. When the antibodies are IgM, it is usually the case that they are auto-antibodies, which recognise lymphocyte membrane structures and are not anti-HLA antibodies. They do not cause acute graft rejection.

INDICATIONS FOR MEASUREMENT

Testing for HLA antibodies is indicated in those who have previously had transplants and is routine for patients awaiting organ allograft, in order to prevent acute graft rejection caused by these auto-antibodies. In particular, they should be looked for 10 days following a blood transfusion and similarly after pregnancy.

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Ask about blood transfusion (and its date).

Ask women about previous pregnancy.

SAMPLE STORAGE AND TRANSPORT

Store serum at +4° C.

Transport at + 4° C.

ASSAY METHODS

– Detection of antibodies against class I HLA by complement dependent micro-lymphocytotoxicity: The patients serum is tested against a panel of mononuclear cells and typed cell lines, which cover the spectrum of HLA specificities present in the general population. The analysis is performed in the absence and then in the presence of dithiotreitol, which depolymerises IgM, thus separating its measurement from that of IgG.

– Antibodies against class II HLA are detected with an identical technique, after extraction of anti-class I antibodies by treating the serum with a pool of platelets, which express HLA antigens of class I but not of class II.

– ELISA and flow cytometry methods are also employed: These are more sensitive and can be employed as second line tests when analysis using micro-lymphocytotoxicity is consistently negative. These techniques can reveal previously unrecognised immunisation.

– A cross match between donor and recipient: Carried out routinely just before transplant surgery. The serum of the recipient is tested against the donor's HLA antigens (cells isolated from lymph nodes, rich in T lymphocytes; and cells isolated from the spleen, rich in B lymphocytes). In this way one can define the antibody specificity (anti-HLA class I, II or anti-lymphocyte auto-antibodies), the Ig isotype (IgG or IgM) and possibly the date of the appearance of antibody in the serum. Micro-lymphocytotoxicity or flow cytometry are the techniques used.

NORMAL EXPECTED VALUES

The results of micro-lymphocytotoxicity are read by counting the number of lysed cells in each well of the test plate (versus the panel). There should be no anti-HLA antibodies in the serum of patients, so there should not be any cell lysis.

INTERPRETATION

The patient can be considered to be immunised if at least one cell is lysed. When positive, the reactivity is expressed as percentage of cells lysed. This can be very high (more than 75% of the cells lysed) in patients, who have had numerous transfusions or previous transplant.

The detection of anti-HLA antibodies in patients awaiting a graft must be reported routinely to the appropriate body. The specificity of these antibodies must be determined. An anti-HLA antibody of a given specificity is a contra-indication for transplantation of a graft that carries that specificity.

Following a cross-match which reveals recipient IgG directed against donor T lymphocytes, organ transplantation is contra-indicated because of the risk of hyper-acute or accelerated rejection of the graft.

Anti-HLA IgM can be found in patients with auto-immune disease. This is not theoretically damaging for the graft and is not a contra-indication to the procedure.

FOR FURTHER INFORMATION

■ Caillat-Zucman S., *Anti-HLA antibodies*, Encycl Med Biol, Elsevier, Paris 2003.

■ Wycknel A., Cohen J.H.M., *Organ transplantation in the year 2000*, Revue française des laboratoires, Nov 2000; 327: 83-90.

ANTI-IA2 OR ANTI-TYROSINE PHOSPHATASE ANTIBODIES

DEFINITION

Anti-IA2 antibodies are auto-antibodies directed against the IA2 antigen *(Insulinoma Associated protein 2)*, related to the family of protein-tyrosine-kinases. These were identified by screening a cDNA expression bank from islet of Langerhans cells, using serum from diabetic patients or at risk subjects. ICA *(Islet Cell Antigen)* antigen 512 is the intracellular domain of the IA2 antigen.

There are two IA2 tyrosine phosphatases (IA2 and IA2–ß or phogrin) coded by different genes, but both expressed in transmembrane secretory granules in the ß cells of the islets of Langerhans, in some cells of the central nervous system and in some other cell types. They are targeted by conformational antibodies involved in the genesis of type-1 diabetes. Anti-IA2 antibodies recognise the intracellular portion of IA-2, the only part implicated in the auto-immune process.

Anti-IA2 antibodies, together with anti-GAD antibodies, anti-insulin antibodies and antibodies against the islets of Langerhans are immunological markers of auto-immune diabetes.

Synonyms: anti-tyrosine phosphatase antibodies, anti-Insulinoma Associated protein 2 (anti- IA2) antibodies, anti-Islet Cell Antigen 512 (ICA 512) antibodies.

INTRODUCTION

Type-1 diabetes (previously known as insulin dependent diabetes) is a disease with an auto-immune component, characterised by almost total disappearance of insulin secretion. It results from a slow and progressive auto-immune process, starting several years before the appearance of the illness and finishing with destruction of the ß cells of the pancreatic islets of Langerhans, the cells which secrete insulin. From the pre-clinical phase of diabetes, auto-antibodies directed against several recognised antigenic targets within the islets of Langerhans, are present in the blood. This means that the disease can be detected pre-clinically. The antibodies involved are directed against GAD-65, the islets of Langerhans, insulin and tyrosine phosphatase. The individual antibody assays do not have sufficient sensitivity and specificity to identify subjects at risk of diabetes. In the majority of clinical situations, it is appropriate to measure two or three of them.

Peripheral T-lymphocytes of diabetic or hyperglycaemic individuals have been shown to react with the intracellular C-terminal fragment of IA-2 protein.

Numerous family studies have demonstrated the predictive value of anti-IA2 antibodies in populations at risk of developing type-1 diabetes.

INDICATIONS FOR MEASUREMENT

The main reason for measuring anti-diabetes auto-antibodies is in screening of states that are pre-diabetic (type-1 diabetes). This screening combines the detection of immunological markers (anti-GAD, anti- islets of Langerhans, and anti-IA2, anti-insulin), with genetic markers (analysis of the degree of homology of HLA DR Q α and β in affected family members) and measurement of insulin secretary capacity of β cells in response to intravenous induction of hyperglycaemia.

- **In subjects at high risk of Type-1 diabetes (first degree relatives):**

  Predictive markers of the risk of developing type-1 diabetes. The possibility of initiating therapy early in the pre-diabetic phase increases the importance of these tests for screening for auto-immune diabetes. In view of their high positive predictive value, they are requested as first-line tests together with anti-GAD, before anti-islet cell antibodies in adults and before anti-insulin antibodies in children.

- **In patients with hyperglycaemia or a diabetic condition strongly suggestive of type-1 diabetes (children, adolescents, young adults):**

  To confirm the auto-immune basis of the condition or, in the absence of antibodies, to suggest another cause of the diabetes (MODY, secondary diabetes, mitochondrial DNA associated diabetes).

- **In subjects presenting with hyperglycaemia, not a priori suggestive of type I diabetes:**

  Relevance of measurement of auto-antibodies as markers of auto-immune aetiology in:

  – Type-2 diabetes: 10% of subjects have detectable antibodies, suggesting that the diagnosis may be slowly evolving type-1 diabetes (LADA).

  – Women presenting with hyperglycaemia in pregnancy: at risk of developing type-1 diabetes.

  – Subjects with another auto-immune condition (such as thyroiditis): in these patients the risk of developing auto-immune diabetes is higher than in the general population.

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not necessary.

QUESTIONS FOR THE PATIENT

Is the patient diabetic or closely related to someone with diabetes? Is he/she on insulin?

SAMPLE STORAGE AND TRANSPORT

Storage of serum: + 4° C 24 h, then freeze at – 20° C.

Transport at + 4° C if moving within 24 hours; if not, frozen.

ASSAY METHODS

Radio-immunological or radio-ligand assay.

NORMAL EXPECTED VALUES

These auto-antibodies are normally not detectable in the blood. The positivity threshold varies according to the laboratory. Example: anti-tyrosine phosphatase antibody < 1 U/ml.

INTERPRETATION

In France, the prevalence of anti-IA2 antibodies in the general population is between 0.5% and 5%, depending on the survey. In type-1 diabetes the prevalence of these antibodies is around 50% at the time of diagnosis.

In first generation relatives of a type-1 diabetic with positive islet cell antibodies, the prevalence of anti-IA2 antibodies is between 20 and 70%. The risk of developing diabetes within 5 to 7 years would be close to 80% in subjects with a high level of these antibodies when first measured. In at risk populations, the combined presence of both anti-GAD and anti–ICA antibodies confers a positive predictive value between 70 and 100% for the development of diabetes within 1 to 5 years (see table).

In gestational diabetes, at least one of the antibodies (anti-GAD, ICA or anti-IA2) is present in about 18% of cases. The risk of developing type-1 diabetes within two years of delivery is 30% if the antibodies are positive and 2% when they are negative.

There is a close association between anti-IA2 and HLA-DR4, a haplotype, which confers a predisposition to type-1 diabetes. This suggests that the anti-IA2 antibodies are more specifically targeted for ß cell destruction than are the anti-GAD antibodies. Their presence seems to be associated with more aggressive and more rapidly progressive disease.

Sensitivity and positive predictive value of immune markers for diabetes (percentage), Verge et al. Diabetes 1996 ; 45(7): 926-33.

	Sensitivity	Positive predictive value – 5 years
Anti-GAD	90	52
ICA (>20 JDF units)	74	51
Anti-insulin	76	59
Anti-IA2 (ICA 512 bdc)	64	81
Anti-GAD + Anti-insulin	68	68
Anti-GAD + ICA 512 bdc	52	86
Anti-insulin + ICA 512 bdc	54	100
1 antibody	18	15
2 antibodies	28	44
3 antibodies	52	100

FOR FURTHER INFORMATION

■ Carel J.C., Lotton C., Boitard C., *Diabetes: auto-antibodies*. In: Meyer O., Rouquette A.M., Youinou P., *Auto-antibody markers for auto-immune disease (Autoanticorps marqueurs des maladies auto-immunes)*, Bmd Ed, Paris, 1999: 363-80.

■ Ongagna J.C., Sapin R., Type 1 *diabetes and auto-immunity*, Biotribune, March 2004, n^0 9: 42-3.

■ Humbel R.L., *Auto-antibodies and auto-immune disease*, Collection Option/Bio, Ed Elsevier, Paris, 2nd Ed, 1997: 189-97.

■ Carel J.C., Faideau B., Lotton C., Boitard C., *Autoantibodies in type 1 diabetes*, Encycl Med Biol, Elsevier, Paris, 2003.

ANTI-INSULIN ANTIBODIES

DEFINITION

Anti-insulin antibodies, together with anti-GAD, anti-IA2 and anti-islet of Langerhans cells (ICA) antibodies, are immune markers for diabetes mellitus. Of these, the anti-insulin auto-antibodies are the only ones which are directed against a specific cellular antigen. Approximately 50% of these antibodies recognise only human insulin. The remainder also recognise porcine and bovine insulin.

Anti-insulin antibodies are found before any replacement therapy in about 30 to 50% of type-1 diabetics. This proportion is significantly higher in children aged less than 10 years, reaching a level of 70%. These antibodies are also found in most patients following several weeks of insulin therapy.

Synonyms: Insulin auto-antibodies or IAA.

INTRODUCTION

A direct role of this antibody in the pathogenesis of type-1 diabetes has not been established. However, the presence of anti-pancreatic antibodies in the pre-diabetic stage (phase of accumulation of inflammatory cells and lymphocytes in the islets) and during the clinical development of the disease (phase of destruction of the ß cells of the islets of Langerhans) suggests their involvement in the disease. Moreover, it has been shown that the specificity of the anti-pancreatic auto-antibodies differs in the two phases of evolution of the disease.

INDICATIONS FOR MEASUREMENT

The importance of measurement of anti-insulin antibodies lies in the screening of individuals at risk of developing insulin dependent diabetes. These subjects come from a population selected on the basis of other criteria (family history, metabolic criteria). The objective is to be in a position to administer treatment that might prevent the development of insulin dependence.

- **In subjects at high risk of Type-1 diabetes (first generation relatives):**
  Predictive marker for the risk of developing type-1 diabetes. In view of their low positive predictive value, these antibodies are only useful when combined with other anti-pancreatic auto-antibodies (ICA, anti-GAD and/or anti-IA2), especially in children.
- **In patients with hyperglycaemia or a diabetic condition strongly suggestive of type-1 diabetes (children, adolescents, young adults):**
  To confirm the auto-immune basis of the condition or, in the absence of antibodies, to suggest another cause of the diabetes (MODY, secondary diabetes, mitochondrial DNA associated diabetes).
- **In subjects presenting with hyperglycaemia, not a priori suggestive of type-1 diabetes:**
  Relevance of measurement of auto-antibodies as markers of auto-immune aetiology (in association with other antibodies, see above) in:
  – Type-2 diabetes: 10% of subjects have detectable auto-antibodies, suggesting that the diagnosis may be slowly evolving type-1 diabetes (LADA).
  – Women presenting with hyperglycaemia in pregnancy who are at risk of developing type-1 diabetes.
  – Subjects with another auto-immune condition such as thyroiditis, for example: In these patients the risk of developing auto-immune diabetes is higher than in the general population.
- **In type-11 diabetics on insulin:**
  In very high titres, anti-insulin auto-antibodies can cause insulin resistance.
- **In hypoglycaemia:**
  For screening for a very rare form of hypoglycaemia due to anti-insulin autoimmunity, itself usually secondary to exposure to drugs containing a sulphydryl group. The IAA can cause a hypoglycaemic syndrome by forming IAA-insulin complexes, from which substantial quantities of insulin can be released into the blood. The diagnosis can be made by finding the combination of IAA, generally at a high level, hypoglycaemia and inappropriately elevated free insulin levels.

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Is the patient diabetic or closely related to someone with diabetes? Is he/she on insulin?

SAMPLE STORAGE AND TRANSPORT

Storage of serum: Ambient temperature 8 h; 2 days at + 4° C.

Transport frozen.

ASSAY METHODS

Radio-immunological assay measuring:

Anti-insulin antibodies induced by treatment with human or porcine insulin.

Anti-insulin antibodies present in pre-diabetes before any insulin treatment.

Two measurements can be performed:

– Measurement of free anti-insulin antibodies. This is antibody not bound to circulating insulin;
– Measurement of total anti-insulin antibody, which is free anti-insulin antibody plus antibody bound to serum insulin.

NORMAL EXPECTED VALUES

These auto-antibodies are not normally detectable in the blood. The positivity threshold varies according to the laboratory (example: Anti-insulin antibodies < 5.5%). For ease of interpretation, the reported results are explicit (negative, at the positivity limit, positive).

INTERPRETATION

The prevalence of anti-insulin antibodies in the general population is around 3% for children and 1% in adults.

In the population of at risk individuals, the presence of anti-insulin antibodies does not in itself have much value for prediction of progression towards insulin dependence. Interpretation depends on taking note of other anti-pancreatic antibodies.

In type-11 diabetes, they are present in 30 to 50% of patients at the time of diagnosis before any treatment. This figure is significantly higher in children from 0 to 4 years of age (the anti-insulin antibodies seem to be the first to appear). They are then usually of weak affinity and specific for human insulin. Their presence before insulin therapy (or less than one week after initiation of treatment) is very suggestive of an autoimmune origin of the disease.

The level of these antibodies is inversely proportional to the age of the patient and the high values found in young children might suggest a greater rate of destruction of ß cells of the islets.

The finding of high levels of anti-insulin antibodies (> 40%) in a type-11 diabetic patient on insulin treatment (usually an antibody with high affinity) results in insulin resistance (insulin resistance syndrome = metabolic syndrome or syndrome X or "type-2 pre-diabetes"). Indeed, these antibodies form stable immune complexes with insulin, which reduces bio-availability of exogenous insulin. In addition, they alter total serum insulin levels.

With a clinical picture of hypoglycaemia, the presence of high levels of anti-insulin antibodies suggests the possibility of a syndrome of autoimmune hypoglycaemia.

In the newborn baby with a diabetic mother, anti-insulin antibodies can be falsely positive because of interference of an insulin binding agent in cord blood, which disappears in a few days. In addition, anti-pancreatic antibodies in the mother can cross the placenta and then be measured in the baby up to the age of about 6 months, before disappearing.

FOR FURTHER INFORMATION

■ Carel J.C., Lotton C., Boitard C., *Diabetes: auto-antibodies*. In: Meyer O., Rouquette A.M., Youinou P., *Auto-antibody markers for auto-immune disease (Autoanticorps marqueurs des amladies auto-immunes)*, Bmd Ed, Paris, 1999: 363-80.

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■ Carel J.C., Faideau B., Lotton C., Boitard C., *Autoantibodies in type 1 diabetes*, Encycl Med Biol, Elsevier, Paris, 2003.

ANTI-ISLET CELL ANTIBODIES

DEFINITION

Anti-islet cell antibodies are auto-antibodies directed against cytoplasmic antigens of all or some of the cells of the islets of Langerhans. They were revealed by indirect immunofluorescence (IIF) on sections of human pancreas from blood group O subjects. They were the first auto-antibodies discovered in diabetes and the principal ones used in family studies over many years. They recognise several different antigens; the insulin secreting ß cells of the pancreas, α cells for glucagon, δ cells for somatostatin and PP for pancreatic polypeptides.

Anti-islet cell antibodies, together with anti-GAD, anti-insulin and anti-IA2 antibodies are immunological markers of auto-immune diabetes. When only one of these antibodies is used, there is not adequate sensitivity and specificity for screening of patients at risk of diabetes. In the majority of clinical situations, it is appropriate to measure two or three of them.

Synonyms: *Islet Cell antibody* or ICA.

INTRODUCTION

Type-1 diabetes (previously known as insulin-dependent diabetes) is a disease with an auto-immune component, characterised by the almost total disappearance of insulin secretion. It results from a slow and progressive auto-immune process, starting several years before the appearance of the illness and ending with the destruction of the ß cells of the islets, the insulin-secreting cells. In the pre-clinical phase of diabetes, one can detect into the blood auto-antibodies directed against several recognised antigenic targets within the islets of Langerhans. This facilitates early diagnosis of the illness. The relevant antibodies are anti-GAD 65, anti-islet cell, anti-insulin and anti-tyrosine phosphatase (IA-2) antibodies.

Numerous family studies have demonstrated the predictive value of ICA in populations at increased risk of developing type-1 diabetes.

INDICATIONS FOR MEASUREMENT

The main reason for measuring these auto-antibodies is for screening of prediabetic type-1 states. Such screening combines the detection of immune markers (anti-GAD, anti-islet, anti-IA2, anti-insulin) with that of genetic markers (measures of the degree of homology of HLA DR Q α and β in affected family members) and the measurement of beta cell insulin-secreting capacity in response to hyperglycaemia induced by IV glucose infusion. The individuals who constitute the relevant population for screening are:

- **Subjects at high risk of Type-1 diabetes (first generation relatives):**

  Predictive markers of the risk of developing type-1 diabetes.

  The possibility of initiating therapy early in the pre-diabetic phase increases the importance of these tests for screening for auto-immune diabetes.

- **Patients with hyperglycaemia or a diabetic condition strongly suggestive of type-1 diabetes (children, adolescents, young adults):**

  To confirm the auto-immune basis of the condition or, in the absence of antibodies, to suggest another cause of the diabetes (MODY, secondary diabetes, mitochondrial DNA associated diabetes).

- **Subjects presenting with hyperglycaemia, not a priori suggestive of type-1 diabetes:**

  Relevance of measurement of auto-antibodies as markers of auto-immune aetiology in:

  – Type-2 diabetes: 10% of subjects have detectable antibodies, suggesting that the diagnosis may be slowly evolving type-1 diabetes (LADA).

  – Women presenting with hyperglycaemia in pregnancy: at risk of developing type-1 diabetes.

  – Subjects with another auto-immune condition such as thyroiditis for example: In these patients the risk of developing auto-immune diabetes is higher than in the general population.

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not necessary.

QUESTIONS FOR THE PATIENT

Is the patient diabetic or closely related to someone with diabetes? Is he/she on insulin?

SAMPLE STORAGE AND TRANSPORT

Storage of serum: + 4° C one week, then freeze at – 20° C.

Transport at + 4° C or in the frozen sate if the sample has already been frozen.

ASSAY METHODS

Indirect immunofluorescence (IIF) on human pancreatic sections from blood group O subjects or on monkey pancreas (only sections of monkey pancreas are available commercially).

NORMAL EXPECTED VALUES

Immunofluorescence results are expressed as titre (the dilution for screening is 1/3) or in JDF units *(Juvenile Diabetes Federation)*, defined by reference to an international standard.

INTERPRETATION

In France the prevalence of ICA in the general population is between 1 and 2%.

In type-1 diabetes the prevalence of these antibodies is high, between 60 and 80% at the time of diagnosis. These antibodies can be detected as long as 5 years before the appearance of clinical symptoms. They disappear when all the islets are destroyed, that is 18 months to 2 years after the start of the diabetes itself *(see table)*.

In subjects with a type-1 diabetic first generation relative, the prevalence of ICA is from 2 to 10%. The positive predictive value (risk of developing type-1 diabetes) of ICA in the

general population is low (2 to 6%). It increases, however, with increasing titre and is inversely proportional to the age of the patient. Thus, Bonifacio et al in 1990 showed that all subjects less than 20 years of age with a first degree diabetic relative and with ICA titre greater than 80 JDF units had developed type-1 diabetes within 7 years of follow-up. However, this test does give a high percentage of false positives (all ICA positive subjects at risk of diabetes do not necessarily develop diabetes). It also has insufficient sensitivity and at risk subjects who are initially negative for ICA can develop the disease. Thus, it is appropriate to combine ICA with measurement of other markers.

During gestational diabetes, at least one of the auto-antibodies (anti-GAD, ICA or anti-IA2) is present in about 18% of cases and the risk of developing type-1 diabetes in the two years following delivery is 30% if the antibodies are positive, compared with 2% if they are negative.

Anti-islet cell antibodies are also sometimes encountered in auto-immune polyendocrinopathy.

Sensitivity and positive predictive value of immune markers for diabetes (percentage), Verge et al. Diabetes 1996 ; 45(7): 926-33.

	Sensitivity	Positive predictive value – 5 years
Anti-GAD	90	52
ICA (>20 JDF units)	74	51
Anti-insulin	76	59
Anti-IA2 (ICA 512 bdc)	64	81
Anti-GAD + anti-insulin	68	68
Anti-GAD + ICA 512 bdc	52	86
Anti-insulin + ICA 512 bdc	54	100
1 antibody	18	15
2 antibodies	28	44
3 antibodies	52	100

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ANTI-KERATIN ANTIBODIES

DEFINITION

These belong to the family of anti-citrullinated peptides antibodies. It is now believed that several auto-antibodies belong to this family. The first, described from 1964 onwards, were the *anti-perinuclear antibodies*. The technique used was crude, difficult and non-standardised, so that very few laboratories used it.

In 1979, antibody markers of the rat oesophageal *stratum corneum* were described by Young and Coll and designated **anti-keratin**. It was recognised later that this term was inappropriate, but it remained in use. During the 1990's immunohistochemical studies revealed that the principal target antigen was filaggrin, which bundles up keratin fibres.

The *citrullinated peptides* or *de-iminated fibrin* are recognised as genuine antigenic targets of immunological action and they maintain local inflammation.

Reagents using a *cyclised citrullinated* peptide or *CCP* are available.

Synonyms: Anti-filaggrin antibody/ anti-filaggrin.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

In rheumatoid arthritis (RA) anti-keratin antibodies appear at a very early stage, often preceding the clinical onset of the disease. They are independent of other markers, such as rheumatoid factors (they are present in 1/3 of RF negative RA). They are easily assayed and are predictive for more aggressive disease.

Specificity for RA is greater than 95% and sensitivity is approximately 30% in the first 6 months of RA and nearly 60% in well-established disease.

In practice, anti-CCP antibody assay is now preferred to that of anti-keratin antibodies as the former is more sensitive.

Currently, testing is very useful in early diagnosis of RA, facilitating optimal management in the early stages of the disease which leads to a more satisfactory long term outcome.

INFORMATION

■ SAMPLE

Serum (Dry tube): A fasting sample is not required.

■ SAMPLE STORAGE AND TRANSPORT

Separate the serum within 12 hours of sampling. Store and transport at + 4° C.

ASSAY METHODS

Immunofluorescence: On sections of the middle third of the rat oesophagus.

Only IgG antibodies are specific for RA. Screening uses a cut-off of 1/10.

Immunofluorescence is read visually in the epithelial "stratum corneum", which is the zone nearest to the lumen of the oesophagus. The appearances must be those of a lamellar fluorescence. If this is absent, specificity is impaired and the report must be returned as "uninterpretable". It is then advisable to proceed to assay for anti-citrullinated peptides antibody.

UNITS AND REFERENCE VALUES

Immunofluorescence: The result is expressed as the reciprocal of the dilution.

The positive threshold is 10. The upper titration limit is 320.

FOR FURTHER INFORMATION

■ *Blood markers of inflammatory arthropathies,* Meyer O., supplement RFL n^{0} 329, January 2001.

■ *Auto-antibodies 2003-4.* CD rom produced by Monier J.C., Auger C., Fabien N.

■ *Auto-antibodies and auto-immune diseases,* Humbel R.L., collection Option/Bio, Ed Elsevier, Paris, 2nd Ed, 1997.

ANTI-LKM ANTIBODIES

DEFINITION

The anti-LKM antibodies are antibodies directed against microsomes of the liver and kidney. There are 3 types:

– Anti-LKM1 associated with type II auto-immune hepatitis (AIH 2)

– Anti-LKM2, previously associated with hepatitis caused by tienilic acid, (a diuretic which is no longer available in France).

– Anti-LKM3, which is found in 10-15% of hepatitis D and in less than 10% of Hepatitis C (the detection of these antibodies in rat tissue is irregular).

This document will only focus on anti-LKM1.

Anti-LKM1 antibodies occur in the serum of 85% of patients with type II auto-immune hepatitis, in 3% to 5% of Hepatitis C cases in hepatitis caused by halothane and in graft versus host disease. The main antigen involved is cytochrome P450 2D6 (CYP2D6). Anti-LKM1 antibodies recognise a linear epitope in AIH 2 and conformational epitopes in Hepatitis C.

Antibodies can be absent in the acute phase and in such circumstances, it is essential to repeat the test two months later. They are usually highly elevated in the cirrhotic phase. With effective treatment, they become negative over 1 to 3 years. When antibodies persist, relapse must be considered likely.

Synonyms: anti-LKM/ anti Liver Kidney Microsomal antibody = anti-endoplasmic reticulum antibodies.

INDICATIONS FOR MEASUREMENT

Type II auto-immune hepatitis is a very rare disease (1 to 2 cases per million). It mainly affects girls (90% of cases) aged between 2 and 15 years and it is frequently associated with other auto-immune conditions, such as diabetes and thyroiditis, etc. It often starts like a severe acute hepatitis developing into cirrhosis in 80% of cases in less than three years. The diagnosis depends on the detection of anti-LKM1 antibodies, frequently in association with anti-hepatic cytosol (LC1) antibodies.

Transaminases are greatly increased and hyper IgG is often found.

As anti-LKM1 antibodies are also found in hepatitis C, it is essential to distinguish the two conditions. Immunosuppressants (prednisolone combined with azathioprine), which are the treatment of choice for AIH 2, can worsen a viral infection. Conversely, alpha interferon, used in Hepatitis C can precipitate deterioration of an auto-immune disease. In fulminant hepatitis, cyclosporin is employed and, if this fails, liver transplant is a last resort.

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not necessary.

QUESTIONS FOR THE PATIENT

Patient's age and Hepatitis C status

SAMPLE STORAGE AND TRANSPORT

Store and transport at +4° C.

ASSAY METHODS

Triple substrate immunofluorescence (kidney, liver, rat stomach):

Kidney: Fluorescence of proximal tubule cytoplasm. Very weak positive results or negativity in the distal tubules.

Liver: Intense fluorescence of cytoplasm of the hepatocytes.

Stomach: Negative.

HEp-2 cells: Negative.

If anti-LKM1 and anti-stomach antibodies both test positive, this suggests a wrong diagnosis of anti-mitochondrial disease.

Immunodiffusion: Confirmatory test. In positive cases, an arc of precipitation is produced with an extract that contains endoplasmic reticulum. This arc should be identical when a known positive serum is employed.

ELISA and immunoblot: Confirmatory test using cytochrome P450 2D6 as antigen.

UNITS AND REFERENCE VALUES

Immunofluorescence: The result is expressed as reciprocal of the dilution.

The threshold is 40. The upper titration limit is 640.

ELISA: The result is expressed in arbitrary units as there is no international standard.

Immunoblot: The result is expressed as negative or positive with reference to an internal standard. "+" Quantification is possible.

FOR FURTHER INFORMATION

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■ Humbel R.L., Auto-antibodies and auto-immune disease, collection Option / Bio, Ed Elsevier, Paris, 2nd Ed, 1997.

■ Johanet C. And Ballot E., *Markers for diseases of the liver and biliary tract: recent developments*, Revue Française des Laboratoires (supplement 361 bis) 3rd Colloque du GEAI *(Groupe d'Etude de l'Auto-Immunité = Group for the Study of Auto-Immunity)*, March 2004.

ANTI-MITOCHONDRIAL ANTIBODIES

DEFINITION

Although 10 different types of anti-mitochondrial antibodies have been described (from M1 to M10), in practice only ***type M2*** antibodies have a significant diagnostic interest and will be discussed here. Of the others, anti-M5 is associated with anti-phospholipids and anti M10 with occasional cases of primary biliary cirrhosis.

Type M2 anti-mitochondrial antibodies are the laboratory marker of choice for ***primary biliary cirrhosis (PBC)***. They are found in 95% of patients with PBC and have excellent specificity (98%). Anti-mitochondrial antibodies develop early in disease and they are present then the GGT should be closely monitored and a liver biopsy considered. These antibodies mostly (95%) bind to protein E2 of the pyruvate dehydrogenase multi-enzyme complex (PDH-E2). Their titre does not correlate with disease progression. PDH-E2 is also the target for CD4+ and CD8+ lymphocytes and it is likely that autoantibodies and T lymphocytes which recognise PDH-E2 contribute to the lesions in PBC. They are present in approximately 1% of cases of hepatitis C although are not anti-PDH.

INDICATIONS FOR MEASUREMENT

Primary biliary cirrhosis is a chronic autoimmune disease characterised by progressive destruction of the small and medium calibre intra-hepatic bile ducts; which is responsible for chronic cholestasis. The disease begins with destructive cholangitis with lymphocytic infiltration around the ducts, followed by obstruction of the ducts. It progresses to fibrosis and then cirrhosis and affected people have a high level of total IgM. In most cases anti-M2 IgG particularly, but also IgM anti-mitochondrial antibodies are found. Anti-nuclear membrane antibodies (particularly anti-gp210 and anti-nucleoporin) and anti "multiple nuclear dots" antibodies may be found on Hep-2 cells in 25 to 50% of cases. These are present in half of the cases of PBC without anti-mitochondrial antibodies. The laboratory profile of the cholestasis syndrome in biliary cirrhosis involves a disproportionately larger rise in serum alkaline phosphatase compared to the other biochemical markers of the syndrome (γGT, bilirubin, cholesterol, etc.).

The disease affects *10 women for every 1 man* and affects people between 40 and 60 years old. It has a prevalence of 50 to 100 cases/million people and may remain silent for more than 10 years, beginning with asthenia and pruritus. In established disease, the picture is dominated by hepatomegaly and jaundice before progressing to portal hypertension with a risk of ascites or gastro-intestinal haemorrhage.

The CREST syndrome, systemic scleroderma, Sjögren syndrome, arthropathy and autoimmune thyroid disorders may be associated with PBC.

Immunosuppressants are relatively ineffective and the specific treatment for the disease is with ursodeoxycholic acid which alters the composition of bile, making it less aggressive to hepatic cells. This drug also appears to act on the immune component of the disease. The only option in some cases particularly if the bilirubin is > 100 µmol/l, is liver transplantation.

INFORMATION

SAMPLE

Serum: A fasting sample is not necessary.

DESIRABLE INFORMATION

Information about any increase in liver enzymes and serological status for viral hepatitis B and C.

SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling.

Store and transport at +4 °C.

ASSAY METHODS

Immunofluorescence on triple substrate (rat kidney/liver/stomach):

Kidney: Cytoplasmic tubular fluorescence, the distal tubules being labelled more than the proximal tubules.

Liver: Weaker, granular hepatocyte fluorescence.

Stomach: Parietal cell fluorescence.

A risk of false positive results occurs if anti-stomach anti-brush border or anti-endoplasmic reticulum antibodies are also present.

A zone effect may occur and titration should be performed if any suspicious appearances are seen.

On HEp-2 cells, a rod cytoplasmic appearance suggests anti-mitochondrial antibodies. This must be confirmed on a triple substrate as in 50% of cases these are not anti-mitochondrial antibodies.

ELISA and immunoblot: Confirmatory methods use the pyruvate dehydrogenase (PDH) complex E2 sub-unit as the antigen.

UNITS AND REFERENCE VALUES

Immunofluorescence: The result is expressed as the reciprocal of the dilution with a threshold of 40. The upper titration limit is 640.

ELISA: The result is expressed in arbitrary units; there is no international standard.

Immunoblot: The result is expressed as negative/positive against an internal standard. "+" type quantification is possible.

FOR FURTHER INFORMATION

- *Les Auto-Anticorps 2003-2004,* Cédérom réalisé par J.C. Monier, C. Auger, N. Fabien.
- *Auto-anticorps et maladies auto-immunes,* R.L. Humbel, collection Option/Bio, Ed Elsevier, Paris, 2nd Ed, 1997.
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ANTI-MULLERIAN HORMONE

DEFINITION

Anti-Mullerian hormone (AMH) or *Mullerian Inhibing Substance* (MIS) is a testicular protein factor responsible for regression of the Mullerian ducts in the male foetus. The Mullerian ducts are the embryonic starting point for the uterus, fallopian tubes and upper part of the vagina.

It is a 140 kDa glycoprotein dimer which is part of the TGFβ *(Transforming Growth Factor* β*)* superfamily.

PATHOPHYSIOLOGY

AMH is secreted from the eighth week of pregnancy by foetal testicular Sertoli cells. Together with testosterone it is responsible for virilising the male foetus, testosterone being responsible for the development of the Wolffian ducts, which become the vas deferens and seminal vesicles. AMH prevents female differentiation of the internal genital tract. AMH acts through a receptor, which has been cloned. Both testicular secretions need to converge in order to produce harmonious virilisation.

After birth, AMH is secreted in large amounts by the testes. The role of postnatal secretion is not known.

In girls, small amounts of AMH are secreted by the granulosa cells.

INDICATIONS FOR MEASUREMENT

The indications for measurement follow from the above section:

1) AMH is an excellent indicator of the presence of Sertoli cells, and its measurement therefore is indicated in:

– Ambiguous genitalia of all types,

– Pseudohermaphroditism,

– True hermaphroditism,

– Cryptorchidism,

– Feminising testes (or androgen insensitivity).

2) In women, AMH concentrations are known to correlate with a number of primary follicles and it is therefore believed to be a marker of usable follicular reserve in Medically Assisted Pregnancy programmes.

3) In ovarian tumours, a high level of AMH, whether or not associated with high levels of inhibin A or B, is an indicator of a granulosa cell tumour. Serial measurements after excision can be used to monitor progression. In recurrent disease, the further rise in AMH occurs earlier than estradiol allowing appropriate measures to be taken promptly.

INFORMATION

SAMPLE

AMH may be measured in serum or EDTA plasma.

The sample may be taken at any time during the day.

QUESTIONS FOR THE PATIENT

Current treatment, particularly testosterone in men?

Date of last period?

Monitoring after surgery?

SAMPLE STORAGE AND TRANSPORT

The sample must be centrifuged promptly and serum or plasma frozen within 4 hours of sampling until the time of assay if this is not performed immediately. Samples should be transported frozen.

ASSAY METHOD

Immunometric assay (sandwich) with enzymatic label.

Results are expressed as ng/ml or pmol/l. The conversion factor is 7.13: 1 ng/ml <-> 7.13 pmol/l.

EXPECTED VALUES

The expected values depend on the method used.

Age-related reference values in children have been published by Lahlou *et al* in J Clin Endocrinol Metab 2004 and are shown in the tables below (DSL France method).

Boys

Age	pmol/l	ng/ml
1 - 30 days	120 - 986	16.8 - 138
1 - 2 months	178 - 1250	25 - 175
3 - 6 months	300 - 1450	42 - 203
7 months - 2 years	150 - 1500	21 - 210
3 - 4 years	120 - 1380	16.8 - 193
5 - 7 years	90 - 1190	12.6 - 167
8 - 9 years	80 - 1010	11.2 - 141
10 - 11 years	50 - 890	7 - 125
12 - 13 years	30 - 750	4.2 - 105
14 - 15 years	20 - 586	2.8 - 82
16 - 18 years	11 - 365	1.5 - 51
19 - 30 years	11 - 84	1.5 - 11.8

Reference values produced by Najiba LAHLOU, Saint Vincent de Paul Hospital, Paris

Girls

Age	pmol/l	ng/ml
1 day - 2 years	1.2 - 52	0.2 - 7.3
3 – 9 years	1 - 23	0.1 - 3.2
10 – 13 years	0.4 - 20	< 0.1 - 2.8
14 – 30 years with periods	3 - 45	0.4 - 6.3

In women with active ovaries, concentrations on the 3rd day of the cycle measured using the Immunotech method are 2.2 to 6.8 ng/ml (16 to 48 pmol/l) and vary little during the cycle.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Concentrations are high at birth in boys and rise further during the first year of life, falling very slightly thereafter until pubertal testosterone secretion begins, which almost entirely suppresses AMH secretion.

AMH is usually detectable in women. Concentrations in blood vary little between the follicular and luteal phases during the menstrual cycle and fall markedly after the menopause.

■ PATHOLOGICAL VARIATIONS

In boys, plasma AMH before puberty is a marker of presence, quality and functioning of testicular tissue.

In a boy without palpable testes:

– If the AMH is normal, then the testes are present. Some teams wait for a year to see whether the testes descend and others intervene surgically from the outset.

– If AMH is undetectable, then no functional tissue is present. After stimulation with recombinant hCG, either testosterone rises and the testes must be explored for surgically, or alternatively the diagnosis is that of persistent Mullerian duct syndrome or "man with uterus" syndrome or, if the testosterone remains low, this indicates anorchidism (surgery is of no benefit).

Man with uterus syndrome

This is also called "Persistent Mullerian duct syndrome" or PMDS and involves the presence of a uterus in a normally virilised person due to defective AMH signalling. At birth the child's karyotype is 46, X,Y, with no ambiguous genitalia. This syndrome may be suggested by cryptorchidism or suspected inguinal hernia. In 50% of cases, AMH is normal or above age-related normal values (so called AMH+ patients) and is due to a mutation in the gene coding for the type II receptor and in 50% of cases the AMH is low or undetectable (AMH – patients: mutation in the gene coding for AMH; approximately 150 people described).

Ambiguous genitalia at birth

– If AMH is normal the testes are present. This is usually due to an androgen resistance syndrome, defective testosterone synthesis or secondary hypogonadism.

– A low AMH suggests testicular tissue degeneration (investigate for an abnormality of enzymes or genes involved in sexual differentiation).

– If the AMH is undetectable, testicular tissue is absent. This is usually seen in female pseudohermaphroditism or congenital adrenal hyperplasia (small virilised girl).

In adult men, AMH is normally very low but is greatly increased in androgen resistance. Testosterone, although secreted, does not suppress AMH.

In male sterility, an undetectable AMH in seminal fluid suggests obstructive azoospermia and a very low value suggests non-obstructive azoospermia without spermatogenesis.

AMH in seminal fluid is a potential marker of spermatogenesis and can be useful in non-obstructive azoospermia, although this measurement is difficult.

In women, a high concentration may indicate androgen insensitivity (feminising testes) or tumour secretion (granulosa cell tumour). Concentrations are also raised in polycystic ovarian syndrome.

As part of Medically Assisted Pregnancy (MAP) programmes:

– AMH concentrations correlate with follicular reserve, which determines the success rate in attempted MAP. Authors have reported a good correlation between serum AMH concentrations, the number of oocytes punctured and the success of the MAP attempts in patients following an MAP ovarian stimulation protocol. Following this work, many studies have subsequently been performed showing that the ovarian response is inadequate if AMH is reduced. AMH is a better marker than FSH, estradiol and inhibin B as it is an independent factor and does not exhibit inter-cycle variations.

– AMH is believed to be the best predictive test of ovarian response. When low, ovarian response will be inadequate. Conversely, the higher it is, the larger number of oocytes will be collected for IVF. Some authors do not believe that it correlates with oocyte quality or with fertilisation rates (for the same number of mature oocytes). Others believe that AMH correlates with live birth rates but is not an independent factor (that it is associated with age and the number of oocytes collected).

– AMH also predicts the risk of ovarian hyperstimulation. According to Nakhuda et al. (Fertil Steril 2006), serum AMH concentrations are raised by a factor of 6 in patients at risk of hyperstimulation compared to normal responders (average 3.6 vs 0.6 ng/ml).

POUR EN SAVOIR PLUS

■ Josso N., Di Clemente N., Gouédard L., *Anti-Mullerian hormone and its receptors*, Mol Cel Endocrinol 2001, 179: 25-32.

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■ Kwee J, Schats R, McDonnell J, Themmen A, de Jong F, Lambalk C. *Evaluation of anti-Müllerian hormone as a test for the prediction of ovarian reserve*. Fertil Steril. 2008 Sep;90(3):737-43.

ANTI-MYELIN ANTIBODIES ANTI-MAG ANTIBODIES

DEFINITION

Anti-myelin antibodies are seen in neuropathies associated with IgM monoclonal gammopathy.

This gammopathy is usually benign (monoclonal gammopathy of unknown significance) but is occasionally malignant (Waldenström's disease). IgM concentrations are usually low, < 5 g/l, and the monoclonal band occasionally requires very sensitive techniques in order to be identified.

Anatomically, demyelination of the peripheral nerves as a result of impaired Schwann cell metabolism results in decreased nerve conduction speed because of a disturbance of saltatory conduction of the nerve impulse.

The demyelination is directly attributable to monoclonal IgM activity against target myelin antigens.

Several antigen targets exist:

– ***MAG*** *(Myelin Associated Glycoprotein)*, although a minor quantitative component (0.1% of peripheral nervous system myelin proteins), it represents the target for more than 75% of anti-myelin antibodies. It is a 100 kDa glycoprotein belonging to the immunoglobulin family and plays a key role in adherence of the myelin sheath to the axon.

– **P0** is a compound making up 60% of myelin proteins although very weakly immunogenic.

– **SGPG** (sulfoglucuronylparagloboside) and **SGLPG** (sulfoglucuronyllactosylparagloboside). are neuronal membrane structures which are specific to the adult peripheral nervous system.

Of the anti-myelin antibodies:

– ***70% are anti-MAG and SGPG / SGLPG*** and recognise the same epitope: glucuronyl-3-sulphate, which is present on NK cells and called HNK1. HNK1 is present on MAG and a few other myelin glycoproteins and on SGPG. MAG binds most strongly.

– ***20 %*** only bind to the ***SGPG / SGLPG*** components.

– ***10 %*** are specific for ***MAG***.

INDICATIONS FOR MEASUREMENT

Anti-myelin antibodies are useful in the diagnosis of sensory-motor neuropathies (polyneuritis) which have a very polymorphic clinic expression with predominantly sensory symptoms.

The onset and progression are chronic and slowly progressive. Distal symmetrical sensory nerve involvement is seen. They particularly affect men over 60 years old, beginning with lower limb paraesthesiae and accompanied by tremor of the hands and ataxia.

Treatment of these neuropathies is relatively disappointing and chloraminophen appears to be the best drug although it is effective in no more than 30% of cases. Plasmapheresis treatment is used as an addition in severe disease. High dose immunoglobulins and interferon α are being studied.

INFORMATION

SAMPLE

Serum or CSF.

ESSENTIAL INFORMATION

The presence of an IgM monoclonal band should be reported.

SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling.

Transport at +4°C.

ASSAY METHODS

Immunofluorescence on monkey sciatic nerve section.

Screening is performed 1/10 with serum and undiluted with CSF; the conjugate must be an anti-IgM. The appearances on examination are of diffuse fluorescence over the entire myelin sheath or a double rosette type ring in the internal and external poles of the myelin sheath. The axon is negative.

If the appearance is difficult to interpret or in order to confirm a screening test, an ***ELISA*** assay is performed for anti-MAG (Myelin associated glycoprotein) antibodies, MAG being the major target antigen for anti-myelin antibodies.

A commercial kit uses MAG isolated from the brain by absorption chromatography as the antigen and monoclonal anti-HNK1 antibody. The serum is diluted 1/1000, and the incubations are performed at +4 °C. The kit is revealed with an anti-IgM conjugate.

Anti-MAG can also be tested by ***Immunoblot***: a beef spinal cord protein extract is electrophoresed on polyacrylamide gel before being transferred. The serum is incubated at +4°C and the test is developed with an anti-IgM conjugate.

If anti-myelin antibodies are positive and anti-MAG are negative, testing for anti-SGPG and anti-SGLPG antibodies is performed by ***immunodetection on thin layer chromatography.***

UNITS AND REFERENCE VALUES

Anti-myelin by Immunofluorescence

The result is expressed as the reciprocal of the dilution with a positivity threshold of 10 per serum and 1 for CSF. The upper titration limit is 320.

Anti-MAG

ELISA: The working range is from 1.000 to 70.000 BTU (Bühlmann Titer Units).

< 1.000 BTU: negative.

> 1.000 and < 15.000 BTU: equivocal area because of cross-reactions between glycolipids and glycoproteins.

> 15.000 BTU: anti-MAG / SGPG / SGLPG antibodies present.

Immunoblot: The result is expressed as negative/positive against an internal standard. "+" type quantification is possible.

FOR FURTHER INFORMATION

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■ *Les Auto-Anticorps* 2003-2004, CD-Rom produced by Monier J.C., Auger C., Fabien N.

ANTI-NEURONE ANTIBODIES

DEFINITION

These are used in the diagnosis of paraneoplastic neurological syndromes (1% of cancers) which are defined as the association of a neurological disorder with cancer, but without invasion or metastases. In more than 60% of cases the neurological disorder precedes the discovery of the cancer by several months or years.

In this syndrome, the tumour cells express antigens specific to the nervous system resulting in an anti-neurone immune response with neuronal destruction.

INDICATIONS FOR MEASUREMENT

There is a preferential association between the antibody found and the underlying tumour which, if it is not known or very small in size, can guide its investigation by PET SCAN.

Antibody-anti	Synonym	IF appearance	Associated syndrome	Associated Cancer
Hu	ANNA-1	neuronal nuclei	sensory neuropathy	small cell lung
Yo	PCA1	Purkinje cell cytoplasm	subacute cerebella syndrome	ovary, breast, uterus
Ri	ANNA-2	neuronal nuclei	opsomyoclonus cerebella-ataxia	Breast, small cell lung
Amphiphysin			Stiff-man syndrome	Breast, small cell lung
CV2			cerebellar syndrome	small cell lung
Tr			cerebellar syndrome	Hodgkin's

The most widely found antibody is anti-Hu antibody (3/4 of cases). Other antibodies exist which are difficult to test for routinely (Ma, Ta, CAR, Zic…).

Early diagnosis and treatment of the tumour occasionally stabilises the neurological syndrome.

INFORMATION

SAMPLE

Serum or CSF: A fasting sample is not required.

SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling.

Store and transport at +4°C.

ASSAY METHODS

Immunofluorescence: On monkey cerebellar sections examined for Purkinje cell cytoplasm or neuronal nuclear labelling.

Anti-nuclear antibodies labelled the nuclei of neurones and interfere in the test.

Immunohistochemistry: On rat brain.

Immunodot: A commercial reagent is available with Hu, Ri, Yo and Amphiphysin antigens. This is reliable and straightforward to use.

Immunoblot: On rat brain homogenate.

UNITS AND REFERENCE VALUES

Immunofluorescence

Screening is performed at 1/50 for serum and 1/10 for CSF. The result is not quantitative although any positive or equivocal result is confirmed by immunodot.

Immunodot/immunoblot

The result is expressed as negative/positive against an internal standard. "+" type quantification is possible..

FOR FURTHER INFORMATION

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ANTI-NEUTROPHIL CYTOPLASMIC ANTIBODIES - ANCA

DEFINITION

Anti-neutrophil cytoplasmic antibodies (ANCA) are autoantibodies against antigens contained in the azurophilic granules of neutrophils. They are diagnostic markers of vasculitis, and have changed the diagnosis and classification of these diseases.

They are also useful in the diagnosis of chronic inflammatory bowel diseases (CIBD) and some autoimmune blood disorders. They are screened by immunofluorescence (IIF) on human neutrophil films fixed with ethanol, which defines 3 types of antibodies depending on the site of the fluorescence: c-ANCA (cytoplasmic fluorescence), p-ANCA (perinuclear fluorescence) and x-ANCA (atypical ANCA).

Synonyms: ANCA *(Anti-neutrophil-cytoplasmic antibodies)*, c-ANCA, p-ANCA, x-ANCA (within the x-ANCA, some have been called NANA for *Nuclear anti-neutrophil antibodies*).

INTRODUCTION

The ANCA are associated with different diseases.

Necrotising systemic vasculitis

This is a heterogeneous group of diseases producing inflammatory damage to the vascular wall. Primary (idiopathic) forms are generally classified depending on the nature and size of vessels affected.

- Large vessels: Horton's disease and Takayasu's disease.

- Medium sized vessels: polyarteritis nodosum, Kawasaki syndrome.

- Small calibre vessels: Wegener's disease, Churg-Strauss syndrome, Microscopic polyangitis, Henoch-Schönlein purpura, essential cryoglobulinaemia, cutaneous leukocytoclastic vasculitis.

Some forms of vasculitis can also occur secondary to infections (hepatitis B virus, streptococci), drugs (penicillin, sulphonamides) or some systemic diseases (systemic lupus erythematosus, rheumatoid polyarthritis, etc).

The ANCA are classically found in vasculitis affecting the medium and small calibre vessels. They are excellent diagnostic markers of Wegener's disease (the appearance produced in IIF is that of a c-ANCA, with anti-PR3 specificity).

They are also seen in the Churg-Strauss syndromes, microscopic polyangiitis and focal necrotising glomerulonephritis or necrotising cresenteric glomerular nephritis (the appearance is usually that of p-ANCA with anti-MPO specificity).

Their involvement in the pathophysiology of these diseases has been well documented: passive transfer in the mouse produces the damage. The anti-PR3 are able to activate neutrophils in human beings and contribute to the development of the lesions.

Chronic inflammatory bowel diseases and autoimmune blood disorders

"Atypical" ANCA are found in ulcerative colitis (UC) and when combined with testing for anti-Saccharomyces cerevisae antibodies (ASCA) help in the differential diagnosis between UC and Crohn's disease (cf. corresponding page).

They are also found in primary sclerosing cholangitis which is sometimes associated with UC.

Infections

ANCA are found transiently in various infections (endocarditis, respiratory infections, malaria, HIV infection).

Drugs and toxins

Cases of vasculitis with ANCA have been reported during treatment with various drugs (hydralazine, propylthiouracyl, D-penicillamine, omeprazole, clozapine, minocycline, etc) and after exposure to silica.

INDICATIONS FOR MEASUREMENT

– ***Necrotising systemic vasculitis:*** ANCA testing is indicated in the presence of signs of glomerulonephritis or intra-alveolar haemorrhage. They are also useful to monitor treatment and to diagnose relapses.

– Differential diagnosis of ***chronic inflammatory bowel diseases*** diseases (UC/ Crohn's Disease) and diagnosis of primary sclerosing ***cholangitis*** (second line test).

– NB: for the laboratory, it is important to know whether the ANCA request is being made in the context of vasculitis, in which case examination for c-ANCA and p-ANCA is of interest with additional measurements of anti-PR-3 and anti-MPO specificity, or in the context of chronic inflammatory bowel disease (CIBD) in which the ANCA being tested for (x-ANCA) require specific methods to be used.

INFORMATION

SAMPLE

Empty tube (serum): The sample should preferably be taken fasting (although fasting is not mandatory).

QUESTIONS FOR THE PATIENT

Are you taking any treatment?

Do you suffer from kidney, lung or bowel problems?

SAMPLE STORAGE AND TRANSPORT

Store and transport serum at +4°C.

ASSAY METHODS

Screening for ANCA is performed at a 1/20 threshold by indirect immunofluorescence (IIF) on a neutrophil film fixed with ethanol. If the result is positive the c-ANCA appearance indicated by diffuse, granular cystoplasmic fluorescence, p-ANCA if it is perinuclear and x-ANCA or "atypical" p-ANCA if it is perinuclear with a thin line. x-ANCA can only be verified after a confirmatory test on a formol-fixed film (in which the fluorescence disappears) and a methanol-fixed film (in which the fluorescence returns in the form of a thin perinuclear line).

Even at low titre, anti-nuclear antibodies interfere producing perinuclear fluorescence or labelling the whole nucleus. This appearance is also seen in the rare cases of antibodies specifically against human neutrophils (GS-ANA)

Appearance	Ethanol	Formol	Methanol
c-ANCA	Cytoplasmic granular	Cytoplasmic granular	Cytoplasmic granular
p-ANCA	peri-nuclear	Cytoplasmic granular	negative
x-ANCA	peri-nuclear	negative	peri-nuclear
Nuclear and GS-ANA*	Nuclear / peri-nuclear	Nuclear / peri-nuclear	Nuclear / peri-nuclear

** Granulocyte specific – ANA*

In 95% of cases the target for c-ANCA is proteinase 3 (PR3). More rarely it is the cap57 antigen (or BPI: *bacterial permeability increasing protein*). In most cases p-ANCA are directed against myeloperoxidase (MPO) or other antigens such as lactoferrin, cathepsin G or elastase. MPO and PR3 specificities are only tested routinely using ELISA, Dot Blot or flow immunofluorimetry on the Bioplex®. Demonstration of other specificities whilst technically possible, has no diagnostic value.

The x-ANCA do not have a clearly identified antigen target and can only be tested by IIF

NORMAL EXPECTED VALUES

The positivity thresholds vary between laboratories between 20 and 40 by IIF.
There is no international standard and the ELISA results are expressed in arbitrary units.

INTERPRETATION

ANALYTICAL INTERFERENCES

- **By IIF:** It is difficult to distinguish typical appearances from the appearances associated with anti-nuclear antibodies or which develop during some infections with hypergammaglobulinaemia or in the presence of circulating immune complexes. Positive results must be confirmed by anti-MPO and PR3 measurement.

  False negatives also occur. When the clinical index of suspicion is high a negative IIF result can be confirmed by ELISA.

POSITIVE ANCA AND AUTOIMMUNE DISEASE

- **Diagnostic utility**

  High titres of c-ANCA are mostly found in Wegener's granulomatosis and usually have anti-PR3 specificity. They are highly specific (97%) and sensitive (81%) markers of this disease. Note however that they may be negative at the beginning of the disease when it is localised and relatively inactive.

Les c-ANCA are also found in approximately 30% of cases of microscopic polyangiitis, 10% of cases of Churg-Strauss syndrome and less than 10% of cases of polyarteritis nodosum. They are not found in Takayasu arteritis.

Les p-ANCA are found in various diseases which may or may not be associated with signs of vasculitis: microscopic polyangiitis, glomerulonephritis, Churg-Strauss syndrome, polyarteritis nodosum, Wegener's disease, systemic lupus erythematosus, rheumatoid polyarthritis.

Les x-ANCA are mostly found in the CIBD (in 50 to 70% of cases of ulcerative colitis and 2 to 20% of cases of Crohn's) and in 40 to 70% of cases of primary sclerosing cholangitis.

MONITORING IN VASCULITIS

The anti-PR3 titre usually correlates with disease activity in Wegener's granulomatosis where the titre falls after treatment is started and becomes negative over a few months. It rises again in relapse either in parallel to the development of clinical signs or before when it provides advance notice of a relapse.

The differential diagnosis between relapse and intercurrent infection in patients treated with immunosuppressants when the ANCA titre rises may require further tests to be performed (C-reactive protein, etc).

ANCA positivity in the main systemic vasculitis *(from LH Noël)*

	ANCA+ anti-PR-3 (%)	ANCA+ anti-MPO (%)	ANCA+, indeterminate (%)	ANCA negative (%)
Wegener's granulomatosis	75	15	5	5
Churg-Strauss syndrome	10	60		30
Microscopic Polyangiitis	45	45	5	5
Polyarteritis nodosum		15		85

FOR FURTHER INFORMATION

- Noël L.H., Les autoanticorps « *anti-cytoplasme des polynucléaires*», ANCA, Biotribune March 2004, n°9: 34-5.
- Rouquette A.M., ANCA: *auto-anticorps anti-MPO, auto-anticorps anti-PR3*, Encycl Med Biol, Elsevier, Paris, 2003.

ANTI-NUCLEAR ANTIBODIES

DEFINITION

The anti-nuclear antibodies are autoantibodies which bind to various cell nucleus constituents (DNA, histones, centromeres, soluble nuclear antigens, etc). They are linked to the antibodies against constituents present in the cytoplasm originating from the nucleus. More than fifty anti-nuclear antibodies have been described although the clinical significance of a large number of these has not been established.

Synonyms: ANA, Anti-nuclear antibodies.

INDICATIONS FOR MEASUREMENT

Testing for these antibodies is the essential diagnostic screening test for non-organ specific autoimmune diseases (or the connective tissue disorders/systemic autoimmune diseases) (Table 1). This test is indicated when more or less specific clinical signs are present (arthralgia, dry mouth and eyes, skin signs etc). Further tests (anti-nuclear antibodies, antibodies against soluble nuclear antigens and double stranded DNA) must be performed to determine the specificity found in Indirect Immunofluorescence (IIF) screening in order to provide a more accurate guide to the diagnosis if any positive ANA result is found (fig 1).

Table I: *Clinical association of the main ANA*

Auto-antibodies	Associated disease (sensitivity %)
Anti-ds DNA	Systemic lupus erythematosus (80%)
Anti-RNP	SHARP syndrome (100%) DLE (25%)
Anti-Sm	DLE (10%)
Anti-SSA	Sjogren syndrome (50%), DLE (30%)
Anti-SSB	Sjogren syndrome (80%), DLE (5%)
Anti-PCNA	DLE (3%)
Anti-Scl70	Diffuse scleroderma
Anti-centromere	CREST syndrome (80%)
Anti-Jo1	Polydermatomyositis
Anti-Pm/Scl	Scleroderma, polymyositis

In addition to the connective tissue diseases the ANA may be found occasionally at high titre in many other pathological and physiological situations:

- Organ-specific autoimmune diseases (autoimmune hepatitis, thyroiditis, etc.)
- Viral infections
- Cancers
- Various disorders (vasculitites, sarcoidosis, renal insufficiency)
- Drug-induced (beta blockers, isoniazide, interferon, minocycline, chlorpromazine, procainamide, etc.)
- Age > 60 years old (predominantly women).

Interpretation of a positive anti-nuclear antibody titre therefore requires very close clinical-laboratory correlation taking account of the results of further tests, clinical signs and the patient's age.

INFORMATION

SAMPLE

Serum (Empty tube): A fasting sample is not required.

ESSENTIAL CLINICAL INFORMATION

Report any medical drugs being taken.

SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling.

Store and transport at 4°C.

ASSAY METHODS

Antinuclear antibodies are tested by indirect immunofluorescence (IIF) on HEP-2 or HEP-2000 cells and results are expressed as the reciprocal of the dilution. Any positive ANA test (≥1/80) is followed by quantitative testing for anti-ds DNA and testing for antibodies against soluble nuclear antigens (ENA). If the second test is negative, anti-histone antibodies may be measured.

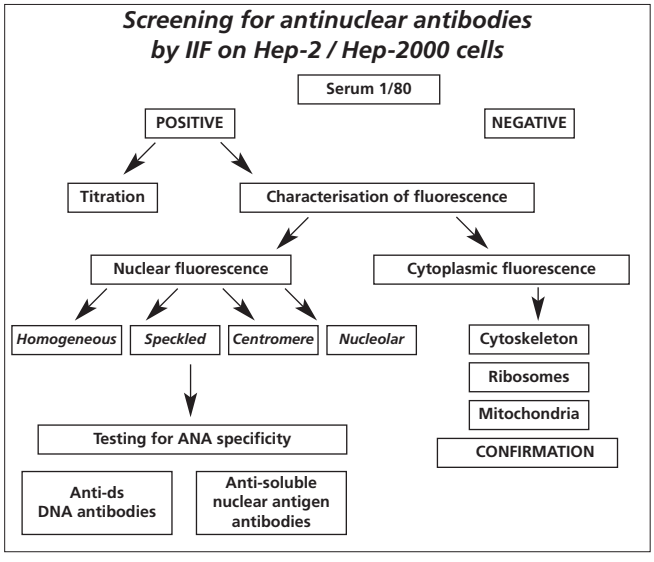
The main nuclear fluorescence patterns on HEp-2 or HEp-2000 are homogeneous, speckled, membranous, centromere and nucleolar. These different appearances provide a guide as to the nature of the target auto-antigen (Table II), although are not sufficient to make a diagnosis as several antinuclear antibodies with different specificities may be present at the same time in the same serum.

Cytoplasmic fluorescence patterns (suspected antimitochondrial, actin, ribosomal, Jo1, synthetase antibodies etc.) are confirmed on a triple substrate (liver, kidney, stomach) or using an appropriate immunoenzymatic method.

Table II: *Main antibodies suggested by their appearance on HEp-2 cells.*

Fluorescence appearance	Suggested specificity
Homogeneous	Anti-ds DNA, anti-histone, anti-nucleosome
Speckled	RNP, Sm, SSA, SSB, Scl70
Nucleolar	Anti-Pm/Scl,

Figure 1: *Diagnostic testing approach to ANA*



UNITS AND REFERENCE VALUES

Immunofluorescence: The results are expressed as the reciprocal of the dilution. The screening dilution is 1/80 or 1/100 on HEp-2 cells; they are not generally titred beyond 1/1280.

FOR FURTHER INFORMATION

■ ROUQUETTE AM. *Les anticorps anti-nucléaires*. In : MEYER O., ROUQUETTE A.M., YOUINOU P. Auto-anticorps marqueurs de maladies auto-immunes. Paris : BMD, 1999 : 71-91.

ANTI-NUCLEOSOME ANTIBODIES

DEFINITION

The nucleosome, which is the basic subunit of chromatin, consists of 4 pairs of histones H2A, H2B, H3 and H4, around which a double strand (native) DNA of mean length 146 base pairs is rolled, closed by histone H1. Within the chromatin the nucleosomes are bound together like pearls in a necklace by a double-stranded DNA fragment.

The nucleosome is generated during apoptosis and leads to cleavage of the chromatin into nucleosome mono- or oligomers. During this process, autoantibodies may be produced. These form a very large family of autoantibodies including:

– Antibodies against conformational determinants of the nucleosome and not recognising either DNA or purified histones: restricted antinucleosome antibodies

– Antibodies against the DNA part of the nucleosome

– Antibodies against the nucleosome histones.

INTRODUCTION AND SEARCH INDICATIONS

Several studies are now available on the clinical significance of anti-nucleosome antibodies measured using readily available commercial kits. Antinucleosome IgG are found in 56 to 85% of patients suffering from systemic lupus erythematosus and even more in the active phase (100%). They are not however, absolutely specific as they are found in other connective tissue diseases including scleroderma (5 to 46% of cases) and mixed connective tissue disorder (20 to 45% of cases), two disorders which are not associated with anti-ds DNA antibodies. Anti-nucleosome IgG appear to be good markers of progression and a good prognostic indicator. A significant correlation exists between these antibodies and with progressive lupus and with glomerular involvement and psychiatric disorders.

INFORMATION

SAMPLE

Serum: A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Report any autoimmune or other known diseases.

SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling. Transport at + 4°C.

DETECTION METHODS

Several commercial ELISA kits are available. The nucleosomes used as antigens are obtained from chromatin extracted from mammal cell nuclei or synthetically in vitro from DNA and histones.

REFERENCE VALUES

These depend on the ELISA kit used.

FOR FURTHER INFORMATION

■ *Les anticorps anti-nucléosomes.* GEAI 2000 Congress. Auto-anticorps Actualité. Spectra Biologie. June 2000.

ANTI-OVARIAN ANTIBODIES

DEFINITION

Anti-ovarian antibodies are circulating autoantibodies against various ovarian structures revealed by indirect immunofluorescence and involved in human disease *(cf. table)*.

Structure labelled	Target cells	Pathology
Theca	Steroid cells	Ovarian failure
Granulosa	Progesterone cells	Endometriosis
Zona pellucida	Sulphated glycoproteins	Female subfertility

INTRODUCTION

Organ-specific autoimmunity frequently affects the endocrine glands and 5 to 7% of the population are believed to have autoimmune endocrinopathy. The immunopathological mechanism is generally of direct effect on the hormone secreting cells (as applies to the anti-ovarian antibodies), although more rarely involves neutralisation of the hormone by an anti-hormone antibody.

Several endocrine glands can be affected concomitantly in the same patient, producing a picture of autoimmune polyendocrinopathy.

Anti-ovarian antibodies have been described as being responsible for gonadal failure (amenorrhoea, sub-fertility) through autoimmune destruction of the ovaries, found in 30% of cases of type-1 polyendocrinopathy, also called Whittaker's or Blizzard's disease or APECED *(Auto-immune Polyendocrinopathy, Candidiasis, Ectodermal Dystrophy)*. This autosomal recessive disorder occurs after the age of 15 years old. Its major clinical signs are adrenal failure, hypoparathyroidism and muco-cutaneous candidiasis, occasionally associated with pernicious anaemia, vitiligo, chronic active hepatitis, type-1 diabetes or thyroiditis. The diagnosis is made when at least two of these disorders co-exist and is confirmed by finding AIRE *(auto immune regulator)* gene mutations. Anti-steroid cell antibodies found in this disease bind to the adrenal cortex and steroid-producing cells in the gonads.

Anti-ovarian antibodies are also described in type-2 polyendocrinopathy or Schmidt's disease (in approximately 10% of cases), which is characterised by autoimmune dysthyroidism, adrenal failure and/or type-1 diabetes.

Several types of autoantibodies are found in these complex diseases, directed in particular against steroid biosynthesis enzymes in the cytochrome P450 system including 21-hydroxylase (anti-P450 C21), 17-alpha-hydroxylase (anti-P450 C17) or 20-22 desmolase (anti-P450 SCC). Anti-P450 C17 and anti-P450 SCC antibodies bind to the adrenal cortex, ovarian follicular internal theca cells, testicular Leydig cells and to placental syncytiotrophoblast cells.

Anti-ovarian antibodies are also found in some cases of female subfertility when an immunological disorder is thought to be present and in this case their target antigens are the ova or different ovarian zona pellucida glycoproteins. This area surrounding the ovocyte consists of several sulphated proteins called 2P, 2P2 and 2P3, the latter (and the most abundant), acting as a receptor for the spermatozoa. These antibodies are also believed to block the adhesion and penetration of spermatozoa through the zona pellucida.

Finally, anti-ovarian antibodies are found in endometriosis, in which case they are directed against the progesterone secreting ovarian follicular granulosa layer cells. They are also believed to be responsible for repeated failure of medically assisted fertilisation in these patients. Injection of gonadotrophins causes ovarian hyperstimulation and repeated handling of the ovocyte during *in vivo* fertilisation tests generate microtrauma explaining the exposure of proteins from the internal ovarian layers, giving rise to an autoimmune response.

SEARCH INDICATIONS

Diagnostic investigation of early ovarian failure, whether or not associated with type-1 (juvenile) or 2 (adult) polyendocrinopathy, female subfertility or endometriosis, particularly following repeated failure of *in vivo* fertilisation.

INFORMATION

SAMPLE

Serum (Empty tube): A fasting sample is not required.

SAMPLE STORAGE AND TRANSPORT

Storage of serum: One week at +4°C then freeze at –20°C.

Transport at +4°C if sample can be analysed within 1 week or send sample frozen.

ASSAY METHODS

Indirect immunofluorescence (IIF) on primate ovarian sections.

REFERENCE VALUES

These autoantibodies are normally undetectable in blood. Positivity thresholds can vary between laboratories.

INTERPRETATION

These autoantibodies are circulating markers of an immunological disorder and can be detected before hormonal abnormalities or clinical signs develop.

In gonadal failure

Anti-ovarian antibodies have been implicated in some forms of early ovarian failure (before the age of 40 years old) characterised by functional ovarian deficiency with amenorrhoea associated with raised serum gonadotrophin concentrations causing subfertility. This type of premature ovarian failure is found in 10 to 40% of patients with autoimmune adrenal failure and anti-steroid cell antibodies (anti-P450 C21, anti-P450 C17 or anti-P450 SCC). It sometimes forms part of a type-1 or 2 polyendocrinopathy in which case it is often associated with autoimmune dysthyroidism (Hashimoto's thyroiditis or Graves' disease).

Anti-P450 C17 and anti-P450 SCC antibodies bind to the adrenal cortex, ovarian follicular internal theca cells, testicular Leydig cells and to placental syncytiotrophoblast cells and are found in 5 to 9% of cases of isolated Addison's disease, in approximately 50% of cases of type-1 polyendocrinopathy and in approximately 35% of cases of type-2 polyendocrinopathy.

In endometriosis and/or immunological female sub-fertility.

The presence of anti-ovarian autoantibodies must be interpreted depending on clinical and laboratory findings.

FOR FURTHER INFORMATION

■ Humbel RL. *Endocrinopathies auto-immunes*. In : Auto-anticorps et maladies auto-immunes. Collection Option/Bio, Ed Elsevier, Paris, 2nd Ed, 1997 :180-207.

■ Fabien N. *Endocrinopathies auto-immunes*. Biotribune March 2004 n°9 :36-8.

ANTI-PARATHYROID ANTIBODIES

DEFINITION

The first anti-parathyroid antibodies described were autoantibodies against the principal parathyroid cells which secrete parathormone (PTH). They are identified by indirect immunofluorescence on human or primate parathyroid sections and are found particularly in the serum of patients suffering from idiopathic hypoparathyroidism.

More recently, antibodies against the extracellular part of the calcium-sensitive receptors have been described on the surface of parathyroid cells. These antibodies called anti-CaSR *(calcium sensing receptor)* are responsible for the hypoparathyroidism seen in most cases of type-1 and occasionally type-2 polyendocrinopathies. They are also found in various situations of hypo- or hypercalcaemia occurring as a result of auto-immunisation against the parathyroid. The antibodies are not however specific for the parathyroid (they exist and are functional on other cell types).

INTRODUCTION

The anti-parathyroid principal cell antibodies may cause destruction of the gland giving rise to hypoparathyroidism.

The calcium ion sensitive receptors present on the surface of parathyroid cells send a signal which allows enables the gland to control the production of parathyroid hormone (PTH) depending on circulating ionised calcium concentrations.

The anti-CaSR receptors can:

– Have an agonist effect on the receptors, whereby they activate the target receptors and behave like calcium, leading to inhibition of PTH production and therefore hypocalcaemia. These antibodies are found in clinical situations of idiopathic hypoparathyroidism, more commonly in young children and women. The symptoms are a combination of neuromuscular signs and signs of hypocalcaemia with tetany. The hypocalcaemia is associated with hyperphosphataemia.

– Obstruct access of calcium to its receptors, whereby the calcium signal is no longer communicated to parathyroid cells the synthesis of PTH is increased resulting in hypercalcaemia.

– These antibodies have also been described in two families suffering from a hypocalciuric hypocalcaemia syndrome which is different from primary hyperparathyroidism (circulating PTH concentrations are normal) and from familial hypocalciuric hypocalcaemia (absence mutation in the gene coding for the extracellular part of the calcium sensitive receptor, giving rise to functional inability of the receptor to bind calcium). In these patients who are predisposed to autoimmunisation, anti-CaSR antibodies obstruct calcium access to the receptor causing poor negative feedback control of calcium on PTH secretion. Serum calcium is then no longer appropriately regulated by PTH and increases.

INDICATIONS FOR MEASUREMENT

Testing for these autoantibodies is indicated in the second line diagnostic investigation of hypo- or hypercalcaemia and in some cases of hypoparathyroidism, particularly in the context of type-1 or type-2 polyendocrinopathy.

INFORMATION

SAMPLE

Serum (Empty tube): A fasting sample is not required.

SAMPLE STORAGE AND TRANSPORT

Storage of serum: One week at + 4°C; then freeze at–20°C.

Transport the serum at + 4°C if the sample can get to the reference laboratory within 5 days or send the sample in its frozen state if it is already frozen.

ASSAY METHODS

Anti-parathyroid Antibodies: Indirect immunofluorescence (IIF) on primate parathyroid or human parathyroid adenoma sections.

Anti-CaSR Antibodies: Radio-immunoprecipitation or immunotransfer, the latter technique being more sensitive.

NORMAL EXPECTED VALUES

These autoantibodies are normally undetectable in blood.

INTERPRETATION

Anti-parathyroid autoantibodies revealed by IIF on parathyroid sections and directed against the main parathyroid cells are found in approximately 40% of cases of idiopathic hypoparathyroidism. Note that these antibodies must be distinguished from antimitochondrial M2 antibodies which label all parathyroid cells (principal cells and oxyphilic cells) and produce granular cytoplasmic fluorescence whereas the anti-parathyroid antibodies produce homogeneous fluorescence only on the principal cells. An anti-parathyroid antibodies result must not be reported without also testing jointly for antimitochondrial M2 antibodies.

The anti-parathyroid antibodies found in type-1 or type-2 polyendocrinopathies appear to be principally anti-CaSR. Type-1 polyendocrinopathy (PEA 1), also called Whittaker's disease or Blizzard's disease or alternatively APECED *(Auto-immune Polyendocrinopathy, Candidiasis, Ectodermal Dystrophy)* is an autosomal recessive disorder occurring before the age of 15 years old. Its major clinical signs are adrenal failure, hypoparathyroidism and a muco-cutaneous candidiasis occasionally associated with pernicious anaemia, vitiligo, chronic active hepatitis, type-1 diabetes or thyroiditis. The diagnosis is made when at least two of these disorders are present and are confirmed by identifying AIRE gene *(auto-immune regulator)* mutations. Type-2 polyendocrinopathy (PEA 2) or Schmidt's disease is characterised by autoimmune dysthyroidism, adrenal failure and/or type-1 diabetes. It is more rarely associated with hypoparathyroidism. Several types of antibodies are found in these complex diseases, particularly anti-steroid cell antibodies (anti-21 alpha hydroxylase, anti-17 alpha hydroxylase, etc.), anti type-1 diabetes antibodies and intrinsic factor antibodies.

The anti-parathyroid antibodies are found in approximately 80% of type-1 PEA, and less often in type-2 PEA. The sensitivity of anti-CaSR for type-1 PEA is reported to be 12.5%.

Anti-CaSR antibodies are found in some hypocalciuric hypercalcaemia syndromes with normal PTH secretion and are also described in hypercalcaemic syndromes with hyperparathyroidism.

FOR FURTHER INFORMATION

■ Humbel RL. *Auto-anticorps et maladies auto-immunes*. Paris : Elsevier Collection Option/Bio, 2nd Ed, 1997:205.

■ Monier JC, Auger C, Fabien N. *Les auto-anticorps 2003-2004*, CD-Rom Pharmacia Diagnostics.

■ Fabien N. *Endocrinopathies auto-immunes*. Biotribune Mars 2004 n°9 :36-8.

■ Monier JC, Fabien N. *Les autoanticorps anti-parathyroïde*. RFL, March 2004, supplement to no. 361, 11-15.

ANTI-PF4/HEPARIN ANTIBODIES

DEFINITION

Anti-PF4/heparin antibodies are IgG, IgA or IgM antibodies directed against platelet factor 4 in the presence of heparin. In a large majority of cases, they are found in the serum of patients treated with heparin (standard or low-molecular weight heparin) who develop a rare but serious complication of the treatment, which is known as "heparin-induced thrombocytopaenia (HIT) type II", previously known as "immuno-allergic thrombopaenia".

Synonym: HPIA test.

INTRODUCTION

HIT is the most serious complication of heparin treatment and occurs in 0.5 to 2% of cases. A result of the immune mechanism, it classically occurs 5 to 21 days after treatment commences, with a peak incidence between the 7th and 10th days. It can, however, occur earlier (before the 5th day, or even within the first 24 hours if the patient has previously received heparin) or later (after the 21st day), particularly in the case of treatment with Low Molecular Weight Heparin (LMWH).

HITis suspected when the absolute number of platelets drops suddenly (below 100 G/l) and/or in cases of a relative reduction in the platelet count of more than 40% of the initial value.

If there is any suspicion of HIT, the heparin treatment must cease immediately, since HIT causes intense platelet activation and an activation of coagulation and the risk is the occurrence of life-threatening venous and/or arterial thrombosis. This means that it is essential not to underrate the diagnosis, nor to exaggerate it, which would deprive the patient of future heparin treatment.

The laboratory diagnosis of HIT is based on two types of test i.e. functional tests and immunoenzymatic tests.

Functional tests on the plasma can reveal the presence of IgG antibodies causing an activation of control platelets. These are platelet aggregation tests (PAT), and tests for the marked release of serotonin (the reference test) which are carried out in highly specialised laboratories.

Immunoenzymatic (ELISA) tests detect IgG's, IgA's or IgM's directed against platelet/heparin factor 4 complexes. These antibodies are detectable in the majority of patients who develop HIT, although a negative test does not exclude the condition. In reality, in fewer than 20% of cases, the antibodies recognise other targets, notably complexes combining heparin with interleukin-8 (IL-8) or with *neutrophil activating peptide-2* (NAP-2).

At the present time there is a consensus for recommending that an immunoenzymatic test and a platelet aggregation test should be combined in evaluating the diagnosis of HIT in a patient, since both these tests have their limits, including the generation of false negatives *(see table)*.

Laboratory diagnosis of heparin-induced thrombopaenia

Nature of the antibodies involved	Platelet aggregation tests	ELISA tests
Anti-PF4/heparin IgG's	+	+
Anti-PF4/heparin IgA's or IgM's	-	+
Anti-NAP-2/heparin IgG's or anti-IL8/heparin IgG's	+	–
Anti-NAP-2/heparin IgM's or IgA's or antiIL8/heparin IgM's or IgA's	-	–

INDICATION FOR MEASUREMENT

Measurement is indicated in a clinical context which suggests HIT *(see above)*.

INFORMATION

■ SAMPLE

Collect in citrate at a concentration of 3.2% (0.109 M) at 1/10 (0.5 ml for 4.5 ml of blood). Tubes citrated at 3.8% (0.129 M) are acceptable.

The sample does not need to be collected with the patient fasting (a light, fat-free snack is permitted). For further information, please refer to the document entitled "General pre-analytical conditions in haemostasis".

If HIT is suspected, the sample must be collected as soon as heparin is stopped, optimally within the first 48 to 72 hours. In practice, the antibodies may disappear in the weeks following the incident. On a longer time scale (a few weeks or months), they may no longer be found, even when HIT is actually present.

■ QUESTIONS FOR FOR THE PATIENT/DOCTOR

Current treatment? Unfractionated heparin or low-molecular weight heparin? Since when? Previous heparin treatment?

Are there any concomitant treatments with other drugs which may produce thrombopaenia (antibiotics, diuretics, antirhumatics, etc.)? Clinical and/or surgical context?

■ SAMPLE STORAGE AND TRANSPORT

2 to 4 hours at room temperature, 1 week at -20° C, -80° C for longer periods.

Rapid thawing in a water bath at 37° C is recommended.

Ensure the sample arrives at the laboratory well within 2 hours as it must be centrifuged, decanted then frozen within a 2 hour period from the point the sample is drawn from the patient.

Transport sample to the reference laboratory in its frozen state.

ASSAY METHODS

ELISA method: The *Heparin Platelet Induced Antibodies* (HPIA) test is the most commonly used and the results are expressed in optical density or OD. An *anti-factor 4 platelet/polyanion* test (HAT) is also available.

REFERENCE VALUES

There are no anti-PF4/heparin antibodies in healthy subjects.

PATHOLOGICAL VARIATIONS

Anti-PF4/heparin antibodies are detectable in a majority (approximately 80%) of patients who develop HIT. The ELISA tests used for detecting them have their limitations, however:

False positives have been described in cardiac surgery in a context of extracorporeal circulation, as well as in various clinical situations (e.g. pregnancy or diabetes) in patients exposed to heparin who present no thrombopaenia and not the slightest functional sign suggesting HIT. The specificity of a positive ELISA test is therefore only high in a clinical context which suggests HIT.

Tests may be negative when HIT is actually present (false negatives). If the target of the antibodies is not the PF4/heparin complex but NAP-2 (Neutrophil Activating Peptide) or IL8 (interleukin-8) (see table) or if the ELISA test may have been performed too soon. It is probable that in the acute stage of HIT, the anti-PF4/heparin antibodies are bound to the antigenic membrane medium and therefore are less available for detection. Because of this, and in a context which suggests HIT, the test can be repeated approximately 3 days later or when the platelet count rises again after heparin administration ceases. The test is positive in approximately 20% of patients, particularly when the initial optical density (OD) in the ELISA test was close to the positive threshold. Nevertheless, some 20% of false negatives remain, even in patients who in fact have HIT.

■ INTERPRETATION OF LABORATORY TESTS

Interpretation should in theory take account of the results of functional and immunoenzymatic tests:
– If both tests are positive, the probability of HIT is high.
– If both tests are negative, the probability of HIT is low. If, however, the clinical probability is high, HIT can not be definitively excluded.
– If only one test is positive: If only the ELISA test is positive, there may be IgM or IgA isotype antibodies which do not activate the platelets in vitro (negative platelet aggregation tests); in a context of cardiac surgery, however, a positive ELISA test is not sufficient on its own to determine a diagnosis of HIT.
HIT is a very particular drug-induced thrombopaenia, with a relatively rapid dynamic of antibody disappearance. Based on ELISA testing, the antibodies reach an undetectable level within an average period of 50 to 85 days.
In overall terms, this diagnosis remains difficult and it is based on considerations which are chronological, clinical and biological (anti-heparin antibodies found after a rigorous search for other causes of thrombopaenia). Clinical-laboratory scoring has also been suggested. A rise in platelet count after heparin treatment is halted is a strong argument in favour of a diagnosis of HIT.
In any event, the results of the laboratory testing must not delay the early treatment of patients.

■ THERAPY FOR HIT

Treatment for HIT is complex and must involve close clinical and laboratory collaboration with the support of a specialised unit (haematology, cardiology, vascular, etc).

A decision to stop heparin administration and replace it with a different antithrombotic must be taken as soon as there is a suspicion of HIT. The substitute treatment should be initiated subject to daily monitoring of the platelet count, which remains the best criteria for a good response to the treatment. Replacement by LMWH is to be prescribed, due to the existence of cross-reactions in almost 100% of cases.

Two therapies have been widely evaluated and are permitted in France when HIT is indicated. These are danaparoid sodium and lepirudin (recombinant hirudin).

Although danaparoid is simple to use, cross-reactions have been detected in vitro and in vivo in approximately 2% of cases. Recombinant hirudin is an interesting alternative, since it retains the advantage of presenting no cross-reactions with heparin.

The reintroduction of heparin in a patient who has had HIT is not absolutely contraindicated. It may be considered by certain experienced teams, subject to the following conditions:
– A highly sensitive test (ELISA) eliminates immunological sensitivity
– This must a long time after the last heparin exposure
– The utilisation of heparin must be of short duration (during extracorporeal circulation)

FOR FURTHER INFORMATION

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ANTI-PHOSPHOLIPID ANTIBODIES

DEFINITION

Anti-phospholipid (aPL) antibodies form a highly heterogeneous family revealed by coagulation tests (see "circulating anticoagulants"), or by ELISA immunological tests. Anti-phospholipid antibodies recognise either isolated phospholipids, both anionic (phosphatidylinositol, phosphatidylserine) and neutral (phosphatidyléthanolamine), or phospholipid/protein cofactor complexes (β_2 glycoprotein I, prothrombin, annexin V, etc.), or cofactors alone. Anti-phospholipid antibodies include anti-cardiolipin antibodies in their composition.

See "Anti-cardiolipin antibodies".

ANTI-PITUITARY ANTIBODIES

DEFINITION

Anti-pituitary antibodies are circulating auto-antibodies directed against various pituitary structures and are demonstrated by indirect immunofluorescence (IIF). They are involved in several human diseases *(see table)*.

Labelled structure	Target cell	Disease
Posterior pituitary	Anti-diuretic hormone	Diabetes insipidus
Anterior pituitary	Prolactin Growth hormone ACTH	Primary sterility Dwarfism Cushing's disease

INTRODUCTION

Organ specific auto-immune disease often affects the endocrine glands and upto 5 to 7% of the general population will present with auto-immune endocrinopathy. The immunopathological mechanism is usually through a direct attack on the hormone secreting cells. More rarely, the hormone is neutralised by an anti-hormone antibody. Anti-pituitary antibodies have been identified as playing an auto-immune role in the pathogenesis of certain conditions of pituitary insufficiency. The antibodies may be directed against various cells, either a single type or multiple types.

INDICATIONS FOR MEASUREMENT

Investigation of the cause in endocrinopathies, such as diabetes insipidus, dwarfism, Cushing's disease or in primary sterility (second line test, or when the patient has an immune disorder).

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not necessary

SAMPLE STORAGE AND TRANSPORT

Storage of serum: One week at + 4° C; then freeze at – 20° C.

Transport at + 4° C or if the sample is already frozen then transport in its frozen state.

AVAILABLE ASSAY METHODS

Indirect immunofluorescence (IIF) on pituitary sections.

NORMAL EXPECTED VALUES

These auto-antibodies are not normally detectable in the blood. The threshold for positivity varies according to the laboratory.

INTERPRETATION

The auto-antibodies are markers of an immune disorder. They are detectable before the appearance of hormonal abnormalities or clinical signs

Anti-pituitary antibodies have been identified as playing a role in the pathogenesis of:

– Certain cases of female primary sterility (anti-prolactin cell antibodies).

– Certain cases of diabetes insipidus (anti-vasopressin cell antibodies).

– Certain cases of Cushing's disease with ACTH deficiency (anti-ACTH cell antibodies).

Their presence is rare and must be interpreted in the clinical and biochemical context

FOR FURTHER INFORMATION

■ Humbel R.L., *Auto-immune endocrinopathies*. In: Auto-antibodies and auto-immune disease (Auto-anticorps et maladies auto-immunes), Collection Option/Bio, Ed Elsevier, Paris, 2nd Ed, 1997: 180-207.

ANTI-SACCHAROMYCES CEREVISIAE ANTIBODIES

DEFINITION

Anti-*Saccharomyces cerevisiae* antibodies (ASCA) have recently been described as a marker for Crohn's disease. The use of the marker is recommended in order to differentiate between Crohn's disease and haemorrhagic rectocolitis.

In the 1980's, research revealed the presence of antibodies directed against food antigens and yeasts. In 1988, *Saccharomyces cerevisiae*, or "brewers' yeast", attracted particular attention and in 1996 peptido-mannan was isolated from its walls; this antigenic target gives the best serological results.

The sensitivity of tests is approximately 60%, while specificity is close to 90%. Various studies have reported positive predictive values of around 96% for ASCA in Crohn's disease. Other workers have shown that combined detection of the two IgG and IgA isotypes of ASCA increased the sensitivity and specificity in diagnosing Crohn's disease. Specificity can be improved to more than 95% by combining ASCA with ANCA, in which case the prescription should specify that the ANCA (anti-neutrophil cytoplasmic antibodies) measurement is for use in diagnosing inflammatory bowel disease (x-ANCA, *cf* corresponding document).

ASCA are also an early marker, appearing before the clinical signs in one third of cases.

Crohn's disease	Sensitivity	Specificity
ASCA positive	61%	88%
ASCA positive with ANCA negative	49%	97%

According to Quinton et al.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

Crohn's disease and haemorrhagic rectocolitis mainly affect young subjects (a peak at 20-30 years of age, with extremes at 5 to more than 60 years) and evolves in bouts.

In Crohn's disease, digestive symptoms are relatively non-specific (diarrhoea -rarely haemorrhagic-, abdominal pain, nausea and vomiting). It is associated with fever and weight loss. Rhumatological, cutaneous, ocular and hepatobiliary manifestations are not rare. Lesions can affect any part of the digestive tract, from mouth to anus. They are segmented and reach the depth of the wall. Histology shows lymphoid nodules and granulomas in one third of cases, but tuberculosis, yersiniosis and Behçet's disease can produce similar lesions.

The prevalence of Crohn's disease is 1/1000 and seems to be rising in industrialised nations. The suspected involvement of certain microorganisms *(Mycobacterium paratuberculosis, listeria, yersinia, etc.)* in the aetiology of the disease remains controversial.

Smoking increases the risk and seriousness of the disease. Family forms frequently occur. Groups HLA DR7, DRB3*0301 and DQ4 are positively associated with the disease. A gene for susceptibility, CARD 15, has been identified. However, the risk of an infected mother transmitting the disease to her child is only 1%.

No treatment cures the disease, although the bouts can be controlled. Use will be made in the first instance of salicylates, corticoids, antibiotics or even artificial nutrition. In severe forms, azathioprine, methotrexate, and now infliximab (anti-TNF), will be introduced. In 80% of patients, surgery will be used at least once during the evolution of the disease.

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not required.

ESSENTIAL INFORMATION

Clinical orientation. Results of ESR or CRP.

SAMPLE STORAGE AND TRANSPORT

Decant the serum within half a day of collection and store at +4° C.

Transport at +4° C.

ASSAY METHODS

Immunofluorescence on smear sample of Saccharomyces cerevisiae.

ELISA on the purified peptido-mannan.

NORMAL EXPECTED VALUES

In immunofluorescence, the usual positive result thresholds are 1/1000 for IgA and 1/1000 for IgG.

For ELISA testing, there are no international units and each kit has its own units and reference values.

FOR FURTHER INFORMATION

■ *Les Auto-Anticorps 2003-2004,* Cédérom réalisé par Monier J.C., Auger C., Fabien N.

■ Humbel R.L., *Auto-anticorps et maladies auto-immunes,* collection OptionBio.

■ Site Internet de l'Association François Aupetit: www.afa.asso.fr

ANTI-SKIN ANTIBODIES

DEFINITION

Anti-skin antibodies are a group of autoantibodies involved in autoimmune bullous dermatoses (AIBD). These antibodies are responsible for the formation of bullae in the epidermis (pemphigus group) or dermo-epidermal junction (pemphigoid group).

Two families of autoantibodies have been identified, depending on their target:

– **Anti-basal membrane antibodies:** Involved in sub-epidermal AIBD, the most common of which is bullous pemphigoid.

– **Anti-intercellular substance antibodies:** Involved in intra-epidermal AIBD or pemphigus.

Their antigen targets are shown in the tables below.

Synonyms: anti-epidermal antibodies, anti-basal membrane antibodies, anti-intercellular substance antibodies (or anti-ICS).

Specificity of anti-intercellular substance autoantibodies in pemphigus

		TARGET PROTEIN	MOLECULAR WEIGHT
PEMPHIGUS VULGARUS	Desmosomes	Desmoglein 3	130
PEMPHIGUS FOLIACAE	Desmosomes	Desmoglein 1	260
PARANEOPLAS TIC PEMPHIGUS	Desmosomes	Desmoplakin 1 Desmoplakin 2 Desmoglein 3 and 1	250 210
IgA PEMPHIGUS	Desmosomes	Desmocollin 1 Desmocollin 2	115 195

Specificity of anti-basal membrane antibodies in pemphigoids

	LOCALISATION	TISSUE	TARGET PROTEIN	MOLECULAR WEIGHT
BULLOUS PEMPHIGOID	Lamina Lucida	Hemidesmosomes	BP 180 BP 230	230 180
SCARRING PEMPHIGOID	Lamina Lucida		BPA180 (LamininеV)	105
BULLOUS EPIDERMOLYSIS	Supra Lamina Densa	Anchoring Filaments	Collagen VII	290
BULLOUS LUPUS ERYTHEMATOSUS	Supra Lamina Densa	Anchoring Filaments	Collagène VII	290
HERPES GESTATIONIS (pemphigoid of pregnancy)	Lamina Lucida	Hemidesmosomes	BP180	180
CHRONIC IgA BULLOUS DERMATOSIS/Type1	Lamina Lucida	Hemidesmosomes		97
CHRONIC IgA BULLOUS DERMATOSIS/Type 2	Lamina Lucida	Hemidesmosomes	LAD1	120

BP Ag : Bullous pemphigoid Antigen

(from Humbel R.L.): Specificity of anti-basal membrane and anti-intercellular substance antibodies.

INTRODUCTION

CLINICAL FEATURES

The suggestive clinical symptoms are chronic, relapsing, lesions involving bullae on healthy or erythematosus skin, which may or may not be associated with mucosal lesions (which are occasionally the first to appear). After excluding all other causes such as drug-induced, infectious, cutaneous porphyria, then the diagnosis of AIBD must be considered.

The intra-epidermal AIBD

Pemphigus:

Different clinical forms are described, the most common being pemphigus vulgaris (or deep pemphigus). This is a rare disease (1.5 to 5/10 million people) particularly affecting middle aged adults (40 to 50 years old) with no sex difference. It usually begins with mucosal erosions (particularly buccal), followed by skin disease which may become generalised.

The bullae are flaccid and break easily, leaving erosions which do not scar.

Pemphigus foliacae or superficial pemphigus is characterised by the absence of mucosal lesions.

Paraneoplastic pemphigus:

This is a rare form of pemphigus associated with various particularly lymphoproliferative and neoplastic disorders.

Sub-epidermal AIBD

Bullous pemphigoid:

Bullous pemphigoid is more common than pemphigus and is a very itchy dermatosis occurring in the elderly. The disease begins with intense diffuse itching associated with eczematiform or urticarial lesions. The bullae are tense and relatively inextensive occurring on erythematous plaques, generally sparing the mucosal membranes.

Treatment is with immunosuppressants (corticosteroid therapy).

Scarring pemphigoid:

Scarring pemphigoid is characterised by selective involvement of the oral, ocular, and genital mucosa, mostly affecting the elderly.

Acquired bullous epidermolysis:

In the classical form the lesions are mechanical bullae affecting rubbing areas on the extremities (elbows, knees, back of hands and feet) with no erythema. The disease may also mimic scarring pemphigoid with bullous or erosive involvement of the oral, nasal and ocular mucosal membranes. Autoantibody testing is an aid to the differential diagnosis.

Dermatitis herpetiformis:

This is very rare in France and affects children over 5 years old and young adults (20-45 years old) and 25% of cases are associated with coeliac disease which is occasionally clinically silent and revealed by the presence of anti-gliadin and anti-endomysial antibodies *(cf corresponding pages)*.

IgA linear bullous dermatosis:

In children this is characterised by bullae on healthy skin. The clinical picture is similar in adult to that of bullous pemphigoid.

■ **Herpes *gestationis* (or gestational pemphigoid):**

This is a particularly rare form of pemphigoid occurring in pregnant women or immediately post-partum (incidence 1/10 000) in the form of vesiculo-bullous lesions. Pruritus which occurs in the peri-umbilical area is a constant finding. The disorder recurs at each new pregnancy and the prognosis for the foetus is good (10% of children will have mild, transient disease).

■ PATHOPHYSIOLOGY

The pathogenic role of the autoantibodies has been clearly demonstrated in the autoimmune bullous diseases. Binding of autoantibodies to their target antigens, results either in loss of cohesion between keratinocytes known as acantholysis (pemphigus group) or dermo-epidermal cleavage (the group of autoimmune bullous diseases of the dermo-epidermal junction, or pemphigoids).

Three arguments reveal the pathogenic role of the autoantibodies in pemphigus:

– The presence of parallelism between circulating antibody titres and disease activity.

– The effectiveness of plasmaphereses.

– Transplacental transmission of neonatal pemphigus in the newborn babies of affected mothers.

SEARCH INDICATIONS

Suspected autoimmune bullous disease, and discrimination between pemphigus and pemphigoids.

INFORMATION

■ SAMPLE

Serum (Empty tube): A fasting sample is not required.

■ QUESTIONS FOR THE PATIENT

Immunosuppressant therapy? Effects of treatment? Corticosteroids?

■ SAMPLE STORAGE AND TRANSPORT

Storage of serum: at +4°C for 1 week then freeze at –20°C.

Transport at +4°C or if already frozen then transport sample in its frozen state.

ASSAY METHODS

Two types of tests are used to diagnose bullous disease:
– Direct immunofluorescence on skin biopsies read and reported by a specialist dermatological Histologist.

– Indirect immunofluorescence (IIF) testing for autoantibodies in serum and possibly if positive, immunoblot or ELISA testing to characterise the autoantibody antigen target.

– Indirect immunofluorescence on monkey oesophagus (other substrates can be used).

– ELISA: Assays for anti-Desmoglein 1, Desmoglein 3 and BP180 (particularly NC16A) antibodies are commercially available.

– Immunoprinting: Performed by a few University Hospitals on dermal or epidermal extracts.

NORMAL EXPECTED VALUES

Immunofluorescence: The result is expressed as the reciprocal of the dilution.

The accepted positivity threshold is 20.

The titre generally correlates with disease activity.

INTERPRETATION

Anti-skin autoantibodies are useful in the diagnosis of autoimmune bullous dermatoses although they are less sensitive than direct immunofluorescence on a skin biopsy. These are also occasionally found in other diseases such as burns or toxiderma.

ANTI-BASAL MEMBRANE ANTIBODIES

– Recognise different dermo-epidermal junction structures in which they produce linear labelling on IIF.

– Are found in 60 to 80% of cases of bullous pemphigoid.

– More detailed analysis of anti-basal membrane specificity can be performed on cleaved human skin, i.e. treated with NaCl 1M (separating the basal membrane between the *lamina densa* and *lamina lucida*). This allows antibodies which bind only to the dermal side characteristic of acquired bullous epidermolysis to be distinguished from those that bind to the epidermal side in bullous and scarring pemphigoids

– In herpes *gestationis* they are found in 20% of cases by the classical IIF method. A three layer method on human skin with anti-complement detects the presence of anti-basal membrane antibodies binding the complement (also called Herpes gestationis factor) with a sensitivity of 90%. It is recommended however that testing is performed for anti-BP180 antibodies which offer greater sensitivity.

ANTI-INTERCELLULAR SUBSTANCE ANTIBODIES

– Produce "bee's nest" labelling by IIF, limited to the epithelium. These are mostly directed against desmosome proteins or integrins.

– They are found in 90% of cases of pemphigus and are generally IgG.

– In IgA bullous dermatosis serum antibodies are rarely found even using anti-IgA antiglobulin (the diagnosis is made by DIF on a skin biopsy).

– Beware of non-specific labelling (false positives): Most of the monkey tissues used for IIF express ABO blood group antigens. The serum may produce "bee's nest", epithelial labelling, falsely suggestive of bullous pemphigoid in blood group O patients with high titres of anti-A and anti-B antibodies. This problem can be resolved by testing positive sera after adsorption onto AB red blood cells or in a solution of soluble AB antigens.

FOR FURTHER INFORMATION

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ANTI-SLA ANTIBODIES

DEFINITION

These were described in 1987 by Manns. Their most likely antigenic targets are cytokeratins 8 and 18, and glutathione-S-transferase. They are wrongly called "soluble liver antigen" whereas in fact they recognise the antigens present in the cytoplasm of other organs, particularly the pancreas (in this case the name is LP for *Liver-Pancreas*). They are highly specific for autoimmune hepatitis.

Synonyms: anti-*Soluble Liver Antigen* antibodies = anti-SLA/LP = anti-*Soluble Liver Antigen/Liver Pancreas* antibodies.

INDICATIONS FOR MEASUREMENT

Some people have proposed calling hepatitis with anti-SLA antibodies, type-3 autoimmune hepatitis. This name is controversial as there is nothing to support it clinically.

Anti-SLA antibodies are present in 10% of cases of type-1 autoimmune hepatitis, associated with anti-actin antibodies and, their key point of interest, in 20% of cases of cryptogenic hepatitis (with no autoantibodies found). These can therefore be reclassified as type-1 autoimmune hepatitis.

Type-1 autoimmune hepatitis is a rare disease (10 to 20 cases/million), mostly of females (80%), affecting people between 10 and 20 or between 45 to 70 years old. It often has a dysimmune background, with thyroiditis, diabetes, or connective tissue disease, etc. Although it rarely starts with severe hepatitic changes and jaundice, it is usually insidious, progressing slowly to cirrhosis. Laboratory findings include raised transaminases (x 5), cholestasis (not always) and polyclonal raised IgG. Treatment is long, with corticosteroids and azathioprine.

INFORMATION

TYPE OF ANTICOAGULANT

Serum (dry tube): A fasting sample is not required.

SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of the sample being taken.

Transport at +4 °C.

ASSAY METHODS

ELISA and immunoblot: These techniques use the purified rat liver cytosolic fraction or recombinant proteins. Whilst ELISA produces a quantitative result the immonoblot has the advantage of being able to be used for individual tests.

EXPRESSION OF RESULTS

ELISA: the result is expressed in arbitrary units. There is no international standard.

Immunoblot: the result is expressed as negative/positive against an internal standard. «+» type quantification is possible.

FOR FURTHER INFORMATION

- *Les Autoanticorps 2003-2004,* CD Rom produced by Monier J.C., Auger C., Fabien N.
- Humbel R.L., *Autoanticorps et maladies autoimmunes,* collection Option/Bio, Ed Elsevier, 2nd Ed, Paris, 1997.
- Revue Française des Laboratoires (supplement 361 bis), 3rd GEAI Congress, March 2004.

ANTI-SMOOTH MUSCLE ANTIBODIES

DEFINITION

Anti-smooth muscle antibodies are the screening test for type-1 autoimmune hepatitis. It is a sensitive test (90%) although in order to be specific the test must identify whether the antibodies are of anti-actin specificity.

Only anti-actin specific anti-smooth muscle antibodies of high titre (>160) are associated with type-1 autoimmune hepatitis. Anti-smooth muscle antibodies of other specificity (often anti-vimentin) are not indicative of any disease and may be seen in different situations, including 20% of viral infections (HCV, HBV, HIV, EBV, CMV, etc) or in drug-induced hepatitis.

INDICATIONS FOR MEASUREMENT

Type-1 autoimmune hepatitis is a rare disease, mostly of women (80%) which affects people either between the ages of 10 and 20 years old or 45 to 70 years old. There is commonly a dysimmune background with thyroiditis, diabetes, connective tissue disease etc. At first stage, it is not very symptomatic, but is usually insidious with a slow progression towards cirrhosis.

Laboratory findings include raised transaminases (x5), cholestasis (not invariably), raised polyclonal IgG and anti-smooth muscle antibodies, with anti-actin specificity. Homogeneous anti-nuclear antibodies without anti-native DNA are found in 40% of cases and led to this form of the disease being called lupoid hepatitis. Anti-SLA (soluble liver antigen) antibodies are reported in 5 to 10% of cases and are highly specific.

Treatment is long and involves corticosteroids and azathioprine.

INFORMATION

SAMPLE

Serum: A fasting sample is not required.

DESIRABLE INFORMATION

Information about any increase in liver enzymes and serological status for viral hepatitis B and C.

SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling.

Store and transport at +4 °C.

ASSAY METHODS

Immunofluorescence in triple substrate (rat liver/kidney/stomach):

Kidney: Blood vessel fluorescence, needles present with anti-actin Ab.

Liver: Reticulated fluorescence for anti-actin antibodies. If not, negative.

Stomach: fluorescence of the muscularis mucosae, musculosa and blood vessels.

UNITS AND REFERENCE VALUES

Immunofluorescence: The result is expressed as the reciprocal of the dilution.

The threshold is 80, a result of 40 being of relatively limited significance. The upper titration limit is 640.

FOR FURTHER INFORMATION

- *Les Auto-Anticorps 2003-2004,* Cédérom réalisé par J.C. Monier, C. Auger, N. Fabien.
- *Auto-anticorps et maladies auto-immunes,* R.L. Humbel, collection Option/Bio, Ed Elsevier, Paris, 2nd Ed, 1997.
- Revue Française des Laboratoires (supplement 361 bis) 3rd GEAI Congress, March 2004. *Acquisitions récentes dans les marqueurs des maladies du foie et des voies biliaires.* C. Johanet, E. Ballot.

ANTI-STOMACH ANTIBODIES

DEFINITION

Gastric parietal cells secrete hydrochloric acid and intrinsic factor into the stomach, thus participating in the digestion of food and the absorption of vitamin B12. Anti-stomach antibodies are markers for gastritis and pernicious anaemia. They are also found in a population with a dysimmune diathesis (Graves's disease, Hashimoto's thyroiditis, diabetes, vitiligo, Sjögren's syndrome, lupus, etc). The antigenic target for these antibodies is H+/K+ ATPase (proton pump), an enzyme causing hydrochloric acid to be secreted by exchange with K+ ions.

Synonyms: anti-gastric parietal cell antibody, anti-H+/K+ ATPase antibody and PCA.

INDICATIONS FOR MEASUREMENT

Gastritis: A common condition with various clinical manifestations, gastritis can be asymptomatic. It can also produce dyspepsia and air swallowing, or pains such as burning sensations.

It can be caused by gastric reflux, damage by toxins (e.g. alcohol) or drugs (such as aspirin and anti-inflammatory compounds), an autoimmune mechanism or a bacterial infection. A bacterial cause is common and involves Helicobacter pylori, which can also induce autoimmunisation.

Pernicious anaemia: This is an evolved form of gastritis with atrophy of the cells in the fundus which secrete intrinsic factor.

The prevalence of anti-stomach antibodies correlates with the degree of damage:

– 15% in the superficial form.

– More than 50% in the deep form.

– Over 90% in pernicious anaemia.

– 30% in dysimmune patients.

The follow-on effects of the reduction in gastric acid in pernicious anaemia is a rise in gastrin, which increases tenfold (it is multiplied by 1000 in Zollinger-Ellison syndrome).

INFORMATION

■ SAMPLE COLLECTION

Serum (Dry tube): A fasting sample is not necessary.

■ ESSENTIAL INFORMATION

Knowledge of gastritis or anaemia (macrocytic or iron deficient).

■ SAMPLE STORAGE AND TRANSPORT

Decant the serum with half a day of sample collection and store at +4° C. Transport at +4° C.

ASSAY METHODS

Immunofluorescence on triple substrate (rat kidney/liver/stomach):

Kidney: Negative.

Liver: Negative.

Stomach: Fluorescence of parietal cells, negative result from principal cells.

NB: Aspects exist which may be difficult to interpret, such as:

– The presence of heterophilic antibodies: Marking is observed of parietal cells and brush edges of proximal tubules of the kidney. This aspect, present in 10% of the population, is not associated with pathology.

– The presence of anti-mitochondrial antibodies: marking is observed of parietal cells and distal tubules of the kidney.

ELISA or immunodot: Confirmation methods using H+/K+ ATPase (proton pump) as antigen.

UNITS AND REFERENCE VALUES

Immunofluorescence: the result is expressed as the reciprocal of dilution.

The threshold is 40.

The upper titration limit is 640.

FOR FURTHER INFORMATION

■ Humbel R.L., Olsson N.O., *Mise en évidence des anticorps anti-cellules pariétales et anti-facteur intrinsèque*, GEAI l'info, n° 7, mai 2005.

■ *Les Auto-Anticorps 2003-2004*. Cédérom réalisé par Monier J.C., Auger C., Fabien N.

■ Humbel R.L., *Auto-anticorps et maladies auto-immunes*, Collection Option/Bio, Ed Elsevier, 2e Ed, Paris, 1997.

ANTI-TESTICULAR ANTIBODIES

DEFINITION

Anti-testicular Antibodies are autoantibodies that act against various testicular structures.

Structure labelled	Target cells	Disease
Seminiferous tubules	Basal membrane	Male subfertility
Germ cells	Spermatocytes	Male subfertility
Sertoli cells		Male subfertility
Leydig cells	Specific steroid cells (testosterone)	Type I or II polyendocrinopathy (women) Testicular atrophy

INTRODUCTION

Organ-specific autoimmunity frequently affects the endocrine organs and 5 to 7% of the general population are believed to have autoimmune endocrinopathy. The immunopathological mechanism is generally a direct attack on the hormone-secreting cells (as applies to the anti-testicular antibodies); more rarely they involve neutralisation of the hormone by an anti-hormone antibody.

Several endocrine organs can be affected concomitantly in the same patient, producing a picture of autoimmune polyendocrinopathy.

– Anti-testicular antibodies have been described as being responsible for hypogonadism causing autoimmune male subfertility. These autoantibodies can be against different testicular targets: the Sertoli cells, seminiferous tubule basal membrane or germ cells.

– Autoantibodies against interstitial cells or Leydig cells have been found in some cases of testicular atrophy due to spermatic cord torsion.

– Autoantibodies against Leydig cells have also been found in women suffering from gonadal failure. These antibodies, called "anti-steroid cells" recognise enzymes involved in steroid biosynthesis in the cytochrome P450 system, including 21-hydroxylase (anti-P450 C21), 17-alpha-hydroxylase (anti-P450 C17) or 20-22 desmolase (anti-P450 SCC). Anti-P450 C17 and anti-P450 SCC antibodies bind to the adrenal cortex, the ovarian follicular internal thecal cells, testicular Leydig cells and placental syncytiotrophoblast cells.

– Autoimmune destruction of the testes may (rarely) occur before or after puberty in type 1 polyendocrinopathy, also called Whittaker or Blizzard's disease or alternatively APECED *(Autoimmune Polyendocrinopathy, Candidiasis, Ectodermal Dystrophy)*. This autosomal recessive disorder occurs before the age of 15 years old. Its major clinical signs are adrenal failure, hypoparathyroidism and muco-cutaneous candidiasis, occasionally associated with pernicious anaemia, vitiligo, chronic active hepatitis, type 1 diabetes or thyroiditis. The diagnosis is made when at least one of the two disorders are present and is confirmed by identifying AIRE gene *(autoimmune regulator)* mutations. Anti-steroid cell antibodies found in this disease bind to the adrenal cortex and to steroid-producing cells in the gonads.

These antibodies are also described in type II polyendocrinopathy or Schmidt's disease (in approximately 10% of cases), characterised by autoimmune dysthyroidism, adrenal failure and/or type 1 diabetes.

INDICATIONS FOR TESTING

Diagnostic investigation of gonadal failure in men (or more rarely in women), giving rise to subfertility whether (or not) in a context of type I (juvenile) or type II (adult) polyendocrinopathy.

INFORMATION

SAMPLE

Serum (Empty tube): A fasting sample is not required.

SAMPLE STORAGE AND TRANSPORT

Serum storage: one week at +4 °C; then freeze at –20°C.

Transport at +4°C.

ASSAY METHODS

Indirect immunofluorescence (IFI) on monkey testis sections.

NORMAL EXPECTED VALUES

These autoantibodies are normally undetectable in blood. Their positivity thresholds vary between laboratories.

INTERPRETATION

Autoantibodies are circulating markers of an immunological disorder. They can be found before the hormonal abnormalities or clinical signs develop.

Anti-testicular antibodies have been implicated in the pathogenesis of some forms of male and female subfertility (anti-Leydig cell antibodies). Anti-steroid cell antibodies (anti-P450 C17 and anti-P450 SCC bind to the adrenal cortex, ovarian follicular internal thecal cells, testicular Leydig cells and to placental syncytiotrophoblast cells) and are found in 5 to 9% of cases of isolated Addison's disease, in approximately 50% of type I polyendocrinopathies and in 35% of type II polyendocrinopathies.

Their presence must be interpreted depending on the clinical and laboratory context.

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ANTI-TETANUS ANTIBODIES

DEFINITION

Anti-tetanus antibodies are antibodies against tetanus toxin, which is responsible for the main symptoms of the disease caused by *Clostridium tetani*. They are called tetanus antibodies, anti-tetanus toxin antibodies, anti-tetanospasmin antibodies or tetanus antitoxin. Tetanus is a notifiable disease in France.

INTRODUCTION

Clostridium tetani is a spore-forming Gram positive obligate anaerobic bacterium. It is naturally soil - borne. Infection can occur in human beings when the skin barrier is broken (gardening, care to the umbilicus at birth in Asia/Africa, and more rarely after a burn or dog bite etc). Protected from oxygen in the wound, the bacterium produces exotoxins facilitating it to become established through local ischemia and tissue necrosis. Tetanospasmin is one of these toxins and is neurotropic, reaching the central nervous system by retrograde transport along neurones. The incubation period of the disease is 3 to 15 days.

The toxin then inhibits alpha motor neurones, blocking the release of inhibitor neuromediators and causing paroxysmal muscle spasms. A dose of 2.5 ng/kg is rapidly fatal in human beings. The initial symptoms are torpor, inability to grasp things, walking difficulties and piloerection. The disease, "tetanus" refers to these spasms becoming permanent and the resultant problems such as trismus (one of the presenting symptoms, spasm of the Masseter muscles preventing the jaw opening) and opisthotonos (generalised spasms, predominantly of the extensor muscles with the result that the body is curved with limbs extended). Complications are often fatal (thrombo-embolic events, cardiovascular problems) and the mortality rate is 25% to 50%. Tetanus infection does not confer immunity as the amount of toxin which spreads in the body is insufficient to cause effective immunisation.

The only way of protecting against the disease is vaccination. This involves a formol-inactivated tetanus toxin called "tetanus anatoxin". Vaccination is in the region of 80 to 100% effective. In the initial vaccination the injection must be repeated three times at regular intervals to obtain protective levels of immunoglobulins. This vaccination is mandatory before the age of 18 months old in France.

INDICATIONS FOR MEASUREMENT

– Assessment of immunity from circulating antibodies against tetanus toxin. Measurement is particularly useful to assess post-vaccination protection in immunodeficient subjects (HIV, diabetes, immunological disease, haematological cancers, etc) and children.

– The epidemiological assessment of vaccine coverage in the population. It is estimated that 40% of people in France presenting at hospital emergency services are inadequately immunised.

– Measurement of anti-tetanus immunoglobulins in an injectable preparation for therapeutic or preventative purposes.

INFORMATION

SAMPLE

Serum (Empty tube): Haemolysis and severe lipaemia invalidate samples as they may interfere with the immunological assay methods.

QUESTIONS FOR THE PATIENT

Date of last tetanus booster (as the combined DTP vaccine). Immunity falls with age. Boosters are recommended every 10 years.

SAMPLE STORAGE AND TRANSPORT CONDITIONS

The serum is stable for 12 hours at +15-25°C, 5 days at +2-8°C and for at least one month between -15 and -20°C.

ASSAY METHODS

The reference technique is an animal experimentation method involving neutralisation of toxin activity by patient serum. The mixture is then injected into mice. Interpretation techniques use time to death, death rate and nature of paralysis. Many factors influence results including the type of toxin used or weight of the mice.

A simple rapid passive haemagglutination test exists. This is more sensitive to IgM immunoglobulins than the neutralisation technique but is less precise for low concentrations of antitoxin.

The assay methods used in laboratories are either radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). ELISA methods correlate well with the neutralisation technique. They are less sensitive for patients with partial vaccination (low antibody avidity). The ELISA techniques used are either "indirect" or "competitive" and produce comparable results.

Fast latex-based or immunochromatographic tests are also available allowing bedside diagnosis.

NORMAL EXPECTED VALUES

– Less than 0.1 KUI/L: no antibodies: full vaccination required.

– From 0.1 to 0.5 KUI/L: low level antibodies: booster vaccination recommended.

– From 0.6 to 2.0 KUI/L: antibodies present: serological retest in 2 years.

– From 2.0 to 5.0 KUI/L: antibodies present: serological retest in 5 years.

– Above 5.0 KUI/L: antibodies present: booster vaccination in 10 years.

PATHOPHYSIOLOGICAL VARIATIONS

There is no effective natural immunisation against tetanus toxin. A few cases of incomplete anti-tetanus immunisation which may be associated with bowel carriage of the bacterium have been reported in developing countries. Apart from these situations, vaccination is the only means of providing protective levels of anti-tetanus antibody.

In the absence of an indication for vaccination, revaccination and treatment with anti-tetanus immunoglobulins are systematically offered in high risk situations such as road traffic accidents.

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ANTI-THYROGLOBULIN ANTIBODIES

DEFINITION

Anti-thyroglobulin antibodies (anti-Tg) are antibodies that act against specific antigenic epitopes on thyroglobulin. They are found in a small number of healthy people and in autoimmune thyroid disease, usually associated with anti-thyroid peroxidase antibodies. They are mostly tested for in patients suffering from differentiated thyroid cancer, the follow up to treatment of which includes thyroglobulin measurement, as the antibodies can interfere with this marker.

Synonyms: anti-Tg. The term anti-thyroid antibodies usually groups together the anti-thyroid peroxidase (anti-TPO) and anti-Tg antibodies.

PATHOPHYSIOLOGY

Thyroglobulin is a glycoprotein synthesised by the thyrocytes and stored in the thyroid follicle colloid. In 80% of cases anti-Tg antibodies are IgG (occasionally IgA or IgM) and are usually polyclonal. Anti-Tg antibodies bind only weakly to complement and are not cytotoxic. They form immune complexes with thyroglobulin although their pathogenic role is still not understood.

INDICATIONS FOR MEASUREMENT

– Validation of thyroglobulin measurements is used to monitor patients suffering from differentiated thyroid cancer after thyroidectomy, and usually following radiotherapy; their measurement is essential for the interpretation of thyroglobulin results and/or for monitoring the efficacy of treatment.

– In suspected or overt thyroid diseases (second line if anti-TPO are negative): in early hypothyroidism (normal or subnormal laboratory function tests) to support the diagnosis of autoimmune thyroid disease or in overt hypothyroidism to confirm the autoimmune origin and possibly start early treatment.

– In non-thyroid autoimmune diseases when treatment carries a risk of reducing tolerance (second line if the anti-TPO are negative) to identify possible concomitant autoimmune thyroid disease.

INFORMATION

SAMPLE

Serum, heparinised plasma or EDTA plasma.

A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Are you taking any of the following treatments? These medicines can result in anti-Tg antibodies being present: amiodarone, lithium carbonate, IL-2, interferon alpha or GM-CSF.

SAMPLE STORAGE AND TRANSPORT

Sample storage and transport: 24 hours at +4 °C; several months at–20°C.

Avoid repeated freeze/thaw cycles.

AVAILABLE ASSAY METHODS

Immunoassay: competitive or "sandwich" assays; enzymatic, luminescent, fluorescent or isotopic markers.

International reference standard: WHO 65/93.

NORMAL EXPECTED VALUES

These antibodies are "normally" absent from serum. Positivity thresholds vary depending on the laboratory. As an indication: Anti-Tg < 40 U/ml.

Prevalence in "healthy" people: 1 to 5% (at low concentrations).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Serum anti-Tg concentrations fall during pregnancy and rise with age.

The presence of these antibodies in serum from "healthy" people may give advance notice of autoimmune disease developing several years later.

PATHOLOGICAL VARIATIONS

In the follow-up of differentiated thyroid cancer after thyroidectomy and usually radiotherapy:

Anti-Tg antibodies are found in the serum of 20 to 40% of patients suffering from thyroid cancer. When present, they interfere with the measurement of thyroglobulin which is used as a treatment follow-up marker in these patients. They usually cause a reduction in the serum concentration of thyroglobulin and are therefore responsible for false negative results to detect relapse of the cancer. In this situation, clinicians use scintigraphy if they are positive. The change in serum antibody concentrations can also be a monitoring factor in these patients: disappearance of thyroid tissue after total surgery +/- radiotherapy could be associated with the antibodies becoming negative. Any rise in their concentration during follow up must be investigated for possible relapse.

■ **In autoimmune thyroid disease:**

– Anti-Tg antibodies are present in approximately 60 to 80% of cases of Hashimoto's thyroiditis and in approximately 30% of cases of Graves' disease. They are less frequent, however, than the anti-TPO antibodies and develop later and their titre is lower. For this reason, anti-Tg measurement is only generally used second line if anti-TPO antibodies are negative (anti-Tg are believed to be present in isolation in approximately 5 to 10% of cases).

– Anti-Tg are not responsible for thyroid diseases in newborn infants.

	Anti-TPO	Anti-Tg	Anti-TSHR
"Healthy" people	3 to 10%	1 to 5%	
Graves' disease	70 to 85%	30%	**80 to 90%**
Hashimoto's thyroiditis	**90 to 98%**	**60 to 80%**	20%
Non-thyroid autoimmune disease	20 to 40%	10 to 40%	

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ANTI-THYROID HORMONE ANTIBODIES

DEFINITION

Anti-T3 and anti-T4 antibodies are immunoglobulins (Ig), usually polyclonal IgG, which bind to tri-iodothyronine (T3) or to thyroxine (T4). They arise in some patients with thyroid disorders, but also de novo. They can be responsible for under or over-estimates of levels of the thyroid hormones, T3 and T4, both total and free. They do not usually disturb the biological activity of the hormones. However, they can cause problems in adjusting hormone substitution therapy in a hypothyroid patient.

Anti-TSH antibodies are directed against thyroid stimulating hormone (TSH), more usually against bovine TSH rather than human. They can be found in euthyroid individuals (very rarely) or with Graves' disease (rarely). The TSH within TSH-anti-TSH antibody complexes might retain some of its biological activity.

Synonyms: Anti-tri-iodothyronine (anti-T3), anti-thyroxine (anti-T4) or anti-thyroid stimulating hormone (anti-TSH) auto-antibodies.

PATHOPHYSIOLOGY

In fact, anti-thyroid hormone auto-antibodies seem to form a sub-population of the anti-thyroglobulin antibodies (anti-TG). In 2/3 cases these auto-antibodies co-exist with anti-TG. The hypothesis, which might explain this association, is that T3 and T4 coupled to thyroglobulin may be recognised by the immune system as foreign antigens.

The production of anti-TSH auto-antibodies might result from deregulation of the idiotype system.

The pathological nature of these auto-antibodies is uncertain. However, it is important to know of their existence, because they can interfere with measurement of T3, T4 and TSH in serum. In addition, anti-T3 and T4 antibodies behave in the serum like additional binding proteins, which can affect thyroid hormone treatment.

INDICATIONS FOR MEASUREMENT

When thyroid hormone measurements do not reflect the clinical picture, anti-T3 or T4 antibodies should be checked. Similarly, they should be requested in hypothyroid patients when the adjustment of the replacement dose is proving difficult and requiring substantial doses.

Anti-TSH antibodies should be looked for in patients with inappropriate serum TSH levels, usually in a context of auto-immune disease. Their presence can be suspected when the dilution test is anomalous.

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not necessary.

QUESTIONS FOR THE PATIENT

Is the patient taking alpha interferon? This drug can induce anti-T3 and/or T4 auto-antibodies.

SAMPLE STORAGE AND TRANSPORT

Storage and transport of serum: at + 4° C over 48 hours.

ASSAY METHODS

Radio-immunological assay.

Incubation with serum, then polyethylene glycol precipitation followed by measurement of the percentage of tracer which is bound.

NORMAL EXPECTED VALUES

These auto-antibodies are not normally detectable in blood. The threshold for positivity differs according to laboratory.

Example:

Anti-T3 < 15% Anti-T4 < 10% Anti-TSH < 18%.

INTERPRETATION

ANTI-T3 AND / OR ANTI-T4 ANTIBODIES

The prevalence of these antibodies in the general population is very low, of the order of 0.1%. In patients with auto-immune thyroid disease, the prevalence lies between 1 and 9%, around 5% in Graves' disease and 9% in Hashimoto's thyroiditis. These auto-antibodies are also found in patients with other auto-immune diseases (7.5%). In general, there is a female predominance (3/4 cases). In around one case in two the antibody is directed against T3, in one case in four against T4 and in one case in four against both hormones.

These auto-antibodies can also be found as a transitory phenomenon in the newborn babies up to the age of 3 months (via placental transfer of maternal auto-antibodies), during post-partum thyroiditis (up to one year after delivery), in patients on alpha interferon (up to a year after cessation of treatment) and in patients who have had fine needle aspiration cytology of the thyroid (up to one year after the procedure).

Anti-T3 and/or anti-T4 auto-antibodies can be responsible for artefacts in thyroid hormone measurement. The error varies according to the technique employed. With methods using a labelled antibody (SPALT methods, *Solid Phase Antigen Linked Technique*), recognition by the auto-antibody of bound ligand, in the solid phase, results in an artefactual increase in measured hormone level. These auto-antibodies do not affect two stage assays.

In hypothyroid patients on l-T4 replacement, when anti-T4 auto-antibodies are present, exogenous thyroxine must saturate the antibody before replacement can become effective. The amounts of hormone taken are, therefore, often substantial and the dose has to be adjusted according to the intra-individual variability of the level of auto-antibody.

ANTI-TSH ANTIBODIES

These are extremely rare, especially in the healthy subject. They are found in about 0.3% of cases of Graves' disease. Their

clinical effects are ill-understood. They do not seem to be associated with a particular form of Graves' disease. They can cause discordance between the results from different assay techniques. This can result in a diagnostic problem in around 1 in 150,000 cases.

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ANTI-THYROID PEROXIDASE ANTIBODIES

DEFINITION

Anti-thyroid peroxidase antibodies (anti-TPO) are autoantibodies against thyroid peroxidase, the major antigen in the microsomal fraction involved in thyroid autoimmune disease. Measurement of anti-TPO is replacing anti-microsomal antibody measurement (they are more specific and the results correlate completely). Anti-TPO antibodies are better markers of thyroid autoimmunity as they always reflect the extent of lymphoid and plasmocyte infiltrate within the thyroid.

Synonyms: anti-TPO, anti-thyroid microsomes (ATM) (old assay). The term anti-thyroid antibodies conventionally groups together anti-TPO and anti-thyroglobulin (anti-Tg) antibodies.

PATHOPHYSIOLOGY

Thyroid peroxidase is a membrane protein located on the apical pole of the thyrocytes. It is a key enzyme in the organification of iodine and, is responsible for the iodination of thyroglobulin and is involved in the synthesis of thyroid hormones. Anti-TPO are polyclonal antibodies, mostly IgG, which bind complement and play a major role in antibody-dependent cellular cytotoxicity. Symptomatically, anti-TPO antibodies are associated with euthyroidism in 50% of cases, subclinical hypothyroidism in 25 to 50% of cases and overt hypothyroidism in 5 to 10% of cases.

INDICATIONS FOR MEASUREMENT

– In suspected or overt thyroid disease: In early hypothyroidism to make the diagnosis of autoimmune thyroid disease or in overt hypothyroidism to confirm autoimmune disease.

– In non-thyroid autoimmune disease or with treatment liable to induce a possible co-existent autoimmune thyroid disease.

INFORMATION

■ SAMPLE

Serum, heparinised plasma or EDTA plasma.

A fasting sample is not required.

■ QUESTIONS FOR THE PATIENT

Are you taking any of the following as these medicines can result in anti-TPO antibodies being present?

Amiodarone, Lithium carbonate, IL-2, alpha interferon and GM-CSF

■ SAMPLE STORAGE AND TRANSPORT

24 hours at +4 °C or several months at –20°C.

Avoid repeated freeze/thaw cycles.

ASSAY METHODS

Immunoassay: Competitive or "sandwich" assays; enzymatic, luminescent, fluorescent or isotopic markers.

International reference standard: WHO 66/387.

NORMAL EXPECTED VALUES

These antibodies are "normally" undetectable in blood. Their positivity threshold varies depending on the laboratory.

As an indication: Anti-TPO < 35 U/ml.

Prevalence in "healthy" people: 10% in women; 3% in men (at low concentrations).

INTERPRETATION

Anti-TPO antibodies are sensitive, specific diagnostic markers of autoimmune thyroid disease:

– Development of anti-TPO is the first abnormality seen in ***Hashimoto's thyroiditis*** even before the development of clinical signs and they are present in 90 to 98% of cases. Anti-thyroglobulin antibodies (anti-Tg) are also usually raised although the increase in serum anti-TPO concentration generally occurs earlier and is larger. During the course of the disease they may rise to extremely high concentrations in serum.

–Anti-TPO antibodies are also found in 70 to 85% of cases of ***Graves' disease*** although anti-TSH receptor antibodies are better to confirm or monitor the course of the disease.

– In early pregnancy, anti-TPO is a predictor of a high risk (approximately 50%) of developing ***post-partum thyroiditis***.

The presence of these antibodies suggests ***thyroid dysfunction during treatment with*** Amiodarone, Lithium Carbonate, IL-2, interferon or GM-CSF.

Other situations: Low serum concentrations of these antibodies are found in non-thyroid autoimmune diseases (systemic lupus erythematosus, type 1 diabetes etc.) and in people with a family history of autoimmune dysthyroidism. They have also been described in chronic hepatitis C, sarcoidosis, breast cancer and in women who have had repeated miscarriages. Anti-TPO are not responsible for transient thyroid disease in newborn babies.

	Anti-TPO	Anti-Tg	Anti-TSHR
"Healthy" people	3 to 10%	1 to 5%	
Graves' Disease	70 to 85%	30%	**80 to 90%**
Hashimoto's thyroiditis	**90 to 98%**	**60 to 80%**	20%
Non -thyroid autoimmune disease	20 to 40%	10 to 40%	

FOR FURTHER INFORMATION

■ Izembart M., *Les anticorps*. In: Bounaud M.P., Duron F., IngrandJ., Izembart M., Piketty M.L., Talbot J.N., L'exploration de la thyroïde, Bioforma Ed, Paris 1999:95-101.

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ANTI-TISSUE ANTIBODIES

DEFINITION

The term "anti-tissue antibodies" is vague and is used to describe antibodies associated with liver diseases. It therefore includes the following antibodies:

– **anti-mitochondrial,**

– **anti-smooth muscle or anti-actin,**

– **anti-endoplasmic reticulum or anti-LKM,**

– **anti-hepatic cytosol or LC1** *(see respective pages).*

These antibodies are tested for by a single immunofluorescence examination using a substrate combination of rat liver, kidney and stomach sections. This test is clearly described in the official French medical laboratory procedures listing.

INDICATIONS FOR MEASUREMENT

Anti-tissue antibodies are an excellent screening test and can be used for the diagnosis of the main autoimmune liver diseases in a single test:

- **Primary biliary cirrhosis or PBC** is a chronic cholestatic liver disease characterised by progressive destruction of small and medium sized intra-hepatic biliary ducts followed by fibrosis leading to severe cirrhosis. Patients have a raised concentration of serum IgM. Anti-M2 anti-mitochondrial antibodies are found in most cases (95%). The disease affects 10 women for every man, between the ages of 30 and 65 years old. It has a prevalence of 50 to 100 case/million population. The disease can be clinically silent for long periods of time and generally has a long course over more than 10 years.
- **Type 1 autoimmune hepatitis:** this affects people between 10 and 20 or between 45 and 70 years old. It often has an insidious onset and progresses slowly towards cirrhosis. The disease is characterised by raised IgG and the presence of anti-actin and anti-smooth muscle antibodies.
- **Type II autoimmune hepatitis:** this is 5 to 6 times rarer than type I and mostly affects girls under 15 years old. It often begins with severe acute hepatitis and progresses rapidly to cirrhosis within two years. The diagnosis is based on the finding of anti-LKM1 and/or anti-LC1 antibodies. Raised IgG is common.

INFORMATION

SAMPLE

Serum: A fasting sample is not required.

DESIRABLE INFORMATION

Information about any increase in liver enzymes and viral hepatitis B and C status.

SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling. Transport sample at +4°C.

ASSAY METHODS

Immunofluorescence on triple substrate (rat kidney/liver/stomach). Antibodies are identified depending on the positive structures by seeing fluorescence at low magnitude (x 20 or x 30 magnification).

Antibodies	kidney	liver	stomach
Anti-mitochondrial	distal > proximal	granular	Parietal cells
Anti-smooth muscle			Muscularis, blood vessels
Anti-LKM	proximal	bright	
Anti-cytosol		bright	

REFERENCE VALUES

Immunofluorescence:

The result is expressed as the reciprocal of the dilution.

The threshold is 80 for anti-smooth muscle antibodies and 40 for others.

The upper titration limit is 640.

Confirmation: refers to the antibody found.

FOR FURTHER INFORMATION

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ANTI-TISSUE TRANSGLUTAMINASE ANTIBODIES

DEFINITION

Transglutaminase (tTG) is the main target antigen recognised by anti-endomysial antibodies. IgA anti-endomysial antibodies are the most specific diagnostic marker for coeliac disease or gluten intolerance and the identification of tTG has improved understanding of the pathophysiological mechanisms of coeliac disease. tTG is a ubiquitous intracellular enzyme which can deaminate glutamine groups into glutamic acid. Gliadin, which is rich in glutamine is a preferred substrate for tTG. The protein complexes created form new antigens provoking firstly the formation of anti-gliadin and anti-tTG IgA antibodies and secondly activation of gliadin specific T lymphocytes leading ultimately to an inflammatory reaction causing destruction of the intestinal mucosal villi.

Synonym: anti-tTG Ab.

INTRODUCTION

Coeliac disease is an intolerance to gluten causing an enteropathy resulting in malabsorption in genetically predisposed people (95% of patients express HLA class II DQ2).

There are two different clinical forms:

– Childhood coeliac disease: This has a fairly typical clinical presentation and usually presents before the age of 2 years old and has a prevalence of approximately 1/2500 in France. Classically the symptoms are a combination of chronic diarrhoea and relatively severe signs of malnutrition. It is associated with constant anorexia and vomiting in 50% of cases. Deterioration in general health is a constant finding. This leads quickly to growth retardation and a falling off of the weight curve.

– In adults, the classical form is only seen in 20% of cases. In all of the other cases the clinical features may only reflect malabsorption and present with low grade non-specific symptoms (bone demineralisation, arthralgia, neurological problems, fertility disorders) which may make it difficult to diagnose. Laboratory findings in this situation may include those of malabsorption (anaemia with iron, folate and vitamin B12 deficiency, deficiency of vitamin K dependent factors, etc.).

The treatment of coeliac disease involves a strict lifelong gluten-free diet. All foods containing wheat, barley or rye must be excluded from the diet. Laboratory and clinical findings improve often spectacularly over a few months on a gluten-free diet. This is the only means of preventing the long term complications of the disease (notably small bowel lymphoma).

The disease is diagnosed by initial serological testing followed by confirmation from histological examination of a small bowel biopsy to confirm the presence and extent of villous atrophy.

INDICATIONS FOR MEASUREMENT

Coeliac disease is associated with antibodies in response in eating gluten. The antibodies which are tested for are anti-transglutaminase IgA and anti-endomysial IgA (± IgG), anti-gliadin IgG and IgA antibodies. Anti-reticulin Ab testing is now no longer recommended.

Anti-tTG antibodies are considered to be the most specific indicators of coeliac disease. They have a sensitivity of 88% to 100% and a specificity of 100%. The disappearance of these antibodies over a few months confirms good compliance with a gluten-free diet.

INFORMATION

SAMPLE

Serum: A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Check that the patient is not already following a gluten-free diet.

SAMPLE STORAGE AND TRANSPORT

Separate serum within half a day of sampling. Transport at +4°C.

ASSAY METHODS

Diagnostically, the identification of tTG has allowed automated ELISA serological tests to be developed which may replace IFI anti-endomysial antibody testing which is occasionally difficult to read and is subjective. The first tests used guinea pig tTG and those which are currently available use human tissue or recombinant tTG.

Immunoblot tests are also available.

REFERENCE VALUES

By ELISA, these are expressed in arbitrary units which vary depending on the kit.

By immunoblot: the result is expressed as negative/positive against an internal threshold. «+» type quantification is possible.

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ANTI-TSH RECEPTOR ANTIBODIES

DEFINITION

Anti-TSH receptor antibodies are IgG autoantibodies directed against the TSH thyroid receptors present on the surface of thyrocytes. After binding to a receptor, they usually behave as stimulating antibodies (they mimic the action of TSH) and are the cause of the hyperthyroidism seen in Graves' disease. In certain cases they have a blocking action and inhibit the biological effect of TSH, result in hypothyroidism, with hypotrophy of the gland. The antibodies, due to their passage through the placenta, may also be responsible for transient dysthyroidism (hypo- or hyperthyroidism) in newborn babies. Various names have been given to these antibodies:

– ***LATS*** *(Long acting thyroid stimulator): this was the original name used following their discovery by MacKenzie*.

– ***TRAK*** *TRAK (TSH-Rezeptor-Antikörper) or* ***TRAC*** *(TSH récepteur anticorps) are the names used in test kits*.

– ***TSAb*** *(thyroid stimulating antibody) or* ***TSI*** *(thyroid stimulating immunoglobulin) only apply to antibodies which have a stimulating effect*.

– ***TBIAb*** *(thyrotropin binding inhibitory antibody) or* ***TBII*** *(thyrotropin binding inhibitory immunoglobulin)* correspond to inhibitory antibodies.

Synonym: Anti-TSHR antibodies.

INDICATIONS FOR MEASUREMENT

GRAVES'DISEASE

Diagnosis

Measuring anti-TSH receptor antibodies is of no value in diagnosing Graves' disease. It may, however, be useful in certain atypical clinical forms such as isolated Graves' opthalmopathy with euthyroidism.

Monitoring and prognosis of Graves' disease

Their concentration is of prognostic interest during therapeutic monitoring of the disease, since it correlates in fact with the probability of relapse after treatment with synthetic antithyroid drugs. Measurement is recommended for diagnosis (aetiology + prognosis) and if the concentration is very high, the measurement should be repeated at intervals of 3 to 6 months and when treatment ceases (generally at 18 months). If the concentration is only slightly elevated, a measurement should be performed at least at the cessation of treatment.

IN PREGNANT WOMEN: SUSPICION OF NEONATAL DYSTHYROIDISM

The interest of measuring the antibodies in the mother lies mainly in assessing the risk of dysthyroidism in the newborn baby. These antibodies pass through the placental barrier and may result in neonatal dysthyroidism, which are usually of a transient nature but necessitate very early diagnosis and treatment. This is why a measurement is useful in the weeks preceding parturition.

Measurement is indicated during the 3rd quarter of pregnancy:

– Mother suffering from Hashimoto's thyroiditis

– Graves' disease discovered during pregnancy

– History of neonatal dysthyroidism in a previous child.

IN CHILDREN OF A MOTHER SUFFERING FROM AN AUTOIMMUNE THYROID PATHOLOGY

This investigation allows a diagnosis of dysthyroidism caused by anti-TSH receptors passing through the placenta.

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not required. Please ensure the sample is not haemolysed.

QUESTIONS FOR THE PATIENT

Current treatment?

Pregnancy?

SAMPLE STORAGE AND TRANSPORT

Separate the serum within half a day of collection and store at +4° C.

Transport at +4° C.

ASSAY METHODS

Radioimmunology

Depending on the nature of the TSH receptor, two techniques are available:

– TRAK assay: Test using the receptor extracted from pig thyroid

– Human TRAK assay: Test using human TSH receptor. The same specificity when compared with TRAK, clinical sensitivity increased by approximately 20% for the diagnosis and therapeutic monitoring of patients with Graves' disease.

NORMAL EXPECTED VALUES

TRAK assay: N < 15 UI/l (zone of doubt from 12 to 15 UI/l)

Human TRAK: N < 1 UI/l (zone of doubt from 1 to 1.5 UI/l). These are indicative values only (the decision threshold can vary).

PATHOLOGICAL VARIATIONS

GRAVES' DISEASE

Diagnosis

Stimulatory anti-RTSH antibodies are detected in 80 to 90% of cases of Graves' disease.

Monitoring and prognosis in Graves' disease

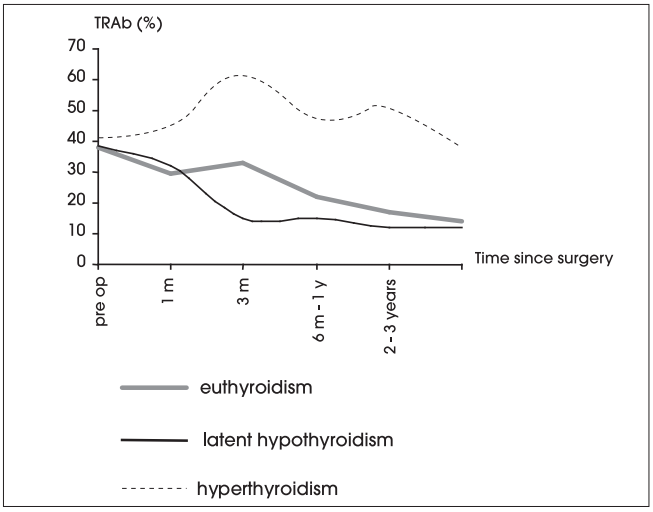
The concentration of anti-TSH receptor antibodies is an important prognostic consideration in patients treated for Graves' disease.

After treatment by synthetic antithyroid drugs, a measurement of anti-TSH receptor antibodies is the principal prognostic criterion after the initial treatment for Graves' disease. An

elevated value is synonymous with a virtually inevitable relapse, with this occurring earlier with increasing concentrations of anti-TSH receptor antibodies.

Anti-TSH receptor antibodies can also be a criterion for ceasing medical treatment if their concentration returns to normal.

After surgery, anti-TSH receptor antibodies also represent a factor for predicting success.



After treatment with radioactive iodine, the antibody concentration is lower in euthyroid patients than those who relapse with hyperthyroidism.

■ HYPOTHYROIDISM

A high concentration of anti-TSH receptor antibodies is found in approximately 20% of patients with Hashimoto's thyroiditis. The hypothyroidism observed in these patients is though not to be due to classical lymphocytic destruction of the gland, but to the inhibitory action of the antibodies on thyroid function.

■ NEONATAL DYSTHYROIDISM

– If anti-TSH receptor antibodies are present in the mother during the 3rd quarter of pregnancy, the newborn baby must be monitored (risk of dysthyroidism).

– In the baby: The TRAK test remains positive for some 50 days after birth (maternal antibodies transmitted to the child), with occasional clinical signs of hyperthyroidism, such as dehydration, tachycardia, hyperthermia, etc.

FOR FURTHER INFORMATION

■ M. Izembart et coll., *L'exploration de la thyroïde*, Le Cahier BIOFORMA N°14 (mai 1999).

ANTI-XA ACTIVITY

DEFINITION

Measurement of anti-XA activity, incorrectly called blood heparin is used for the laboratory monitoring of treatment with heparins and heparin derivatives, such as unfractionated heparin or UFH, low molecular weight heparins or LMWH, danaparoid, pentasaccharide or fondaparinux.

Anti-Xa activity provides a measure of the anticoagulant activity of UFN, LMWH and danaparoid. It can also be used to assess the anti-Xa activity of different anti-thrombotic agents which act on this parameter: indirect anti-thrombin (AT) dependent agents such as fondaparinux or potentially direct agents such as rivaroxaban (test to be adapted).

UFH is the natural sulphate of polysaccharide extracted from beef lung or pork intestine. It is formed from a heterogeneous mixture of molecules of a molecular weight ranging from 3000 to 30000 Daltons. It has a half-life of approximately 90 minutes and it is catabolised by a hepatic heparinase and partially removed in the inactivated form by the kidney, explaining the risk of overdose, mostly in hepatic insufficiency.

The LMWH obtained by enzymatic or chemical fractionation of UFH are formed from a mixture of polysaccharide fragments of a molecular weight between 1000 and 10000 Daltons. They have a half-life in the region of 4 hours.

They have excellent bioavailability (> 90%) and bind little, if at all, to plasma proteins, endothelial cells or macrophages. They are removed by the kidney, hence the risk of accumulation in renal insufficiency. They are contra-indicated in severe renal insufficiency (creatine clearance < 30 ml/min).

The heparins act by accelerating the speed of action of anti-thrombin (AT) to which it binds through a pentasaccharide structure. Binding results in conformational change, of the AT which becomes able to inactivate the enzymes generated during coagulation, which are sensitive to its action (IIa, Xa, etc.), approximately 1000 times faster.

The longer heparin chains inhibit both thrombin and factor Xa (FXa), whereas the shorter chains (< 5,400 Daltons) only inhibit FXa. UFH is mostly formed from chains which inhibit both factor IIa (thrombin) and factor Xa (Xa/IIa ratio = 1), whereas the LMWH contain a variable number of molecules which inhibit thrombin and a larger proportion of chains which only inhibit FXa (Xa/IIa ratio still > 1, ranging from 1.5 to 4 depending on the molecule).

Fondaparinux has a half-life of 17-18 hours and has exclusively anti-Xa activity. It is only removed in the kidney, hence its contra-indication in patients with renal insufficiency.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

The heparins are indicated for use prophylactically and as curative treatment of venous thrombo-embolic disease. They are the anticoagulants of choice when rapid anticoagulation is desired as they have immediate intravenous action. The peak activity is obtained after 3 to 6 hours, following subcutaneous administration.

It is not easily possible to measure circulating concentrations by a chemical method. The only method available is therefore one of their biological activity. The most sensitive measurement is the rate of factor Xa inhibition by the patient's plasma or anti-Xa activity. Anti-Xa activity is measured against a specific calibration line and the results are expressed in IU anti-Xa/ml for UFH, LMWH and danaparoid and in µg/ml for synthetic drugs (fondaparinux and rivaroxaban).

Measurement can be used to adjust doses in order to ensure effective anti-coagulation (sufficient dose) at the same time monitoring for the risk of haemorrhage (overdose).

INDICATIONS FOR ANTI-XA ACTIVITY MEASUREMENT

Prophylactic treatments

Laboratory monitoring of the LMWH is of no use in prophylactic treatment, except in the elderly and/or in patients with renal insufficiency, patients of extreme body mass and pregnant women, in order to prevent the risk of accumulation (risk of haemorrhage).

UFH monitoring is also not recommended except in high risk situations (orthopaedic surgery) when this is usually based on measurement of the Activated Partial Thromboplastin Time (APTT) and not on measurement of anti-Xa activity (recommended APTT 1.2 to 1.3 times the control for a sample taken midway between two injections 8 hours or 12 hours apart). The APTT must be interpreted with caution in view of all the circumstances liable to alter the APTT independently of heparin (increased factor VIII due to inflammation, presence of circulating antibodies).

Curative treatments

Monitoring of standard heparin therapy is based on the APTT or on measurement of anti-Xa activity. In practice the dosage is usually adjusted according to the APTT, although anti-Xa activity is essential for therapeutic monitoring of patients treated with UFH who have another reason for the ACT to be altered (deficiency of intrinsic coagulation pathway factor or the presence of a circulating lupus anticoagulant, inflammation, etc.).

Measurement of anti-Xa activity to monitor curative LMWH treatment is not mandatory, as firstly the dosage is adjusted according to the patient's weight and secondly the inter-individual variability in response to these treatments is small. It is however, indicated in patients with chronic renal insufficiency, in those of extreme body weight, after either haemorrhagic events or extension of thrombosis or in longer-term treatment beyond 5 to 8 days, the average length of treatment for deep vein thrombosis and pulmonary embolism. It is important to be aware that anti-Xa activity values approximately 4 hours after injection of an LMWH vary between different pharmaceutical preparations. *(cf. table)*.

INFORMATION

These have been precisely defined and must be observed strictly for the results to be correctly interpreted.

SAMPLE

Taken into citrate, concentration 3.2% (0.109 M) at 1/10 (0.5 ml per 4.5 ml of blood). 3.8% citrated tubes (0.129 M) are acceptable. The sample must be correctly identified and dated (date and time of sample).

Blood can also be collected into a CTAD tube (citrate, theophylline, adenine, dipyridamole) which allows the sample to be stored better (this should be preferred when the transport time for the tube is more than 2 hours). No other anticoagulants must be used.

The sample does not need to be taken fasting and patients may have a light low fat snack. For further information refer to the *"General haemostasis pre-analytical conditions"* page.

The time of sampling is particularly important:

Unfractionated heparin:

- Continuous IV injection of UFH: sampling time after treatment for 4 hours.

- Subcutaneous (SC) injections: "peak" sample taken midway between 2 injections (NB: In some situations it may be useful to take a "trough" measurement immediately before the next SC injection).

LMWH:

The sample for anti-Xa activity measurement is taken at the peak, 3 to 4 hours after subcutaneous injection if the compound is administered as two daily injections or 4 to 6 hours after the injection if it is administered as one injection/24 hours.

QUESTIONS FOR THE PATIENT

Name and dose of drug being administered? Time and route of administration?

SAMPLE STORAGE AND TRANSPORT

The sample must be centrifuged and separated within an hour of sampling.

The test is performed on low platelet plasma obtained by centrifuging (2000 to 2500 g, for 15 minutes at between 10 and 20°C). If the test is to be performed later, the sample should be frozen after a second centrifugation stage (separating the plasma from the first centrifugation into a polypropylene or PET tube).

Separated plasma can be stored for 1 month at – 20°C and for approximately 3 months at - 80°C.

Transport the plasma frozen at – 20°C.

ASSAY METHODS

Anti-Xa activity by an amidolytic chromogenic method is the most widely used (calibrated with the substance in question: UFH, LMWH, danaparoid, pentasaccharide). Kits are available which use a single calibration for UFH and LMWH.

Chronometric methods: Heptest® – Pict®, are not widely used.

Usual anti-Xa activity 3 to 4 hours after subcutaneous injection of enoxaparin, dalteparin or nadroparin (preventative or curative treatment with 2 daily injections), or 4 to 6 hours after subcutaneous injection of curative dose nadroparin, 1 injection/24h or tinzaparine

	Indication	Dose	Anti-Xa activity (IU anti Xa / ml)
(Enoxaparin INN)	Prevention of moderate risk in surgery	2000 IU/24 hours (20 mg/24 hours) (1 injection/24 hours)	0.18 ± 0.04
LOVENOX® (Enoxaparin INN)	Prevention of high risk in surgery or prevention in medicine	4000 IU/24 hours (40 mg/24 hours) (1 injection/24 hours)	0.43 ± 0.11
LOVENOX® (Enoxaparin INN)	Curative treatment of established deep vein thrombosis Unstable angina Non Q wave myocardial infarction	100 IU/kg/12 hours (1 mg/kg/12 hours) (2 injections/24 hours)	1.20 ± 0.17 After the 7th injection
FRAGMINE® (Dalteparin INN)	Prevention of moderate risk in surgery	2500 IU/24 hours (1 injection/24 hours)	0.15 to 0.25
FRAGMINE® (Dalteparin INN)	Prevention of high risk in surgery	5000 IU/24 hours (1 injection/24 hours)	0.30 to 0.45
FRAGMINE® (Dalteparin INN)	Curative treatment of established deep vein thrombosis	100 IU/kg/12 hours (2 injections/24 hours)	from 0.59 to 0.69 ± 0.25 mean values from D2 to D10 of treatment
FRAGMINE® (Dalteparin INN)	Unstable angina, Non Q wave myocardial infarction	120 IU/kg/12 hours (maximum dose: 10 000 IU/injection)	0.6 to 1.2
FRAXIPARINE® (Nadroparin INN)	Prevention of moderate risk in surgery	2850 IU/24 hours (1 injection/24 hours)	0.25 to 0.35
FRAXIPARINE® (Nadroparin INN)	Prevention of high risk in surgery	38 IU/kg/24 hours for 3 days then 57 IU/kg/24 hours (1 injection/24 hours)	0.25 to 0.35
FRAXIPARINE® (Nadroparin INN)	Curative treatment of established deep vein thrombosis	83 IU/kg/12 hours (2 injections/24 hours)	1.01 ± 0.18
FRAXIPARINE® (Nadroparin INN)	Unstable angina, Non Q wave myocardial infarction	86 IU/kg/12 hours (2 injections/24 hours)	1.01 ± 0.18
INNOHEP® (Tinzaparin INN)	Prevention of moderate risk in surgery	2500 IU/24 hours (1 injection/24 hours)	0.10 to 0.15
INNOHEP® (Tinzaparin INN)	Prevention of moderate to high risk in surgery	3500 IU/24 hours (1 injection/24 hours)	0.15 to 0.20
INNOHEP® (Tinzaparin INN)	Prevention of high risk in surgery	4500 IU/24 hours (1 injection/24 hours)	0.35 to 0.45
INNOHEP® (Tinzaparin INN)	Curative treatment of established deep vein thrombosis Treatment of pulmonary embolism	175 IU/kg/24 hours (1 injection/24 hours)	0.87 ± 0.15
FRAXODI® (Nadroparin INN)	Curative treatment of established deep vein thrombosis	171 IU/kg/24 hours (1 injection/24 hours)	1.34 ± 0.15

**Generally, the anti-Xa activity at the peak during curative treatment for established venous thrombosis must be below 1.5 IU anti-Xa/ml, although varies according to the LMWH used.*

Monitoring of anti-Xa activity is reserved for certain patients (renal insufficiency, pregnant women, elderly or patients with extreme weight) particularly to confirm lack of accumulation.

Laboratory monitoring must also include a platelet count before treatment or no later than 24 hours after starting treatment and then twice weekly for the usual duration of treatment. Heparin-induced thrombocytopenia (HIT) should be suspected if the platelet count is < 100 000/mm³ and/or with a relative fall in platelet count of 30 to 50%.

Danaparoid - Usual anti-Xa activity values seen during danaparoid treatment

Indication*			Dosage		Expected anti-Xa activity
Prophylactic treatment of thrombo-embolic disease (patients with no past history of HIT**)			750 anti-Xa units twice daily, subcutaneously, for 7 to 10 days or until the risk of thrombo-embolism has fallen		0.2 unit/mL on day 1 (for a sample taken midway between 2 injections). 0.2 to 0.4 unit/ml on day 5 (for a sample taken midway between 2 injections). Plasma concentrations must not exceed 0.4 unit/ml. The steady state is usually reached after treatment for 4 to 5 days.
Prophylactic treatment of thrombo-embolic events (patients with acute HIT without thrombosis or with a past history of HIT)	Patients with acute HIT without thrombosis	Weight ≤ 90 kg	750 anti-Xa units, 3 times a day, subcutaneously, for 7 to 10 days or until the risk of thrombo-embolism has fallen		
		Weight > 90 kg	1250 anti-Xa units, 3 times a day, subcutaneously, for 7 to 10 days or until the risk of thrombo-embolism has fallen		
	Patients with a past history of HIT	Weight ≤ 90 kg	750 anti-Xa units, twice daily, subcutaneously, for 7 to 10 days or until the risk of thrombo-embolism has fallen		
		Weight > 90 kg	1250 anti-Xa units, twice daily, subcutaneously, for 7 to 10 days or until the risk of thrombo-embolism has fallen		
Curative treatment of thrombo-embolic events (in patients suffering from acute HIT or with a past history of HIT)		Weight ≤ 55 kg	1250 anti-Xa units as intravenous bolus followed by	i.v. regimen: 400 units/h infusion for 4 h, then 300 units /h for the next 4 hours then maintenance infusion of 150 to 200 units /h for 5 to 7 days. This treatment regimen is particularly recommended for patients at high risk of extension of thrombosis and for patients weighing more than 90 kg. s.c. regimen: • 1500 anti-Xa units twice daily for patients of weight ≤ 55 kg • 2000 anti-Xa units twice daily for patients of weight > 55 kg and ≤ 90 kg 1750 anti-Xa units 3 times a day for patients of weight > 90 kg if it is not possible to use the i.v. administration route for 5 to 7 days.	i.v. regimen: • 0.5 to 0.7 units/mL when measured 5 to 10 minutes after the bolus • Must not exceed 1 unit/mL during the adjustment phase and must be between 0.54 and 0.8 units/mL during maintenance treatment (i.e. 3 to 5 days after starting treatment) s.c. regimen: • 0.4 to 0.8 unit/ml on day 3 of treatment (midway between two injections)
		55 < Weight ≤ 90 kg	2500 anti-Xa units as intravenous bolus followed by		
		Weight > 90 kg	3750 anti-Xa units as intravenous bolus followed by		

Where applicable, doses must be adjusted for extent of renal insufficiency in the elderly.
In patients with renal insufficiency, specific anti-Xa activity must be monitored if anti-Xa activity increases and/or serum creatinine rises above 220 µmol/l, the daily dose of danaparoid should be reduced in order to maintain the desired anti-Xa activity.
For prophylactic treatment apart from specific situations, anti-Xa activity does not need to be measured.
Generally, monitoring of plasma anti-Xa activity during curative treatment in patients suffering from HIT or with a past history of HIT treated with danaparoid for thrombo-embolic events is not necessary. Monitoring may be considered in certain clinical situations, particularly in overweight cachexia, renal insufficiency or high risk of haemorrhage.
A platelet aggregation test should be performed if possible with danaparoid before starting treatment (treatment may however be started without waiting for the results of this test but should be replaced by another anticoagulant if the test is positive).
Danaparoid should be administered subject to daily platelet monitoring.
**For other indications, refer to the dictionnaire Vidal®.*
***HIT: type 2 heparin induced thrombocytopenia.*

NORMAL EXPECTED VALUES

The expected values depend on the indication for preventative (low, moderate or high risk) or curative treatment, the heparin used (standard or low molecular weight heparin or heparin derivatives) and method of administration (number of injections/day, intravenous or subcutaneous).

The recommended therapeutic margins are regularly updated by international experts.

UFH

Heparin activity (anti-Xa): therapeutic range for curative treatment with unfractionated heparin (UFH).

Heparin (anti-Xa activity in IU/ml)	Methods of administration and sampling
0.3 to 0.6 IU/ml	midway between two S.C. injections at any time as soon as a plateau has been achieved for IV infusion
0.15 IU/ml	trough level immediately before the next S.C. injection.

LMWH

- Preventative treatment: the expected values for different LMWH are in the region of 0.1 to 0.3 IU anti-Xa/ml of plasma for prophylaxis of low to moderate risk and 0.3 to 0.6 IU anti-Xa/mL of plasma for prophylaxis of high or very high risk.

- Curative treatment: *cf. table on next page.*

Fondaparinux:

Expected values 4 hours after injection are in the region of 0.10 to 0.40 µg/ml of plasma for preventative treatment and approximately 1.4 µg/ml of plasma for treatment of deep vein thrombosis or pulmonary embolism.

INTERPRETATION

It is essential firstly to ensure that the sample has been taken at the correct time and that the pre-analytical conditions have been followed. In addition, it should be ensured that anti-Xa activity has been measured within the linear range of calibration (if not it should be re-measured diluting the test plasma in a normal plasma pool).

In general, anti-Xa activity below the recommended range indicates that the dose administered is inadequate and that doses should be increased and monitoring continued. Anti-Xa activity above the upper limit of the therapeutic range may indicate accumulation of the substance or an excessive dose, suggesting a very likely increase in the risk of haemorrhage.

FOR FURTHER INFORMATION

■ Boutières-Albanèse B. *Mesure de l'héparinémie par activité anti-Xa*. Encycl Med Biol, Elsevier, Paris, 2003.

■ Samama MM. Desnoyers PC. *Héparines*. Encycl Méd Chir (Elsevier Paris), Cardiologie-Angéiologie, 11-912-A-10, Hématologie, 13-022-D-10, 1996, 8p.

■ *Logiciel d'autoformation des biologistes en hémostase*, Bioforma CD Rom 2004.

ANTIBODIES AGAINST ETHYNIL ESTRADIOL AND PROGESTERONE

DEFINITION

Synthetic hormones used for oral contraception can, in some women, elicit specific antibodies. The first of these antibodies was discovered in 1975 and were directed against ethinyl-estradiol. Other antibodies were subsequently found in certain patients on synthetic sex hormones (both steroid and non-steroid) and they recognise synthetic progestins derived from nortestosterone and cyproterone acetate, or synthetic, non-steroid oestrogens (like diethylstilbestrol which is used in the treatment of prostate cancer, and clomifene citrate which is used to induce ovulation). In contrast, the endogenous hormones (17ß-estradiol, progesterone) and semi-natural derivatives (conjugated horse oestrogens) used for post-menopausal hormone replacement therapy are not immunogenic.

Synonyms: anti-oestrogen antibodies, anti-EE.

INTRODUCTION

Antibodies against ethinyl-estradiol were first detected in a woman on an oral contraceptive who had experienced a pulmonary embolism associated with a "benign" monoclonal gammopathy, with antibodies directed against ethinyl-estradiol by monoclonal immunoglobulin. Such antibodies were then detected in about 30% of all women on oral contraceptives, and in 80-90% of those who had experienced vascular complications (associated with other risk factors, notably smoking). It was hypothesised that these antibodies had pathogenic effects at the walls of blood vessels.

SEARCH INDICATIONS

The indication that was proposed originally was screening for vascular risk in women using an oral contraceptive with a view to identifying the population at high risk of thrombosis. This recommendation was based on epidemiological findings but it has never been demonstrated that these antibodies have any activity in vivo and the systematic screening of women using oral contraceptives is unjustified.

INFORMATION

SAMPLE

Serum (Dry tube): The patient does not necessarily have to be fasting.

After clotting at 37°C for 3 hours, centrifuge for 10 minutes; all traces of fibrinogen have to be removed to avoid falsely positive results.

SAMPLE STORAGE AND TRANSPORT

Store serum at +4°C for up to 48 hours, and then freeze at -20°C.

Transport at +4°C or frozen if the sample is already in its frozen state.

ASSAY METHODS

Antibodies against ethynil-estradiol are detected in a radio-immunological assay based on tritiated ethynil-estradiol. Cross-reactions can occur with other steroid sex hormones such as the progestagens and endogenous hormones, notably oestrogens.

NORMAL EXPECTED VALUES

No antibodies detected.

INTERPRETATION

Such antibodies can appear within three weeks of the beginning of a course of oral contraception and persist for years after withdrawal of the contraceptive. Their serum concentration depends on neither the administered dose of the oral contraceptive nor the duration of the course of treatment. Their presence constitutes a vascular risk factor.

Antibodies against ethinyl-estradiol have been implicated in the triggering of systemic lupus erythematosus but their true role in this pathogenic process has yet to be demonstrated.

The main risk attributed to these antibodies is that they may trigger thrombosis in women on oral contraceptives. However, the reality of this risk has never been demonstrated in controlled studies conducted in sufficiently large populations. In consequence, such a test is not justified at this time, neither in women on an oral contraceptive nor in an "aetiological work-up" to investigate thrombosis.

FOR FURTHER INFORMATION

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- Beaumont V, Lemort N, Beaumont JL. *Oral contraceptives, sex steroid-induced antibodies and vascular thrombosis: results from 1318 cases*. Europ Heart 1991; 12:1219-24.
- Beaumont V, Gioud-Paquet M, Kahn MF, Beaumont JL. *Antiestrogen antibodies, oral contraception and systemic lupus erythematosus*. Clin Physiol Biochem 1989; 7:263-8.

ANTIBODIES AGAINST INTRINSIC FACTOR

DEFINITION

Cells of the stomach wall secrete hydrochloric acid and intrinsic factor (IF) into the stomach to promote digestion and vitamin B12 absorption in the small intestine. IF carries a specific binding site for vitamin B12 and the resultant complex can only be assimilated in the terminal ileum. Once the complex has been endocytosed, the vitamin B12 is released.

Pernicious anaemia is due to impaired vitamin B12 absorption as a result of the inactivation of IF by auto-antibodies which can be of two different types:

– Type I or blocking antibodies which prevent the formation of the IF-vitamin B12 complex.

– Type II or precipitating antibodies which prevent binding of the complex to the ileal receptor.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

Pernicious anaemia is a megaloblastic anaemia which is relatively silent until the vitamin B12 deficiency has become profound when the symptoms combine those of the anaemia (tiredness, shortness of breath, etc.) with gastritis (abdominal pain, anorexia and vomiting) and neurological problems (paresthaesia, impaired sensory function and coordination).

This autoimmune condition is often associated with other immunological disorders, such as Graves' disease, Hashimoto's disease, diabetes, vitiligo, Sjögren's syndrome, and lupus).

Differential diagnosis focuses on dietary deficiency, exuberant bacterial proliferation in the gut, gastrectomy, folate deficiency, hyperthyroidism and increased requirements during pregnancy.

INFORMATION

■ SAMPLE

Serum : The subject does not need to be fasting. The subject should not have recently taken a vitamin B12 supplement.

■ QUESTIONS FOR THE PATIENT

Do you have any symptoms of gastritis?

Full blood count: Macrocytic anaemia? Hyper segmented neutrophils?

Differential bone marrow cell count: Hyperactivity with large cells present?

Results of vitamin B12 and folate assays?

Have you recently taken a vitamin B12 supplement?

■ SAMPLE STORAGE AND TRANSPORT

Separate the serum within half a day of blood drawing.

Store and transport at +4°C.

ASSAY METHODS

Radio-immunology was the first technique used. The patient's antibodies are allowed to compete with immobilised, pure IF for the binding of [57Co] radio-labelled vitamin B12. This test only detects Type I antibodies and the result will be distorted if the patient recently took a dose of vitamin B12 for therapeutic or diagnostic (Schilling test) reasons. Any vitamin B12 present in the serum will bind the test IF and this can give rise to falsely positive results.

ELISA and immunodot methods: Competitive assays suffer from the same weaknesses as radio-immunology. Classic sandwich methods detect both types of antibody and are not interfered with by vitamin B12. The antigen used is purified porcine IF or recombinant human IF. The results are highly specific and sensitivity approaches 90%.

UNITS AND REFERENCE VALUES

There are no internationally accepted units: Every kit has its own units and reference values.

FOR FURTHER INFORMATION

■ Humbel R.L., Olsson N.O., *Mise en évidence des anticorps anti-cellules pariétales et anti-facteur intrinsèque*, GEAI l'info, n° 7, mai 2005.

■ *Les Auto-Anticorps 2003-2004*, Cédérom réalisé par Monier J.C., Auger C., Fabien N.

■ Humbel R.L., *Auto-anticorps et maladies auto-immunes*, Collection Option/Bio, Ed Elsevier, Paris, 2e Ed, 1997.

ANTIDEPRESSANTS

DEFINITION

Antidepressants are defined by their common action on mood disorders, although they are a very structurally heterogeneous group of molecules which are grouped into several subclasses:

– The tricyclic antidepressants (TCAD): Imipramine type (imipramine, clomipramine, desipramine, etc.). dibenzocycloheptadiene type (amitriptyline, amineptine) and other derived molecules (maprotiline, mianserin, etc.).

– Monoamine oxidase inhibitors (MAOI): Iproniazide, moclobemide and toloxatone.

– Selective serotonin reuptake inhibitors (fluoxetine, fluvoxamine, etc.).

We will only consider the TCAD here, restricted to imipramine, clomipramine, and amitriptyline, which are the longer standing compounds, the best studied toxicologically and the most concerning, as they are amongst the most toxic.

Pharmacologically, the antidepressant effect of the TCAD involves inhibition of serotonin and noradrenaline re-uptake. They also have a sedative and vasodilating effect and may cause seizures and anticholinergic effects through partial blockade of the alpha adrenergic, gabaergic, muscarinic and histaminic receptors. The TCAD are indicated for the treatment of major depressive episodes. They are widely prescribed in France, explaining common overdose problems, usually accidental in children or in attempted suicide in adults. Acute TCAD poisoning makes up 17 to 37% of intensive care hospitalisations for intentional poisoning and approximately half of the life-threatening drug overdoses. It carries a mortality rate of approximately 3%.

Various emergency screening methods are available for blood, urine or gastric fluid but precise identification of the molecule in question and quantification requires chromatographic techniques in blood.

PHARMACOKINETICS

Following oral ingestion, the TCAD are generally rapidly and almost completely absorbed. Their bioavailability however is only 30 to 60% because of a major first pass hepatic effect. They are extensively bound to plasma proteins (> 85%) in the circulation and have a wide tissue distribution. They are metabolised in the liver by demethylation and hydroxylation reactions forming primary active metabolites, some of which have a longer half-life than the parent molecule and then by conjugation with glucuronic acid to inactive metabolites. Their excretion in the urine varies depending on the compound (< 10% for clomipramine and 80% for amitriptyline and desipramine).

International non-proprietary name	Metabolites	Half-life (T1/2) of excretion	Therapeutic range (trough) For reference
Imipramine (IMI) Desipramine (DMI)	Desmethyli-mipramine (desipramine or DMI)	IMI: 9 to 20 h- DMI: 22 h	IMI+ DMI: 150 to 250 ng/ml DMI: 115 to 250 ng/ml
Clomipramine (CMI)	Desmethylclo-mipramine (DCMI)	CMI: 21 h DCMI: 25 to 50h	90 to 250 ng/ml Norclomipramide: 150 to 300 ng/ml. CMI + DCMI: 250 to 500 ng/ml
Amitriptyline (AMI)	Nortriptyline (NOR) hydroxylated derivatives	22 to 40 h	Total AMI + NOR (FPIA): 120 to 250 ng/ml

Finally, their pharmacokinetics varies depending on the presence of concomitant diseases (renal insufficiency, hepatic insufficiency), alcoholism (increases plasma imipramine and clomipramine concentrations), smoking (reduces plasma imipramine and clomipramine concentrations) and therapeutic interactions *(cf. table)*.

Main therapeutic interactions

Mechanism	Molecule (INN)	Effects
Induction of metabolism	Carbamazepine	↓ IMI (40–50 %), ↓ CMI and/or DCMI, ↓ AMI
Inhibition of metabolism	Fluoxetine, paroxetine, sertraline,	↑ IMI + DMI (x 2 to 4), ↑ DCMI, ↑ AMI
	Fluvoxamine, Citalopram	↑ IMI, ↑ CMI (x 3 to 4), ↑ AMI ↑ DMI, ↑ AMI
	Haloperidol, thioridazine	↑ IMI + DMI (x 2)
	Cimetidine	↑ IMI, ↑ DMI (x 2) and ↑ CMI (x 3 to 4) and ↓ DCMI (x 1.5 to 3)

IMI: imipramine, DMI: desmethylimipramine; CMI: clomipramine, DCMI: desmethylclomipramine.
AMI: amitriptyline, NOR: Nortriptyline.

INDICATIONS FOR MEASUREMENT

– Aid in the diagnosis of acute poisoning (intentional or accidental): Rapid screening methods are available as a qualitative guide and quantitative methods are available to identify the molecule in question, and the extent of poisoning.

The clinical symptoms of acute isolated TCAD poisoning can be grouped into three major syndromes:

- Anticholinergic effects such as sinus tachycardia, mydriasis, dry mucous membranes, constipation, urinary retention, and delusions.
- Cardiac toxicity (sinus tachycardia, hypotension, collapse, arrhythmia, asystole).
- Central toxicity (drowsiness, coma, agitation, pyramidal syndrome and seizures).

These generally reach maximum severity 4 to 12 hours after ingestion and fatal complications occur at an average of 6 hours after taking the drug.

– Therapeutic monitoring: Pharmaceutical laboratory recommendations are that TCAD therapeutic monitoring of

plasma concentrations is not required. It is however recommended in at risk patients, such as children, the elderly, those with heart, liver or renal disease etc, in polymedicated patients (risk of therapeutic interactions), and in patients who are resistant to treatment or those who have signs of toxicity at usual doses.

There are also strong arguments to recommend monitoring as measurements can help to detect possible under-dosing or failure to comply with treatment, which in depression carries a significant risk of suicide. In addition, measurements are believed to be useful to assess response to treatment, checking that therapeutic concentrations are achieved before concluding that a patient is "resistant to treatment" (which published findings report in between 25 and 40% of patients) if an inadequate effect is obtained. Finally, regular measurements in polymedicated patients should help dosage adjustment to avoid possible overdose (*cf above*, therapeutic interactions).

INFORMATION

■ SAMPLE

– Serum or plasma sample taken into EDTA or heparin. Avoid tubes with separating gel.

Take sample:

- For therapeutic monitoring, in steady state (approximately 1 week for clomipramine, 2 to 3 weeks for desmethylclomipramine, its main active metabolite; 10 days for imipramine) immediately before the next dose (trough concentration),
- As soon as possible for acute suspected or known poisoning.

– Random Urine: Collect sample in a sterile container. TCAD may be tested for in conjunction with other drugs (benzodiazepines, amphetamines, cocaine, opiates, cannabis, etc.). Urine testing is only performed for qualitative analysis in suspected acute poisoning (it has no use in therapeutic monitoring).

■ QUESTIONS FOR THE PATIENT OR THOSE WITH THE PATIENT

What is the context in which the measurement is being requested? Is the testing for therapeutic monitoring, suspected acute accidental poisoning or attempted suicide? If possible, the amount taken, date and time it was taken, and substances taken at the same time? Alcohol and other central nervous system depressants (other psychotropic agents, opiates, etc.) increase toxicity plus see above for the main therapeutic interactions.

■ SAMPLE STORAGE AND TRANSPORT

Qualitative testing: Centrifuge and decant the sample promptly. Store the serum/plasma and urine at + 4°C.

Clomipramine measurement in whole blood can be stored for a few hours at room temperature (avoid haemolysis); freeze the plasma or serum at – 20°C promptly if analysis is to be performed later.

Imipramine measurement in whole blood can be stored for a few hours at room temperature (avoid haemolysis) 3 days at + 4°C; freeze the plasma or serum at – 20°C promptly if the analysis is performed more than 3 days later.

ASSAY METHODS

- Screening methods (qualitative) in various biological fluids (plasma, serum, gastric fluid, urine): immunological methods: RIA, EMIT, FPIA…

- Identification of the molecule responsible – quantitative assay, high performance liquid chromatography with ultraviolet or electrochemical detection, gas phase chromatography with flame ionisation detector or mass spectrometry.

INTERPRETATION

Therapeutic monitoring: Therapeutic range for trough steady state concentrations *(cf. table on previous page)*. Clinical signs of clomipramine overdose usually occur when serum/plasma CMI + DCMI concentrations are > 500 ng/ml.

Diagnosis and monitoring of acute poisoning: Antidepressants are involved in 25 to 60% of drug poisoning in France and are often serious and an intake of 0.5 g of active substance is potentially toxic, a dose of 1 g can have severe consequences and ingestion of ≥ 2 g may be fatal. These drugs are therefore very commonly tested in emergency toxicology. The samples of choice are blood and urine. Screening methods are available to all laboratories although they have disadvantages, particularly their lack of specificity (possible falsely positive results). In many situations, therefore, an immunochemical method should be considered as a guiding stage and any positive result must be confirmed by a more specific method. Although there is no close relationship between plasma concentrations and clinical symptoms, measurements are still very often requested by clinicians to confirm poisoning in the shortest possible time, as most deaths occur in the initial hours following hospitalisation. Measurements are less useful to monitor poisoning, as this is based mostly on electrocardiographic data. They are however useful to establish that gastro-intestinal absorption has stopped when clumps of tablets are formed, which are not removed by gastric lavage.

The treatment of acute TCAD poisoning is symptomatic with gastric lavage, administration of activated charcoal and sodium bicarbonate to correct the metabolic acidosis.

FOR FURTHER INFORMATION

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ANTIPHOSPHATIDYL-ETHANOLAMINE ANTIBODIES

DEFINITION

Antiphosphatidylethanolamine antibodies belong to the highly heterogenic family of antiphospholipid antibodies. Phosphatidylethanolamine is a neutral phospholipid and a major component of the cell membrane. APE's sometimes recognise a phosphatidylethanolamine/cofactor complex. The cofactor has been identified as high-molecular weight kininogen (HMWK).

Synonym: aPE.

INDICATIONS FOR MEASUREMENT

APE can be found in autoimmune diseases, associated with venous and/or arterial thromboses or repeated miscarriages defining antiphospholipid syndrome (APS). APS is said to be "primary" if it is not associated with any other autoimmune disease or secondary if another such disease (e.g. systemic lupus erythematosus) is present. APS is preferentially associated with antiphospholipids and/or anticardiolipins and/or anti-ß2 glycoprotein I *(cf. corresponding documents)*. APE antibodies are searched for in the second intention in cases where there is a strong suspicion of APS and the above-mentioned antibodies are absent. They are not specific to APS, but various studies agree in finding them more frequently associated with thromboembolic accidents or repeated miscarriage in lupus patients.

The experience of certain French groups shows a higher frequency of the IgM isotype in patients who have had miscarriages, but also in cases of unexplained thrombosis, while a European investigation mainly finds IgG's, in patients having suffered thrombotic accidents.

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Report any previous history of venous or arterial thrombosis, miscarriage, known autoimmune disease or drug treatment.

SAMPLE STORAGE AND TRANSPORT

Separate the serum within half a day of collection.

Store at +4° C a few days then store at -20° C; Transport at +4° C within a few days (avoid successive freezing/thawing).

ASSAY METHODS

Assays employ ELISA immunoenzymatic techniques. Some highly-specialised laboratories have developed "in-house techniques", although kits have been commercially available since the end of 2008.

REFERENCE VALUES

Reference values vary according to the kit used.

FOR FURTHER INFORMATION

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ANTIRIBOSOMAL ANTIBODIES

DEFINITION

These rare antibodies are found in systemic lupus erythematosus.

The ribosomes are composed of 2 subunits:

– A small subunit comprised of one RNA 18S and at least 33 different base proteins.

– A large subunit comprised of 3 distinct RNA's, approximately 46 different base proteins and 3 phosphoproteins, P0, P1 and P2.

Antiribosomal antibodies mainly recognise the phosphoproteins P0, P1 and P2.

The following less common proteins may also be recognised: L12 protein, L5/5S rRNA complex, S10 protein, Ja protein and RNA 28 S.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

Antiribosomal antibodies with anti-P protein specificity are almost exclusively found in systemic lupus erythematosus (SLE), in 10 to 20% of cases. They have, exceptionally, been described in other contexts (mixed connective tissue disease, scleroderma and rheumatoid arthritis). There is a curious ethnic variation in the prevalence of anti-P protein antibodies in lupus and they occur more frequently in Japanese or Chinese lupus subjects than in Caucasians and African Americans.

Three types of clinical manifestation in particular, associated with the presence of anti-P protein antibodies, have been reported in lupus:

– Hepatic impairment

– Renal impairment

– Depressive neuropsychiatric manifestations

In practice, finding antiribosomal antibodies steers the diagnosis towards SLE. Their presence is generally a sign of the evolution of the disease. On the other hand, although a more frequent association with neurological, hepatic and renal manifestations has been reported, this is too variable to be of real prognostic value.

INFORMATION

■ SAMPLE

Serum (Dry Tube): A fasting sample is not necessary.

■ QUESTIONS FOR THE PATIENT

Report any known pathology, whether autoimmune or not.

■ SAMPLE STORAGE AND TRANSPORT

Decant the serum within half a day of collection and store at +4° C.

Transport at +4° C.

DETECTION METHODS

The classical technique is indirect immunofluorescence on a triple substrate (liver, kidney and stomach of rat).

Antiribosomal antibodies reveal their presence by perinuclear, clumped fluorescence of hepatocytic cytoplasm, with marking of the principal stomach cells.

On HEp-2 cells, anti-P phosphoprotein antiribosomal antibodies produce cytoplasmic fluorescence in only 30% to 60% of cases. The fluorescence is composed of very fine, dense dots, tending towards a homogeneous appearance. This cytoplasmic appearance is often combined with nuclear fluorescence, to a variable degree according to the associated pathology.

Electrosynthetic and western blot techniques using global cytoplasmic extracts have also been used, but they lack sensitivity.

The most sensitive techniques are immunoenzymatic methods using purified ribosomes, synthetic peptides or fusion proteins. Few kits are commercially available and high costs limit their utilisation.

In practice, screening for antiribosomal antibodies can be carried out on HEp-2 cells. Confirmation by Immunofluorescence imaging on triple substrates is indispensable.

UNITS AND REFERENCE VALUES

Immunofluorescence:

The result is expressed as the reciprocal of dilution.

The dilution for screening on HEp-2 is 1:80 and on triple substrates it is 1:40.

FOR FURTHER INFORMATION

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ANTIRETICULIN IgA ANTIBODIES

DEFINITION

Antireticulin antibodies (ARA) were the first antibodies described in coeliac disease (intolerance to gluten). They can be detected by indirect immunofluorescence on sections of rat tissue, but the target antigen is not known. Five types of ARA have been described, although only R1 antibodies are of interest.

INTRODUCTION

Coeliac disease is an intolerance to gluten resulting in enteropathy and a malabsorption syndrome in genetically-predisposed subjects (95% of sufferers present a class II HLA-DQ2 serotype group).

The disease takes 2 distinct forms:

– Childhood coeliac disease, with a somewhat standard clinical presentation:

The condition generally occurs before the age of 2 years. Its prevalence in France is approximately 1/2500. It classically combines chronic diarrhoea and quite severe signs of malnutrition. It is accompanied by constant anorexia, vomiting in 50% of cases and permanent impairment of general health. This leads quickly to growth retardation and a falling off of the weight curve.

– In adults, the classical form is observed in only 20% of cases. In all other cases, the clinical signs merely reflect malabsorption, with mild and aspecific features (bone demineralisation, arthralgia, and neurological and reproductive problems), which can render diagnosis difficult. At the biological level, there may be signs linked to malabsorption (anaemia with deficiency of iron, folates and vitamin B12, and consequent deficiency in vitamin K, etc.).

The treatment for coeliac disease is a lifelong, gluten-free diet, which requires the exclusion of all foods containing wheat, barley or rye. Under a gluten-free diet, a pathological and clinical improvement, sometimes spectacular, is observed within a few months. Only the gluten-free diet can prevent long-term complications (e.g. small bowel lymphoma).

A diagnosis of coeliac disease is reached by serological screening followed by pathological conformation of the illness by a histological investigation of the small bowel in order to verify the presence and degree of villous atrophy.

MEASUREMENT INTEREST

Coeliac disease manifests itself by the presence of antibodies in response to the ingestion of gluten. Anti-endomysial IgA's, anti-transglutaminases and anti-gliadin IgG's and IgA's are the antibodies searched for *(see corresponding chapters)*.

Anti-reticulin antibodies are the least sensitive marker for diagnosing coeliac disease and should be abandoned in favour of anti-transglutaminase and/or anti-endomysial antibodies.

INFORMATION

SAMPLE

Serum (Dry tube): A fasting sample is not necessary.

QUESTIONS FOR THE PATIENT

Check that the patient is not already on a gluten-free diet.

SAMPLE STORAGE AND TRANSPORT

Decant the serum within half a day of collection and store at +4° C. Transport at +4° C.

ASSAY METHODS

The antibodies are assayed by triple-substrate indirect immunofluorescence (sections of liver, kidney and stomach).

In liver sections, fibrillar fluorescence is observed around portal spaces and linear marking around the sinusoids. In stomach sections, the fluorescence is present within the connective tissue. In the case of the kidney, fluorescence is peritubular and periglomerular.

REFERENCE VALUESE

Immunofluorescence:

The result is expressed as the reciprocal of dilution.

The positive threshold adopted is 10.

FOR FURTHER INFORMATION

■ Amouyel P., Bienvenu F., Cellier C., Cerf-Bensoussan N., Fabien N., Ghedira I., Heyman M., Jolivet B., Sakly W., Schmitt J., Schmitz J., *La maladie coeliaque en 2003* : Cahier thématique BioTribune.

■ Johanet C., *Marqueurs sérologiques de la maladie coeliaque*, Spectra Biologie 1998; 17:32-36.

ANTIRETROVIRAL DRUGS

DEFINITION

Antiretrovirals (ARV's) are defined by their common action on the human immunodeficiency virus (HIV), although they are heterogenic molecules in structural terms and grouped into a number of categories:

– Nucleoside reverse transcriptase inhibitors (nRTI's)

– Non-nucleoside reverse transcriptase inhibitors (NNRTI's)

– Nucleotide reverse transcriptase inhibitors (NTRTI's)

– Protease inhibitors (PI's)

– Raltegravir is an Integrase inhibitor

– Enfuvirtide is a fusion inhibitor

– Maraviroc is an antagonist of the HIV CCR5 co-receptor

The availability of ARV's has revolutionised the treatment of patients infected with HIV. The aim of the treatment is both to reduce the viral load until it becomes undetectable (< 50 copies/ml), and to restore immunity (CD4 > 500/mm^3).

AIDS has today become a chronic pathology requiring long-term treatment (over years or even decades) with molecules which are frequently efficacious but not without undesirable effects.

Plasma measurements of antiretrovirals are being used with increasing frequency due to their high "intra" and "inter" individual variability in terms of pharmacokinetics. Good correlation has been observed between plasma concentrations and antiviral efficacy of these drugs (except for nRTI's, cf. interpretation) and/or their tolerance. This means that such measurements allow antiretroviral dosage to be adapted for each patient in order to maintain good tolerance of the treatment, which is useful in ensuring compliance, and improve efficacy in preventing virological and/or clinical failure.

PHARMACOKINETICS AND SCHEDULES FOR PEAK SAMPLING

See Table 2 on next page.

INDICATIONS FOR MEASUREMENT

Measurements are at present indicated for NNRTI's and PI's, in certain situations:

- When drug interaction is expected

- In patients co-infected with HCV or HBV, even in the absence of transaminase elevation, and patients suffering from hepatic insufficiency

- In patients of extreme weight

- In children for molecules without marketing authorisation when the virus presents resistance mutations

- For pregnant women in certain situations; in particular on the initiation of treatment during pregnancy (PI measurement at W30 – W32 and if treatment fails)

- In cases of malabsorption

Table 2

	Bioavailability *per os (%)*	Link to plasma prot. (%)	Tmax (h)	T1/2 for clearance (h) (RI = renal insufficiency)	Exact time of peak sampling (Cmax)
nRTI					
3TC/lamivudine	80-85	16–36	1-1.5	3-7	1 h
ddC/zalcitabine	> 80	< 4	0.8-1.6	1-3 (8 h if RI)	1 h
AZT/zidovudine	52-75	7-38	0.5-1.5	1-2 (8 h if RI)	1 h
ddI/didanosine		< 5	0.8	0.7-2.7 (4 h if RI)	1 h
d4T/stavudine	82-99	< 5	1.2 to 1.6	1.2-1.6 (4 h if RI)	1 h
Abacavir	83	49	1-1.5	1.5	1 h
Emtricitabine / FTC	75-93	< 4	1 to 2	10	1 h
NNRTI					
Efavirenz		> 99	5	52-76	5 h (rarely requested)
Nevirapine	> 90	60	4	17-35	4 h (rarely requested)
Delavirdine	85	98	1	6	1 h
Etravirine		99.9	4	30-40	2.5 - 4 h
Nucleot. RT inh					
Tenofovir	25-40	< 7	1-2	12-18	1h
PI					
Amprenavir and Fosamprenavir	35-90	90	1.1-2.1	2-10	1 h (3h if combined with ritonavir)
AAtazanavir (+ ritonavir)		86	3	8.6	3 h
Darunavir +ritonavir	82	95	2.5-4	15	3 h
Indinavir	60-65	60-65	1.5	2.8	1 h (3h if combined with ritonavir)
Lopinavir		98-99	4	5-6	3 h (5h if combined with ritonavir)
Nelfinavir		>=98	2-4	3.5-5	3 h
Ritonavir	60-75	98-99	1.5-5.5	3-5	3 h
Saquinavir	4	98	2.4-3.8	7-13.2	3 h
Tipranavir	Not known	99.9	1 to 5	4.8 to 6	3 h

NB: In cases where several HIV protease inhibitors are combined, Tmax values are 3h after oral administration.

Table 1: Antiretroviral drugs

Treatment/dosage	Side effects	Contraindications	Recommendations
Nucleotide reverse transcriptase inhibitors			
Tenofovir disoproxil 300 mg/day	Digestive problems, nausea, diarrhoea, flatulence, vomiting, reduction of blood phosphates giving rise to kidney and bone problems	Lopinavir, didanosine, antibiotics in the aminoglycoside family, foscarnet sodium, amphotericine B, vancomycine, pentamidine isethionate, ganciclovir	To be taken with meals: contraindicated if there is a previous history of tenofovir allergy or in cases of renal insufficiency
Protease inhibitor			
Amprenavir 1200 mg 2 times/day	Gastro-intestinal problems, rashes, paralysis around the mouth	Terfenadine, astemizole, cisapride, pimozide, triazolam, diazepam, flurazepam, midazolam, derivatives of rye ergot, proton pump inhibitors, St. Johns wort	To be taken with caution by subjects with renal or hepatic insufficiency
Atazanavir 300 mg once/day	Ocular problems, headaches, insomnia, rashes, vomiting, diarrhoea	Rifampicine, astemizole, terfenadine, cisapride, quinidine, derivatives of rye ergot, proton pump inhibitors, St. Johns wort	Add 100 mg of ritonavir during meals. Do not take if hepatic insufficiency present
Darunavir 600 mg 2 times/day + 100 mg ritonavir 2 times/day	CNS problems, rashes, rare cardiac problems, renal insufficiency, osteoporosis	Rifampicine, amiodarone, bepridil, quinidine, systemic lidocaine, astemizole, terfenadine, midazolam per os, triazolam, cisapride, pimozide, sertindole, simvastatine, lovastatine and derivatives of rye ergot	Contraindicated in cases of severe hepatic insufficiency
Fosamprenavir 700 mg 2 times/day + 100mg ritonavir 2 times/day	Same as amprenavir	Same as amprenavir	Same as amprenavir
Indinavir 800 mg 3 times/day	Renal calculi, dry skin, alopecia (hair loss)	Rifampicine, astemizole, cisapride, dihydroergotamine, ergotamine, terfenadine, ethinyl estradiol	To be taken every eight hours with water before eating. Drink at least 1.5 l of water to avoid renal calculi
Lopinavir+ritonavir 400 mg/100 mg 2 times/day	Diarrhoea, ihypertriglyceridaemia, pancreatitis	Astemizole, terfenadine, midazolam, triazolam, cisapride, pimozide, amiodarone, alcaloids of rye ergot, St. Johns wort, rifampicine, disulfirame, metronidazole	Contraindicated in cases of severe hepatic insufficiency
Nelfinavir > 13 years: 1250 mg x 2/day or 750 mg x 3/day	Diarrhoea, glucose intolerance, diabetes, rashes	Terfenadine, astemizole, cisapride, amiodarone, quinidine, ergot derivatives, pimozide, oral midazolam, triazolam, sildenafil, phenobarbital, carbamazepine, rifampicine, contraceptives with norethindrone or ethinylestradiol, omeprazole	To be taken during meals
Ritonavir 100 to 200 mg/j (booster) or 1000 -1200 mg/day	Digestive problems, diarrhoea, elevated triacylglycerides	Astemizole, cisapride, dihydroergotamine, ergotamine, terfenadine, primozide, rifampicine, efavirenz, midazolam, rifabutine, triazolam	To be taken during meals
Saquinavir 1000 mg, 2 times/day	Few. Poor bioavailability interferes with efficacy	Carbamazepine, efavirenz, nevirapine, phenobarbital, phenytoin, primidone, rifabutine, rifampicine, astemizole, cisapride, terfenadine	To be taken within 2 hours after a meal. Combination with ritonavir (booster 100 mg x2/day) is recommended
Tipranavir 500 mg + ritonavir 2 times/day	Hepatic toxicity, headaches, sleep or mood problems	Rifampicine, St. Johns wort, antiarythmics, antihistaminics, rye ergot derivatives, cisapride, neuroleptics, simvastatine, lovastatine	Aptivus not to be taken by subjects with hepatic insufficiency
Nucleoside analogue reverse transcriptase inhibitors			
3TC, lamivudine 300 mg/day (150mg 2 times/day)	Headaches, fatigue	Zalcitabine	No particular monitoring
Abacavir 600 mg/day	Fatigue, abdominal pain, hypersensitivity, rash, fever, nausea	Ethanol	Hypersensitivity problems which may require treatment to be stopped **Never restart the treatment**
AZT/zidovudine 500-600 mg/day	Anaemia, neutropaenia	Stavudine, ribavirine	Blood monitoring to check for any appearance of anaemia or neutropaenia
d4T/stavudine 60 to 80 mg/day	Neuropathy, pancreatitis	Zidovudine, doxorubicine, zalcitabine	Monitor the risks of neuropathy and pancreatitis. It may be implicated in the appearance of lipoatrophy
ddI/didanosine 400 mg/day	Stomach pain, diarrhoea, pancreatitis, neuropathy, headache	Ganciclovir, zalcitabine	Take when fasting and at a separated from other medicines, avoid alcohol, which increases the risk of pancreatitis
Emtricitabine 1 capsule 200 mg 1 times/day 24 ml (240 mg) drinkable solution	Lactic acidosis, lipodystrophy, neutropaenia	Lamirudine Zalcitabine	Do not combine with drugs eliminated by active tubular secretion

Non-nucleoside reverse transcriptase inhibitor			
Delavirdine 400 mg x 3/day	Skin rash, nausea, headache, diarrhoea, anaemia, neutropaenia, thrombopaenia, renal or hepatic toxicity		Preferably to be taken with acidic drinks (orange drinks)
Efavirenz 600 mg once/day	Central nervous system problems: ebriety, dizziness, nightmares. Sometimes skin rash	Saquinavir,astemizole, cisapride, midazolam, terfenadine, triazolam, ethynil-oestradiol, ritonavir	To be taken when retiring. If nervous system side effects persist, tell your doctor
Etravirine 200 mg x 2/day	Cutaneous toxicity, lipodystrophy, osteonecrosis	tipranavir/ritonavir, nelfinavir, indinavir, clarithromycine, carbamazepine, phenobarbital and phenytoin, rifampicine, diazepam, St. Johns wort	To be taken after breakfast and the evening meal
Nevirapine 200 mg/day/14 days then 200 mg 2 times/day	Fever, severe skin rash	Saquinavir, ketoconazole, ethynil-oestradiol, rifampicine	Contraindicated in cases of severe hepatic insufficiency

NB: The information contained in this table is not exhaustive.

- In cases of renal insufficiency or dialysis
- In cases of virological failure
- When faced with suspected dose-dependent toxicity.

Drug interactions which modify plasma concentrations: the example of AZT

Combinations resulting in a reduction of plasma concentrations of AZT	Combinations resulting in an increase of plasma concentrations of AZT
– clarithromycine – nelfinavir – nevirapine	– atovaquone – cotrimoxazole – fluconazole in high doses – lamivudine: increase in AZT Cmax 39 % – methadone – valproic acid

INFORMATION

■ SAMPLE

Plasma sample collected in heparin or EDTA: Avoid tubes with separator gel.

Sample collection just before the next dose is taken (residual concentration or Cmin): For medicines taken twice per day, collection is usually performed just before the morning dose. Generally speaking, a delay of at least 15 days should be allowed for equilibrium to be reached after treatment starts or is modified (1 week in the case of efavirenz). The measurement primarily allows an evaluation of treatment efficacy and will be repeated depending on clinical, virological and immunological data.

Peak sampling (Cmax): 1 to 5 hours after administration, depending on the molecule. The measurement primarily allows an evaluation of the toxicity of the molecule. It may be requested very rapidly after the appearance of any undesirable effects, in order to identify any overdose.

■ QUESTIONS FOR THE PATIENT

Any request for a measurement of drugs levels must include the following: The reasons for prescribing (identifying efficacy or toxicity); the time of sample collection; the date of commencement of treatment and/or of any change in drug dosage; drug information (quantity administered, frequency and mode of administration); the age, height and weight of the subject where possible.

What other medicines are you taking? There are numerous drug interactions with ARV's.

■ SAMPLE STORAGE AND TRANSPORT

- Storage of the full blood sample: a few hours at room temperature.

Storage and transport of plasma decanted into a polypropylene tube: –20° C (several months).

ASSAY METHODS

LC-MS/MS (liquid chromatography with mass spectrometer in tandem).

INTERPRETATION

The interpretation of plasma antiretroviral measurements requires close collaboration between clinicians and the laboratory scientists and Pathologists, since good knowledge is needed of the clinical, virological and immunological status of the patient.

"Therapeutic ranges" have been established for certain antivirals **(NNRTI's and PI's)** on the basis of studies of concentration/effects relationships (depending on the dose administered, galenical form and measurement method). The minimum concentrations suggested by the laboratories undertaking measurements for these molecules are those below for which there is an increased risk of therapeutic inefficacy, for a sensitive viral strain. Maximum concentrations are difficult to define, bearing in mind the variability of the relationship between concentrations and undesirable effects, depending on subjects and treatments. In addition, when the pharmacokinetic and pharmacodynamic variability of molecules is taken into account, the dose-concentration relationship is not always linear and knowledge of that relationship remains imperfect, particularly in connection with combinations of several antiretroviral molecules, which is generally the case.

In general, the broad outlines of the approach are as follows:

- Plasma concentration of the ARV is within the recommended range: There is no need to modify the dosage or drug. A routine check is recommended a few months later or if there is a significant increase in viral load, a drop in the number of CD4 lymphocytes or an appearance of side effects.

- Plasma concentration of the ARV is below the recommended range: There should be a renewed discussion with the clinician regarding the parameters that might produce this result (drug combinations, treatment

administration, modalities, etc.). It may then be necessary to increase the dose of one or more of the antiretroviral molecules.

- Plasma concentration is higher than the recommended maximum: : If there are side effects which might correspond to overdose, a reduction is generally suggested in the unit dosage or frequency of administration of a molecule, and/or a booster for PI's (a booster is a drug prescribed in combination with a PI to raise bioavailability and thus efficacy; Ritonavir is at present the drug used to boost other PI's). A check is usually made 2 weeks after the dosage modification.

It is important, however, to underline the importance of a clinician/scientist/pathologist discussion in each case.

Example: Although a relationship has been clearly demonstrated between residual concentrations of indinavir and virological efficacy (significant reduction in viral load) in patients receiving tritherapy which includes this molecule (Trilège trial), the Pharmadept study, using a drug combination with ritonavir at an infratherapeutic dose (for its booster effect), showed no clear advantage in pharmacological therapeutic monitoring of the antiretroviral.

Where nRTI's are concerned, the relationship between plasma concentration and therapeutic effect remains difficult to establish, since efficacy is better reflected in concentrations at the action site than in plasma concentrations: in reality, these molecules are only active at cellular level after metabolism of the triphosphate derivatives and no correlation exists between their plasma and intracellular concentrations. This means that for nRTI's in general, the interpretation of measurement results must take account of the following:

– The pharmacokinetic characteristics, in particular the clearance half-life of the molecule.

– Inhibitory concentrations (IC90) measured *in vitro* under standard conditions.

– Treatment being received by the patient, and their virological status (native virus or possible mutated virus).

Further studies, taking the whole treatment into account, should allow therapeutic ranges to be more clearly defined, with improved individual adaptation of posology and antiretroviral treatments.

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ANTISPERM ANTIBODIES

DEFINITION – INTRODUCTION

Antisperm antibodies (ASAb) are found in 2-15% of couples being investigated for fertility concerns. The antibodies are mainly IgG and IgA but IgM antibodies can be indentified on rare occasions. They may be found in men in the seminal fluid bound to spermatozoa in semen or in serum, and in women in cervical mucus and possibly in serum.

They are directed against different antigenic structures on the sperm surface:

– 14, 90 and 110 kDa proteins: common targets in subfertile men with anti-sperm Antibodies.

– *Human nuclear autoantigen sperm protein* (NASP): the target for autoantibodies found in some people who have undergone vasectomy.

These antibodies play an important role in fertilisation, sperm mobility and their binding to the zona pellucida of the oocyte or penetrating the oocyte.

The presence of anti-testicular antibodies in men indicates autoimmunisation and reflects rupture or deterioration of the blood- testis barrier. When present in seminal fluid the autoantibodies can cause agglutination of spermatozoa through the flagella or acrosome and this impedes their movement or fusion with the oocyte. The prevalence of antisperm antibodies in subfertile men ranges from 3 to 15%.

Their presence in cervical mucus in women generally causes sperm immobilisation and they may be responsible for subfertility.

Synonym: ASAb.

INDICATIONS FOR MEASUREMENT

Second line assessment of subfertility

Testing in men is indicated:

– When spontaneous sperm agglutinates are present on the spermogram (particularly if spermatozoa are mobile but not advancing), occasionally associated with asthenospermia.

– When the Hühner test is negative.

– When the patient has a past history of accidental or surgical (vasectomy, etc.) trauma which may have caused damage to the vas deferens, epididymis or testes, or has a past history of repeated genital infections.

The antibodies may be tested for in women in cervical mucus if the Hühner test is negative.

Testing for anti-sperm immunisation may be requested during Medically Assisted Fertilisation treatment (MAF), particularly in the assessment of non-segmentation *in vitro fertilisation*.

INFORMATION

SAMPLE

The semen must be collected within close proximity to the laboratory, into a sterile container after abstinence from sex for three days, after passing urine with scrupulous local cleansing and washing of the hands thoroughly. The patient should also be asked to drink large amounts of fluid before the investigation.

Seminal plasma is collected after centrifuging the semen for 10 minutes at 2000 rpm.

Cervical mucous sampling is generally planned on day 13 or 14 of the menstrual cycle (ovulatory period), after abstinence from sex for 3 days and is performed using an "Aspiglaire catheter".

Blood sample: into empty tube (serum): the patient does not need to be fasting before the sample.

QUESTIONS FOR THE PATIENT

Semen or cervical mucus sampling:

– In men: have you followed the sampling conditions *(cf. above)?*

– In women: how long do your menstrual cycles usually last and if possible what has your temperature been taken in the morning before getting up from the start of the cycle (the temperature curve allows the ideal day to be identified)?

Test submitted on a pre-approved request (France), either:

– Testing for ASAb bound to spermatozoa

– Testing for circulating ASAb in seminal plasma and serum in men or in cervical mucus and serum in women. These tests must be performed simultaneously.

SAMPLE STORAGE AND TRANSPORT

Testing for ASAb bound to spermatozoa:

The test must be performed on "fresh" semen, 30 minutes after collection. The sample cannot be stored for later analysis.

Testing for circulating ASAb:

– Serum: frozen (minimum 0.5 ml).

– Cervical mucus: frozen (minimum 0.25 ml).

– Seminal plasma: frozen (minimum 0.5 ml).

ASSAY METHODS

– Testing for ASAb bound to spermatozoa

Direct method revealing IgG and IgA Antibodies bound to spermatozoa.

Mixed antiglobulin agglutination method (Mixed Antiglobulin Reaction Test or MAR test): IgG antibody - coated latex particles are mixed with anti-IgG antiserum. If specific antibodies bound to spermatozoa are present a mixed agglutinate of latex particles/spermatozoa forms.

– Testing for circulating ASAb

Indirect method to test for free ASAb in different biological fluids: serum, seminal plasma and cervical mucus.

Semen agglutination method:

Agglutination occurs in the presence of antibodies with two active sites which bind different spermatozoa to each site ("witness" spermatozoa). This method derived from the *Tray Agglutination Test* described by Friberg in 1974 is performed on a microtitre plate and provides an ASAb titre. Examining the agglutinates occasionally allows the location of the antigen against which the ASAb is directed to be identified (head to head agglutination or flagellum to flagellum agglutination).

NORMAL EXPECTED VALUES

– Testing for ASAb bound to spermatozoa

The test is deemed to be positive if more than 20% of the mobile spermatozoa are bound to at least one bead.

The result is negative if the percentage binding is less than 10%. The result is equivocal between 10 and 20%.

– Testing for circulating ASAb

In serum:

– Positivity threshold: 1/20.

– 1/20, 1/40, 1/80: low titres, to be interpreted in the clinical context.

– > 1/160: high titres, significant.

In seminal plasma and cervical mucus:

– Positivity threshold: 1/4.

– 1/4, 1/8, 1/16: low titres, to be interpreted in the clinical context.

→ 1/32: high titres, significant.

INTERPRETATION

The management of immunological subfertility depends on the level of immunisation, although this forms part of the overall assessment of the subfertility. The diagnosis of immunological subfertility is strongly suspected if more than 50% of spermatozoa bind to immuno-beads in the MAR test.

Treatment aims to reduce the titre of Ab (corticosteroid therapy is controversial) and generally involves MAF techniques ranging from intra-uterine insemination (if ASAb is found in cervical mucus) to conventional *in vitro* fertilisation with micro-injection when high titres of ASAb are present in semen.

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ANTITHROMBIN

DEFINITION

Antithrombin is a glycoprotein belonging to the Serpine family (Serine proteases inhibitors). It is synthesised by the liver and has a half-life of approximately 3 days.

Synonym: antithrombin III (former name).

PHYSIOLOGICAL ROLE

Antithrombin (AT) is the most potent of the physiological coagulation inhibitors. It mostly inhibits thrombin (activated factor II) and to a lesser extent other coagulation factors (IXa, Xa, and XIa). Its slow action is amplified in the presence of glycosaminoglycans (heparin and heparan sulphates). Binding of antithrombin to a heparin pentasaccharide structure (or heparan sulphates of vascular endothelial cells) results in a change in the conformation of the antithrombin which makes it approximately 3000 times more effective.

INDICATIONS FOR MEASUREMENT

Measurement seeks to establish deficiency of the protein and is indicated in documented deep vein thrombosis (DVT) and/or pulmonary embolism (PE) and/or arterial thrombosis, particularly in the following situations:

– Thrombosis occurring in a young subject (< 40 years old),

– Recurrent thromboses,

– Past family history of DVT/PE, particularly under 40 years old,

– Idiopathic thrombosis (no triggering factor) after a careful clinical investigation.

AT measurement may be performed in the acute phase of thrombosis before oral anticoagulation treatment is started. This allows the possibility to detect antithrombin deficiency which would require administration of AT concentrates jointly with heparin therapy. It can also be performed in an "aetiological assessment of thrombosis" distant to the event (when the patient is not receiving any heparin anticoagulation treatment).

Its systematic use before oestrogen-progestogen oral contraception or hormone replacement therapy for the menopause is not justified unless a well documented history of venous thromboembolism is present in the near family.

INFORMATION

SAMPLE

AT concentrations are normally lower in serum than in plasma (a difference of approximately 30%, varying depending on pathological conditions). Because of this, AT must be measured on 0.109 M 1/10 citrated plasma (0.5 ml per 4.5 ml of blood). 0.129 M citrate tubes are acceptable.

Outside of the emergency situation the sample is generally taken in the morning between 07:00 and 11:00. The subject should preferably be fasting and coffee, tobacco and alcohol must be avoided in the hour before the sample. A small fat free snack is permitted.

Generally, AT measurement must be performed at least 10 days after stopping heparin therapy. It may however be measured in patients receiving unfractionated heparin: in that situation the antithrombin level is very rarely less than 60% and deficiency may be suggested by a markedly reduced level (< 60%). AT may be measured in patients on AVK.

For more information, refer to the *("General haemostasis pre-analytical conditions")*.

QUESTIONS FOR THE PATIENT

Current treatment? Medicines causing a marked reduction in AT (approximately 30%):

– Tamoxifen.

– L-asparaginase for acute lymphoblastic leukaemias, non-Hodgkin's lymphomas, etc.

Medicines causing a slight reduction in AT (approximately 10%):

– Oestrogen-progestogens.

– Unfractionated heparin, low molecular weight heparins (very slight reduction with the latter).

Conversely, androgens and cortisol increase AT synthesis.

SAMPLE STORAGE AND TRANSPORT

Separated plasma can be stored for 2 weeks at – 20°C and 6 months at – 70°C. It is recommended that it be thawed quickly in a water bath at 37° C before measurement.

Transport:

Functional AT activity: Sample frozen within one hour following sampling.

AT antigen (or by weight): Sample frozen within 4 hours of sampling.

ASSAY METHODS

- **Assay of AT antigen:** Immunochemical assay by weight.
- **Assay of AT activity:**

  – Heparin cofactor activity assay is the first measurement performed to test for deficiency as it can detect all types (types I and II, cf. table). This usually involves a chromogenic assay (amidolytic technique).

  – If heparin cofactor activity is low (= 80%), AT antigen (or mass) measurement should be combined to differentiate type I deficiency, in which the antigen concentration is reduced, from type II deficiency (normal antigen concentration and low activity).

- **Assay of progressive activity**

  Any qualitative deficiency must be typed as the risk of thrombosis varies depending on the type of deficiency. Typing requires a so-called progressive activity method to be used measuring antithrombin or anti-factor Xa activity in the absence of heparin (slow reaction).

NORMAL EXPECTED VALUES

– Antithrombin activity: 80 to 120%.

– Antithrombin antigen (immunological method): 0.24 to 0.36 g/l.

Values may vary depending on the methods used.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

AT levels are reduced in pregnancy (approximately 15% reduction) and in newborn babies in whom levels of approximately 60% are found but are not complicated by thrombosis.

Normal values are reached after 6 months to 1 year.

PATHOLOGICAL VARIATIONS

Acquired deficiencies are seen in the following situations:

– Hepatic insufficiency: defective synthesis.

– Nephrotic syndrome: urinary loss.

– Disseminated intravascular coagulation: consumption.

– Septic shock.

– Treatment with tamoxifen, L-asparaginase, estrogen-progestogen with more than 30 µg of ethynil-estradiol, unfractionated heparin and, to a lesser extent, low molecular weight heparins.

Congenital deficiencies

The prevalence of congenital AT deficiencies has been estimated to be between 1/2000 and 1/5000 in the general population and between 1 and 2% in subjects who have had a DVT.

There are two types of deficiency:

– Quantitative deficiencies are the most common (80% of cases) where antigen and activity are reduced in parallel. In almost all cases these are heterozygote deficiencies (levels of approximately 50%) as homozygous status does not appear to be compatible with life.

– Qualitative deficiencies (approximately 20% of cases): 3 types are described in the table below.

All AT deficiencies are associated with a 50 factor increase in risk of venous thrombosis compared to the general population, except for type II HBS deficiency (cf. table) which is associated with a low risk of venous thrombosis in heterozygotes and a significant risk in homozygotes (very rare, only around ten cases described in the literature). AT deficiencies are also associated with increased risk of arterial thrombosis in adulthood and also in children.

Congenital antithrombin deficiencies

	Heparin cofactor activity (%)	Progressive activity (%)	Protein level (%)	thrombo-embolic risk
Type I: quantitative defect	↘	↘	↘	High
Type II: qualitative defect				
– Reactive site abnormality RS	↘	↘	Normal	High
– Heparin binding site abnormality HBS	↘	Normal	Normal	Low
– Heparin binding site and reactive site abnormality (pleiotrophic effect) PE	"limit" 70-80	"limit" 70-80	"limit" 70-80	High

A diagnosis of constitutional antithrombin deficiency must be followed by family cascade screening and a certificate is given to all members of the family with the abnormality by the doctor in France.

If thrombosis occurs in a patient with a deficiency, treatment involves an association of heparin and AT concentrates in order to maintain the AT level at 80% or above. This is switched early to anti-vitamin K agents. Prophylactic treatment for recurrent thrombosis is controversial in patients with asymptomatic deficiency. This is recommended in some at risk situations, particularly pregnancy and in the *post-partum* period.

Raised plasma AT concentration (apart from injection of AT concentrates) does not currently have any known clinical significance.

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APOLIPOPROTEINS

DEFINITION AND SYNONYMS

The apolipoproteins, still called apoproteins, form the protein part of lipoproteins which transport lipids (cholesterol, triglycerides) in blood. They play a role in the structure and metabolic enzyme activation of lipoproteins and are formed from several associated polypeptides, explaining their variety (10 main apolipoproteins are currently described). Their nature and quality vary depending on the circulating lipoproteins.

INTRODUCTION

- **Apolipoproteins A:** Apo A1 and A2 are the commonest Apo A. They are the main protein fraction of HDL and are synthesised by the liver and intestine. Apo A1 is a marker of protection against cardiovascular diseases and returns cholesterol to the liver by activating LCAT (lecithine cholesterol-acyl-transferase). Apo A2 appears to have a limited role (it is believed to be an activator of hepatic lipase).
- **Apolipoproteins B:** At present there are 2 forms of Apo B: Apo B48 which is present in chylomicrons originating from the intestine and Apo B100 in LDL and VLDL originating from the liver. Apo B takes part in the transport and deposition of cholesterol in arterial walls. It is a marker of atherosclerosis, responsible for cardiovascular diseases (particularly myocardial infarction).
- **Apolipoproteins C:** These are synthesised in the liver and exist in several isoforms: Apo C1, Apo C2 and Apo C3. They have a poorly understood pathophysiological role and a relatively complex metabolic role. Apo C2 is believed to be a physiological activator of lipoprotein lipase (LPL) and Apo C3 to be an inhibitor of LPL and hepatic triglyceride lipase.
- **Apolipoproteins E:** Are synthesised by the liver, intestine, brain, kidney and adrenal glands. They have 3 main isoforms: E2, E3 and E4 which play a minor structural role but are involved in the binding and regulation of the catabolism of cholesterol and triglyceride rich lipoproteins. Apo E3 is the commonest form. The E4 isoform is currently believed to be a risk factor for Alzheimer's disease.

INDICATIONS FOR MEASUREMENT

Assessment of cardiovascular risk for Apo A1 and B. These measurements are not justified in the follow up of treated stable hyperlipidaemia for which measurement of cholesterol and triglycerides is sufficient.

Risk factor for Alzheimer's disease for Apo E (E4 allele).

INFORMATION

SAMPLE

Sample of venous blood collected into a dry tube. Plasma samples are not recommended. Patients must be fasting for 12 hours and not have suffered a recent acute illness or not have been pregnant recently.

QUESTIONS FOR THE PATIENT

Age?

Pregnancy?

Dietary habits?

Risk factors (smoking, hypertension, etc)?

Current treatment?

SAMPLE STORAGE AND TRANSPORT

Serum samples can be stored at +4°C for one week, beyond which they must be frozen at –15 and –20°C. Once frozen, samples can only be thawed once.

ASSAY METHODS

Liquid precipitation methods (nephelemetry, turbidimetry) are the most widely used as they are highly sensitive, rapid, automated and can be run in large batches. On the other hand, large amounts of triglycerides on the sample interfere with these methods. They are used above all to measure Apo A1 and B.

Gel precipitation methods (Mancini radial immunodiffusion technique or Laurell electroimmunodiffusion (EID) are easy to perform and are used particularly for Apo E measurement. Hypertriglyceridaemic samples also cause problems with these methods when used to measure Apo A1 and B.

Radioimmunological methods are highly specific.

Enzymoimmunological methods are highly sensitive.

The results of these different methods are still difficult to compare between laboratories. Therefore, measurement standardisation efforts are currently going on internationally (IFCC). Apo A1 and Apo B measurements cannot be interpreted in exclusion from the remainder of the lipid profile.

NORMAL EXPECTED VALUES

For reference:

Apo A1: Men: 1.20 to 1.60 g/l
Women: 1.30 to 2.10 g/l

Apo B: Men/Women: 0.45 to 1.35 g/l

Apo C3: Men/women: 16 to 45 mg/l

Apo E: Child: 0 to 76.0 mg/l
Men: 13.0 to 76.0 mg/l
Women: 0 to 94 mg/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

- **Age:** Apo A1 and Apo B concentrations rise with age.
- **Sex:** Apo A1 concentrations are higher in women and fall after the menopause. Apo B concentrations are lower in women until the menopause.
- **Pregnancy:** Lipid abnormalities must not be investigated during pregnancy or for up to 3 months after delivery.
- **Medicines:** These can influence apolipoprotein measurements, in particular, oestrogens increase HDL levels, progestins reduces HDL and corticosteroids increase HDL and triglycerides.

PATHOLOGICAL VARIATIONS

- **Apo A1 and B:** Apoprotein A1 reflects the concentration of anti-atherogenic lipoproteins. Apo B is a marker of atherogenicity. For this reason the two measurements can not be dissociated.
- **Apo A1** falls in hypertriglyceridaemia and in mixed hyperlipidaemia, in severe liver disease (hepatitis, cirrhosis, cancer), in inflammatory states (major burns, multiple trauma), in chronic renal insufficiency and in hypothyroidism. It rises in familial hyperlipoproteinaemias and hyperthyroidism. A raised Apo A1 is not necessarily a protective factor against cardiovascular risk although low values represent an additional risk.
- **Apo B** increases in primary hypercholesterolemia and mixed hyperlipidaemias, in some secondary hypercholesterolaemias such as type II diabetes or hypothyroidism and in cholestasis (when Apo B rich LPX is present). Concentrations are raised in subjects who have had cardiovascular damage or who are at risk of cardiovascular damage. On the other hand it may fall in acute hepatic insufficiency and hyperthyroidism. Values are very low in hypobetalipoproteinaemia and zero in abetalipoproteinaemia (a rare disease).
- **Apo C3** is low in hereditary deficiencies and in Tangier's disease. It is increased in types III, IV and V hyperlipidaemia.
- **Apo E:** The E2 phenotype is found in subjects suffering from type III hyperlipidaemia

  The E4 phenotype is believed to be a cardiovascular risk factor by promoting LDL removal and deposition. It also appears to be a risk factor for Alzheimer's disease although it is not a predictive factor (Apo E4 has been found in 92% of patients with an early form of the disease and in 60% of patients who develop a late form).

FOR FURTHER INFORMATION

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ARBOVIRUS

DEFINITION

Arboviruses are anthropozoonoses common to human beings and many vertebrates (mammals, birds, reptiles, amphibians). The arboviruses group together more than 500 species which belong to around ten different families: *Togaviridae, Flaviviridae, Réoviridae, Rhabdoviridae, Bunyaviridae, etc*. They are RNA viruses with a heterogeneous structure transmitted by a bite from blood-sucking arthropods, such as mosquitoes, flies, ticks or mites (arbovirus derives from the English "***Ar***thropod ***Bo***rne ***Virus***". Chikungunya, which was recently in the news because of an epidemic infecting la Réunion in the austral summer of 2005/2006, is a tropical infectious disease due to an arbovirus (alphavirus belonging to the *Togaviridae* family) transmitted by mosquitoes from the *Aedes* genus. Two strains are currently described in the world with one in Asia and the other in Africa.

INTRODUCTION

The main reservoirs of the virus are animals (rodents, monkeys and birds) and human beings are an accidental host. The arthropod vector becomes infected when feeding on blood, ingesting the viruses which multiply and concentrate in its salivary glands and are transmitted to a new animal or to human beings at the time of another bite. Arboviruses are present throughout the whole world, particularly in tropical areas but also in temperate areas. Their geographical distribution depends on the vector and animal reservoir. There are indigenous arboviruses and other so called "imported" viruses. Some viruses may be seen in France including the ***TBEV or tick-borne European Encephalitis virus*** in the East and the ***WNV or West Nile virus*** around the Mediterranean basin. The arboviruses develop as endemic or epidemic diseases.

SYMPTOMS

The arboviruses cause acute infections of varying severity, ranging for most, from simple asymptomatic disease to severe clinical presentations. Generally, infection begins with sudden onset of acute fever which resolves spontaneously. The established phase of the disease, which develops after a short of remission phase, varies depending on the arbovirus in question, such as haemorrhagic fevers (yellow fever), encephalomeningeal forms (Japanese encephalitis, West Nile fever etc.), hepatorenal syndrome or a return to an acute fever or flu-like syndrome (dengue fever). The features may be those of an isolated syndrome or several concomitant syndromes. The Chikungunya virus causes an acute painful febrile syndrome. According to the WHO this is a form of Dengue characterised by high fever persistent for 2 or 3 days followed by a maculopapular rash, muscle aches and intense joint pains, mostly affecting the limb extremities (wrists, ankles, phalanges).

SEARCH INDICATIONS

Diagnosis of arbovirosis in a patient returning from a stay in an endemic area presenting with compatible clinical signs.

Differential diagnosis from other viruses responsible for haemorrhagic fever or meningeal disease.

Aetiological diagnosis of benign encephalitis in a patient bitten by a tick in the East of France or by a mosquito in the South Mediterranean area.

INFORMATION

SAMPLE

Cerebrospinal fluid: If the clinical picture is of encephalitis or meningism.

Peripheral blood: To be taken during the acute viraemic phase.

Serum: For serological diagnosis.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

Recent stay in an endemic area?

History of bite from an arthropod?

Recent transfusion?

Vaccination (yellow fever, tick-borne encephalitis, Japanese encephalitis)?

SAMPLE STORAGE AND TRANSPORT

Samples for direct diagnosis are sent to specialist laboratories equipped with L3 or L4 type confinement premises. The sample must be accompanied by an information form. They must be sent in accordance with national and international legal procedures to ensure safety (triple bagging). Blood samples should be sent within a period of 24 to 48 hours. CSF should be sent as soon as possible. Prior to transport, samples should be stored at +4°C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

- **Isolation of virus** by cell culture is performed in a few specialist laboratories from assorted samples guided by clinical symptoms, usually from peripheral blood taken during the viraemic phase. The samples are inoculated into litters of newborn mice.
- **Identification of virus** may be performed using specific immunological reactions. Molecular biology techniques such as testing for viral genome by specific PCR provides a rapid acute phase diagnosis.

SEROLOGICAL DIAGNOSIS

This is the most widely used routine method despite its interpretation limits. The available techniques are: IHA, complement binding, sero-neutralisation but above all, ELISA (immunocapture) to test for specific IgM and IgG.

TREATMENT

Curative treatment is symptomatic only with analgesics and antipyretics given in uncomplicated forms of the disease. Forms of the disease with encephalitis require intensive resuscitation techniques with transfusions of fresh blood or concentrated platelets in haemorrhagic disease. Prevention of arboviroses is based on control of the vectors, monitoring for outbreaks of epidemics, vaccination of exposed people (antimalarial, vaccination against the TBE virus and against the Japanese encephalitis virus) and vaccination of domestic animals.

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ARSENIC

DEFINITION

Arsenic (As) is a metalloid found widely throughout the crust of the earth. Behind lead and mercury, arsenic is the third major environmental poison. Contamination of underground water collections and the water supply with mineral arsenic is a serious public health problem in some parts of the world where arsenicism is endemic.

It is used mostly in industry and in metallurgy arsenic is present as an impurity in many minerals (copper, zinc, lead, tin, cobalt and gold, etc) it is also used or found in glass, semi-conductors, pigments, wood treatments, pesticides and tannery industries.

Acute poisoning with this element is increasingly rare as its use has fallen very greatly over the last few decades, in suicides where people have access to arsenic though their occupation or in its use in crime.

Arsenic is also present in organic form (particularly arsenobetain) in large amounts in shellfish, crustaceans and fish. This form is far less dangerous to health and is easily excreted by the body and any raised result found in biological media, must therefore be confirmed by post-speciation analysis to differentiate non-toxic dietary arsenic from toxic mineral arsenic.

METABOLISM

The absorption of arsenic depends on its solubility, granulometry and chemical species. Approximately 80% of the amount ingested (from dirty hands or diet) is absorbed in the intestine and it can be absorbed through the lung, particularly with occupational exposure from dust and fumes, and to a small extent through the skin. Arsenic is rapidly distributed throughout the body and binds to proteins, accumulating the liver, muscle, skin and integumentary system. Its half life in blood is 7 days.

Approximately 70% of the inorganic compounds are rapidly excreted in the urine in the form of monomethylated (25%), dimethylated (50%) and unchanged (25%) products. Once exposure ceases arsenic concentrations fall by approximately 10 to 20% per day. It is also excreted in the bile and through the integument (its accumulation in these tissues can provide a retrospective diagnosis).

Organic arsenic is mostly excreted unchanged in the urine.

MECHANISM OF ACTION

The trivalent salts ($As^{3}+$) are far more toxic than the pentavalent compounds ($As^{5}+$): $As^{3}+$ binds to thiol groups causing thiol deprivation asphyxia which is responsible for many metabolic and organic effects.

Mineral arsenic derivatives have carcinogenic and mutagenic properties.

SYMPTOMS

■ ACUTE POISONING

Symptoms begin 20 minutes to 12 hours after ingestion. Symptoms are mostly gastrointestinal with abdominal pain, vomiting and, occasionally bloody diarrhoea (arsenical cholera), accompanied by hypotension with water and electrolyte disturbances as a result of water loss. Impaired liver, kidney and heart function also occur with occasionally fatal dysrythmias. Recovery is accompanied by neurological disorders in survivors.

■ CHRONIC POISONING

The main symptoms are skin lesions (melanoderma, palmoplantar hyperkeratosis, skin cancers), mucosal and integument lesions, impaired neurological and liver function (degenerative liver damage which may result in cirrhosis), cardiovascular damage and unequivocal carcinogenic effects.

INDICATIONS FOR MEASUREMENT

Blood arsenic measurement is not widely used in the occupational context (outside of accidental situations). It reflects recent exposure but its correlation with the extent of exposure has not been clearly established.

End of shift and end of week urine arsenic measurements reflect exposure to the inorganic compounds during the week. Speciation (a treatment used to separate different forms of As) is the only means of removing dietary organic As and separating mineral As and the methylated and dimethylated derivatives, the only compounds which correlate well with the extent of exposure.

Urine arsenic measurement however does not allow to assess exposure to gallium or indium arsenide which are only slightly metabolised and excreted extremely slowly.

INFORMATION

■ SAMPLE

5 ml of whole heparinised blood; 20 ml unacidified urine sample.

Whole blood: End of shift sample, regardless of day.

Urine: Sample taken at the end of shift and at the end of week.

■ QUESTIONS FOR THE PATIENT

Information about any shellfish, crustaceans and fish eaten within 48 hours before the sample as this may result in interference from dietary arsenic with the mono and dimethylated arsenic result because of spontaneous partial demethylation of the dietary trimethylated compounds.

■ SAMPLE STORAGE AND TRANSPORT

Whole blood samples can be stored and transported to the laboratory at room temperature.

Urine samples can be stored and transported to the laboratory at room temperature or between + 2 and + 8°C.

ASSAY METHODS

Electrothermal atomisation atomic absorption spectrophotometry (graphite furnace) and Zeeman correction with hydride generation.

Induced plasma source atomic emission spectrophotometry.

Induction coupled plasma mass spectrometry (ICP-MS).

Speciation is performed by high performance liquid chromatography (HPLC).

REFERENCE VALUES

Maximum acceptable concentration in water for human consumption: < 10 µg/l.

Reference value in the general population:

Blood arsenic: < 10 µg/l.

Inorganic arsenic + urinary mono and dimethylated derivatives: < 10 µg/g creatinine or 10 µg/l.

Urinary inorganic arsenic (As 3+ and As 5+) < 2.2 µg/l.

– French guideline value (occupational exposure):

Urinary metabolites of urinary inorganic arsenic = 50 µg/g creatinine at end of week.

– American ACGIH value (BEI) = for exposure to arsenic and its soluble inorganic compounds: inorganic arsenic + urinary methylated metabolites = 35 µg As/l at end of week.

FOR FURTHER INFORMATION

■ Guide BIOTOX 2001, INRS.

■ *Exposition chronique à l'arsenic hydrique et risques pour la santé*, IVS, Département santé environnement. invs.santé.fr

■ Toxicologie industrielle et intoxications professionnelles, *Arsenic*, Lauwerys. R. 3rd edition, MASSON; p 119-128.

ASCARIDIOSIS

DEFINITION

Ascaridiosis is an obligate human parasitosis which is found very widely throughout the world. It is associated with poor hygiene conditions and because of this occurs particularly in tropical areas and in children.

It is due to the presence of a nematode belonging to the ascarididae order: *Ascaris lumbricoides*.

– ***The adult*** is a round worm. Its transversely striated cuticle is white-pink in colour (if alive) and dark and lustrous (if dead). The sexes differ and the male measures 15 to 20 cm and has a cross curved posterior end. The female is longer than the male and may reach 20 to 25 cm and has a rectilinear posterior end.

– ***The egg*** is ovoid, measuring 50 to 75 microns long and 40 to 50 microns wide. It has a double brown coating. The internal coating is smooth and yellowish in colour, the external coating is thick, albuminous and mamillated. A single central germ cell is found inside and it is not embryonated when laid.

INTRODUCTION

EPIDEMIOLOGY

Ascaridiosis is cosmopolitan and is endemic in hot wet third world countries where it is responsible for high infant mortality. Its prevalence is believed to be as high as 90% of the population in tropical countries including 70% of children.

Human contamination occurs in food (vegetables or fruits) in contact with the ground, drinking water or hands soiled with embryonated *Ascaris* eggs.

LIFE CYCLE

Infestation occurs as a result of ingesting eggs containing an L2 infesting larva. Once released in the small intestine, the larva crosses the intestinal mucosa reaching the liver in 3 to 4 days. It then affects the right heart, lung and pulmonary alveoli at around day 8. It rises through the bronchial tree to the pharynx and may then return to the gastro-intestinal tract during swallowing or in a reflex cough.

The larva (L2) undergoes a final casting (L3) and becomes an adult in the small intestine. The adult worms spend 12 to 18 months in the intestine where they feed on intestinal contents. The fertilised females then lay thousands of eggs which are excreted in faeces into the external environment approximately 60 days after contamination. The eggs which are laid are not embryonated, making self-infestation impossible.

In order to become embryonated (L1) and infesting (L2) they need to spend time in a wet external environment at a high temperature (28°C-30°C) for several weeks. The embryonated eggs are highly resistant in the external environment and may remain there for several years.

SYMPTOMS

Clinical symptoms vary. Infestation is generally asymptomatic in temperate countries with good hygiene and is only detected on a systematic stool examination or following a fortuitous finding of a raised eosinophil count.

When expressed clinically, the disease develops in 2 successive phases:

Invasion phase

This represents the period of larval migration and is dominated by pulmonary symptoms constituting Loeffler's syndrome. This is a combination of episodes of coughing occasionally accompanied by slight dyspnoea and mild fever (approximately 38°C). Expectoration contains eosinophilic granulations, Charcot-Leyden crystals and very rarely larvae. It also includes a poorly delineated and above all fleeting pulmonary infiltrate which disappears over 8 to 15 days.

Established disease phase

This represents the presence of adult worms in the small intestine and is dominated by common gastro-intestinal disorders, such as abdominal pain, diarrhoea, nausea or vomiting. Nervous signs such as sleep disorders or irritability are common in children. Finally, allergic symptoms such as pruritus or Quincke's oedema may occur.

Surgical complications may develop with massive infestation, particularly in children, and are due to the erratic location of the worm in the biliary, pancreatic or intra-hepatic ducts or in the appendix causing acute pancreatitis, appendicitis or peritonitis. Large numbers of worms in the small intestine can cause intestinal obstruction.

SEARCH INDICATIONS

Diagnosis of ascaridiosis in a subject living in, or having spent time in a tropical area, presenting with gastro-intestinal and pulmonary disorders.

Diagnosis of ascaridiosis in the assessment of an isolated raised eosinophil count.

Differential diagnosis with other faecal-transmitted parasitoses, such as amoebiasis, giardiasis, teniasis, etc

INFORMATION

SAMPLE

Stool, in order to test for eggs. The sample should be collected into a clean dry container, preferably at the laboratory.

Blood, collected into EDTA for a blood count and differential cell count.

Serum (Dry tube) for serological diagnosis or to measure total IgE.

QUESTIONS FOR THE PATIENT

Clinical symptoms and date of onset?

Return from a stay in a tropical or inter-tropical area?

Current treatment?

SAMPLE STORAGE AND TRANSPORT

Serum: Store at +4°C.

Stool: If the stool sample cannot be collected in the laboratory, it must be sent there as quickly as possible. Avoid exposure to heat or cold when the stool is transported.

DIAGNOSTIC METHODS

■ DIRECT DIAGNOSIS

This involves:

– Direct fresh examination without staining

– Examination after staining, using the sedimentation technique (Bailenger, MIF) or flotation technique (Junod) or by concentration (Kato).

The following are performed:

– Macroscopic examination of stool, examining for adult parasites.

– Microscopic examination of stool, examining for characteristic eggs.

The typical Ascaris eggs are easily recognisable because of their mamillated appearance although atypical eggs such as unfertilised eggs (which vary in size and shape) or eggs lacking their external coating, which are easy to confuse with plant cells or ankylostoma eggs, are commonly seen.

■ INDIRECT DIAGNOSIS

Antibodies develop 1 week after the first contamination and increase, reaching a maximum titre at 15 days to 3 weeks later and then fall, becoming negative as soon as eggs appear in the stools.

The most widely used serological methods are immunoelectrophoresis, electrosyneresis or indirect immunofluorescence. These use the *Ascaris suum* (pig ascaris) antigen and produce many cross-reactions with other helminthiases (toxicariosis, anguillulosis, filariasis, etc.).

■ NON-SPECIFIC DIAGNOSIS

– Raised eosinophil count (hypereosinophilia), which develops 1 week after first infestation reaching a peak at around day 20 and becoming negative in the adult stage.

– Hyperleukocytosis: From 10000 to 20000/mm^3.

– Charcot-Leyden crystals in stools and sputum.

– Increased total IgE reaching extremely high values in extensive infestation or when infestation becomes chronic.

INTERPRETATION

During the invasion phase: Hypereosinophilia, hyperleukocytosis, direct diagnosis impossible, indirect diagnosis can be performed.

During the established disease phase: Only direct diagnosis with stool parisitology examination is possible, approximately 2 months after contamination. The unequivocal diagnosis is made by finding eggs or far more rarely an adult worm in stool or vomit or finding a larva in sputum.

TREATMENT

Medical treatment: This involves anti-parasite chemotherapy with 3 days of flubendazole or a single dose of albendazole, ivermectin or pyrantel.

Surgery: Is required for complications. The effectiveness of treatment is monitored by a stool parisitology examination two months after treatment is completed.

Prophylaxis is based mostly on individual hygiene measures (hand washing, cleaning fruit and vegetables in contact with the ground) and community measures (sanitation measures, regulations on the use of human manure, preventing faecal contamination, mass population treatment).

FOR FURTHER INFORMATION

■ Cassaing S., Morassin B., Magnaval J.-F., *Encycl Med Chir 2003*; VIII003

■ Mougeot G., *Conduite des examens en parasitologie*, editor Masson, Paris, 1995: p. 20 to 21

■ Kamoun P., Fréjaville J.-P., *Ascaris: Ascaris lumbricoides*. Guide des examens de laboratoire, 4th edition, Paris, Flammarion, 2002: p 210 to 214.

ASPERGILLOSIS

DEFINITION

The aspergilloses are opportunistic cosmopolitan mycoses due to proliferation within the body of filamentous fungi from the Aspergillus genus which belongs to the Ascomyceti class and the Aspergillacae family. There are approximately 300 species of Aspergillus, 5 of which are pathogenic to human beings. Aspergillus fumigatus is responsible for 80 to 90% of human aspergilloses; *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Aspergillus nidulans* are involved more rarely.

INTRODUCTION

EPIDEMIOLOGY

The *Aspergilli* are ubiquitous fungi found widely throughout the ground and in decomposing organic material (hay and grain); spores are often present in ambient air.

Human beings are affected mostly through the respiratory tract by inhaling spores carried in the wind and some occupations are particularly exposed such as pigeon rearers, farm workers and millers. Direct infection is possible but rare.

PATHOPHYSIOLOGY

The *Aspergilli* are opportunistic pathogenic fungi and their growth in human beings implies that factors which predispose to their development are present.

– ***Local factors:*** Pre-formed cavity, chronic obstructive pulmonary disease, bronchopulmonary cancer, emphysema.

– ***General factors*** associated with immunosuppression; malignant blood diseases, AIDS, immunosuppressant treatments, corticosteroid therapy, etc.

– ***Environmental factors:*** The number of spores circulating in the air varies by place and by season.

– ***Virulence factors:*** Small spore size, heat tolerance, presence of host protein adhesion molecules, production of toxins and proteases, vascular tropism of the *Aspergilli.*

SYMPTOMS

The *Aspergillus* are above all respiratory pathogens.

Pulmonary aspergilloses:

Immuno-allergic aspergilloses

– *Aspergillar asthma* is due to an allergic reaction to aspergillar spores in an atopic subject.

– *Allergic bronchopulmonary Aspergillosis (ABPA)* occurs in asthmatics or those suffering from cystic fibrosis as asthma-like attacks associated with fleeting pulmonary infiltrates.

– *Extrinsic allergic alveolitis (EAA)* is an alveolar pneumonitis associated with extensive spore inhalation.

Pulmonary aspergilloma

This is the development of a mycelial mass occurring in a bronchus or pre-existing lung cavity (tuberculus cavity, abscess or cyst) in the form of a ball called an "aspergillous truffle". Clinical symptoms are dominated by cough and haemoptysis. Radiological examination shows a characteristic bell sign.

Invasive pulmonary aspergillosis (IPA)

This affects immunosuppressed people, particularly patients receiving aplasia-inducing chemotherapy prior to a transplant (heart, kidney or bone marrow) or patients suffering from malignant blood diseases. The invasive nature of the infection rapidly results in mycelial proliferation throughout the pulmonary or extra-pulmonary parenchyma, or even throughout the body and is often fatal. IPA can be diagnosed early by a chest CT scan.

Extra-pulmonary aspergilloses

Pleural aspergillosis, aspergillar endocarditis, otomycoses, sinus aspergillosis, skin damage, aspergillar keratitis, aspergillar onyxis, impaired cerebral function, bone damage.

SEARCH INDICATIONS

– Diagnosis of classical pulmonary aspergillosis.

– Early diagnosis of invasive form of the disease in an immunosuppressed subject.

– Diagnosis of extra-pulmonary forms of aspergillosis.

– Differential diagnosis from other mycoses (Mucorale, *Candida, Nocardia, Fusarium*).

INFORMATION

SAMPLE NATURE AND DETAILS

– *Nasopharyngeal samples, bronchial secretions, trans-tracheal puncture-aspiration, broncho-alveolar lavage, protected bronchial brushings:* Collected into sterile containers for mycological diagnosis.

– *Bronchial or pulmonary biopsies:* Intended for histological diagnosis.

– *Serum:* To test for antibodies in immunocompetent subjects or antigen in the immunosuppressed.

– *Other samples:* Sinus, urine, CSF, pleural fluid etc, intended for mycological diagnosis.

QUESTIONS FOR THE PATIENT

– Clinical symptoms?

– Reduced immune defences?

– Are cytotoxic drugs being taken?

– At risk profession (size of inoculum)?

– Current anti-mycotic treatment?

SAMPLE STORAGE AND TRANSPORT

The samples must be collected under strict aseptic conditions (to avoid contamination) and transported promptly to the laboratory.

Store serum at + 4°C.

BIOLOGICAL DIAGNOSTIC METHODS

MYCOLOGICAL DIAGNOSIS

Direct macroscopic examination: Provides information about the disease depending on the site of isolation and appearance of the lesions.

Direct microscopic examination: Examination for septated and branched mycelial filaments or more rarely aspergillar heads which are characteristic of the *Aspergillus* genus.

This is a fundamental finding to confirm the diagnosis.

- **Culture:** On Sabouraud medium, with or without chloramphenicol at 37°C and possibly reinseminating the colony onto malt agar (to stimulate fruit body formation). The appearance and colour of the colonies obtained combined with microscopic examination allow to identify the species of aspergillous in question.
- **Antifungigram:** The MIC are measured (minimum inhibitor concentrations) on solid medium (E test®)
- **Molecular biology:** DNA detection from biological samples using gene amplification techniques and strain typing are not yet routinely available.

IMMUNOLOGICAL DIAGNOSIS

- **Testing for specific antibodies:** This is useful in the diagnosis of aspergilloma, in ABPA, EAA and in chronic necrotising pulmonary aspergillosis. The methods used are precipitation or indirect haemaglutination techniques. In practice a haemaglutination or electrosyneresis screen is generally performed, with confirmation of positive results by immunoelectrophoresis (IEP). ELISA tests are available.
- **Testing for circulating galactomannane antigens:** This is useful for the early diagnosis of IPA and is performed by latex particle agglutination or, better, by the ELISA technique which is more sensitive. If deep infection is suspected, the test should be performed once to twice weekly.

GUIDING DIAGNOSIS:

– *Raised eosinophil count (hypereosinophilia)* in an active exacerbation of ABPA.

– *Total and specific IgE is* increased in allergic reactions, such as aspergillar asthma or ABPA.

RESULTS

The diagnosis of aspergillosis is based on clinical, radiological and laboratory findings.

The biological diagnosis of aspergillosis is influenced by the type and quality of the sample and by the patient's clinical context.

The mycological diagnosis can provide information supporting aspergillosis: Positive direct examination, repeated isolation of large numbers of the same strain of aspergillus, isolation of one or more colonies in a neutropenic subject. Finding the fungus in a sterile biological fluid confirms infection.

Serological diagnosis by detecting galactomannan antigen is very useful in IPA. The effectiveness of treatment can also be monitored by monitoring antigenaemia.

The presence of more than three IEP arcs and positive catalase activity can guide the diagnosis of deep aspergillosis.

TREATMENT

– Diffuse forms: IV amphotericin B or fluorocytosine, or both in combination.

– Aspergillar bronchitis, necrotising bronchial aspergillosis, ABPA: Itraconazole

– Bronchial aspergilloma: Surgery, with or without medical treatment.

FOR FURTHER INFORMATION

- O.Morin, *Aspergillus,* Encycl Med Biol, Elsevier Paris, 2003.
- M.Gentilini, *Aspergillose*. Dans: Médecine tropicale, 5th edition, Paris, Flammarion 1993: 268-270.

ASTROVIRUS

DEFINITION

It was in 1975 when Madeley and Cosgrove observed under electron microscopy and described for the first time Astroviruses in diarrhoea stools from children. They are the only genus of the *Astroviridae* family and were named as such because of their five or six pointed star shape morphology. They are unenveloped viruses from 28 to 30 nm in diameter, the capsid of which exhibits icosahedric symmetry. Their genome is a single strand positive polarity and 3', polyadenylated RNA which has 3 open reading cartridges. It codes for non-structural viral proteins (a serine protease and an RNA-dependent RNA polymerase) and 2 to 5 structural proteins which carry the antigenic determinants, some of which are common to all of the human astroviruses (group antigens) and others allowing the 8 currently known human serotypes to be identified, of which serotype 1 is the most common.

Astroviruses are highly resistant and keep their infectious properties for 6 to 10 years when stored at –70°C.

INTRODUCTION

■ EPIDEMIOLOGY – PATHOPHYSIOLOGY

Astroviruses are ubiquitous viruses which are transmitted between human beings faeco-orally (water and food). They are responsible for 2 to 9% of cases of community-acquired acute childhood gastroenteritis and may be responsible for nosocomial infections and infections in communities. The infections are sporadic or epidemic with a winter seasonal peak in temperate countries or during the wet season in tropical countries. They mostly affect children under 5 years old (according to serology prevalence studies, 50 to 80% of children under 5 years old have been in contact with the virus), the elderly (antibodies may disappear late in life) and the immunosuppressed. Their incidence is probably underestimated. Astroviruses infect mature enterocytes (small intestine), M cells and Peyer's patches; the pathophysiology of the infections in human beings is poorly understood.

■ SYMPTOMS

After an incubation period of 3 to 5 days, infection results in vomiting, watery diarrhoea, (non-abundant) and fever in 80% to 100% of cases, accompanied by moderate abdominal pain and rarely by respiratory symptoms. The illness usually recovers spontaneously over 2 to 3 days.

SEARCH INDICATIONS

In the presence of suggestive clinical or epidemiological features, aetiological diagnosis of acute gastroenteritis:

Community-acquired gastroenteritis rarely requires a laboratory diagnosis although it may be indicated when uncertainty of the cause is present, particularly in children who are vulnerable due to pre-existing disease or during hospitalisation.

In hospitalised patients (nosocomial infection).

Food or water origin (food poisoning).

The diagnosis, however, is not widely made and is mostly found from epidemiological studies.

INFORMATION

■ SAMPLES

Stool samples taken during the acute phase of the illness into a sterile pot with no specific transport medium.

■ SAMPLE STORAGE AND TRANSPORT

Can be sent to the laboratory at room temperature. Storage recommended at + 4°C (for 72 hours). Beyond this time the sample can be frozen at - 30°C.

DIAGNOSTIC METHODS

Routine:

– ELISA method: Direct testing for viral antigens in a stool sample.

Others:

– Electron microscopy: This can test for other viruses which cause gastroenteritis (calicivirus, rotavirus, enteric adenoviruses), although it is a cumbersome, expensive and relatively insensitive method.

– Culture: Usually on CaCo-2 cells in the presence of trypsin (used for research).

– RT-PCR: Testing for the viral genome in stool after reverse transcription and gene amplification (epidemiological studies). Astroviruses are usually typed by RT-PCR on samples which are positive or equivocal by ELISA, followed by sequencing.

RESULTS

The finding of an astrovirus in a patient suffering from acute gastroenteritis provides an aetiological diagnosis although it does not exclude the presence of other concomitant pathogens which may also be involved in the infection. Conversely, the negative result does not formally exclude diagnosis as excretion of virus in stools is short-lived. In epidemics of food or water borne gastroenteritis, a full virological investigation is required including identification of the agent responsible in patients' stools, virological analysis of the contaminated water or food, testing for virus in people who took part in preparing the food and comparison of strains. Rotaviruses, caliciviruses, adenoviruses and astroviruses are tested by ELISA or RT-PCR in patients' stools. Once the virus is detected it must be genotyped to compare the strain or strains found in patients to those in the food or water.

There is no specific treatment for astrovirus gastroenteritis. Prevention is based on specific hygiene measures.

FOR FURTHER INFORMATION

■ Garbarg-Chenon A., *Astrovirus*. Encycl Méd Biol (Elsevier, Paris), 2003.

■ Pothier P., *Virologie des affections intestinales*. 19th Corata Congress, Lyon 2002.

BARTONELLOSIS

DEFINITION

Bacteria from the genus *Bartonella* are part of the alpha-proteobacteria, the Bartonellaceae family similar to rickettsia and brucella. They are small aerobic, generally oxidase and catalyse negative (a few are weakly positive) gram negative, polymorphic, non-acid alcohol resistant bacilli or coccobacilli, often immobile except for a few species (*B. bacilliformis, B. clarridgeiae*) which do not metabolise sugars and are difficult to culture. *Bartonella* are facultative intracellular bacteria and obligate human and animal parasites.

31 species of *Bartonella* are currently recognised, several of which have been associated with human infection, primarily *B. bacilliformis, B. quintana and B. henselae (see table)*. The bacteria multiply firstly in vascular wall reticulo-endothelial cells and then pass into the circulation where they infect red blood cells and may spread around the body. The reservoir for the organisms is generally mammals and they are transmitted through a vector. They can cause serious infections if immune defences are reduced.

Species	Reservoir	Vector	Human infection	Location
B. bacilliformis	Human	*Diptra*	Peruvian Oroya Verruga fever (Carrion's disease)	*Andes*
B. quintana	Human	*Body louse*	Trench fever Bacillary angiomatosis Endocarditis	*Worldwide*
B. henselae	Cat	*Cat flea*	Cat scratch disease Bacillary angiomatosis Hepatic peliosis Endocarditis – Retinitis	*Worldwide*
B. clarridgeiae	Cat	*Cat flea*	Cat scratch disease	*Europe* *America*
B. elizabethae	Rat	*Flea (?)*	Endocarditis	*America*
B. grahamii	Mouse	*Flea (?)*	Endocartidis, Uveitis	*Europe*

INTRODUCTION

EPIDEMIOLOGY

Each species of Bartonella appears to have a natural host in which the organisms may cause symptomatic bacteraemia. The geographical distribution is governed by the presence of the reservoir and vector. Human transmission occurs mostly from the host reservoir either directly or through a blood-sucking arthropod vector (mosquito, flea, louse), or, more rarely, from being pricked by an infected spine or splinter as the bacteria are fairly resistant in the external environment.

SYMPTOMS

Infection with *Bartonella bacilliformis*

This is strictly a human infection and is transmitted by haematophagic agents *(Lutzomyia verrucarum or sandfly)* and occurs mostly in the Andes (Colombia, Equateur, Peru, Chile, and Bolivia). After an acute primary infection (fever and myalgia) which often recovers spontaneously, the disease may present in two different forms:

Oroya fever occurs as a result of massive red blood cell infection. This is characterised by intermittent fever with profound malaise, lymphadenopathy, hepato-splenomegaly and above all a very profound anaemia. Its clinical course ends with severe cellular immunodeficiency and without treatment the mortality rate ranges from 40 to 85 per cent.

"Peruvian wart" or "verruga peruana" occurs as a result of infection and then proliferation of endothelial cells. It is characterised by a muco-cutaneous rash containing verrucal, pseudotumour and haemorrhagic components. The *Bartonella* are present either free or in clusters in intracytoplasmic vacuoles. This form of the disease follows the primary infection and more rarely follows Oroya fever.

Infection with *Bartonella quintana*

The only reservoir for *B.quintana* is human beings. The infection is transmitted by lice *(Pediculus humanus corporis)* and still occurs in populations living in poor hygiene conditions.

Trench fever (or five day fever) is a septicaemia of varying severity which may also be asymptomatic. It begins with fever and headaches, followed by pretibial bone pain which recurs in cycles every five days. The lengths of the episodes vary and they repeat for four to six weeks, each new episode being less severe. It has a good prognosis although the disease can be very incapacitating.

Infection with *Bartonella henselae*

Cat scratch disease or "benign inoculation lymphogranulomatosis" is a common infection. The preferred bacterial host is the cat, particularly young animals although dogs can also act as hosts. Infection occurs from a scratch, bite or simply licking and more rarely through being pricked by burrs or spines, etc. The cat flea can act as a vector. Inoculation may also occur in the conjunctiva. The disease particularly affects children and results in large lymphadenopathy in the territory around the initial lesion, groin, axilla, for example. This follows a papule which is usually not recognised, five to thirty days earlier. It may be accompanied by fever, headache and asthenia although usually the person remains in good general health. The lymphadenopathy is worrying because of its volume. It generally disappears over several weeks although may form a fistula. Infected people usually recover without complications.

The Parinaud oculoglandular syndrome is a combination of conjunctivitis and pretragial lymphadenopathy. This follows penetration of the organisms through the conjunctiva (rubbing with contaminated hands).

Complications and severe forms of *Bartonella* infections

Except for Oroya fever due to *B. bacilliformis*, infections with the other *Bartonella*, particularly *B. quintana* and *B. henselae*, are generally mild in immuno-competent people. Pronounced systemic signs may however be seen and the Parinaud syndrome may be complicated by retinitis and signs of encephalopathy have been described.

Complications are common however and may be serious in the immunosuppressed (particularly HIV seropositive).

Cutaneous angiomatosis is a result of vascular proliferation. This results in violet coloured haemorrhagic skin lesions resembling

Peruvian verruga. It may be associated with osteomyelitic lesions.

Bacillary or parenchymal peliosis is a deep, vasoproliferative tissue infection often located in the liver, which results in hepato-megaly accompanied by fever, nausea and vomiting with raised alkaline phosphatase. Splenic, pulmonary, cerebral and bone marrow involvement also occur.

Endocarditis is a feared development in patients with pre-existing valve disease and in alcoholics.

INDICATIONS FOR MEASUREMENT

The infection must be considered in many clinical situations, particularly in the immunosuppressed and especially if contact with a cat is reported.

Possible clinical forms of CSD	
Lymphadenopathy with or without inoculation papule	Parinaud oculoglandular syndrome, retinitis, uveitis
Persistent fever of unknown origin	Encephalitis or neurological disease
Deterioration in general health with fever, malaise, weight loss and diffuse pain	Angiomatosis, erythema nodusum
Hepatic disease of known cause	Non-immune, febrile haemolytic anaemia
Endocarditis	Thrombocytopenic purpura

INFORMATION

SAMPLE

Venous blood for blood culture.

Lymph node biopsy or needle aspiration.

Other samples: CSF, liver biopsy, skin lesion, eye, heart valves.

Serum for antibody testing.

QUESTIONS FOR THE PATIENT

Date of onset and clinical symptoms?

Immune status?

Heart disease?

Contact with a cat or possibly with another animal, dog or rodent?

SAMPLE STORAGE AND TRANSPORT

Blood for blood culture transported as quickly as possible at ambient temperature.

Biopsy or needle aspirate (without preservative or histological fixing agent) to identify the organism or its components.

Other samples: Kept at + 4°C.

DIAGNOSTIC METHODS

NB: The diagnosis of *B. bacilliformis* infections (which are not seen outside of South America) is not discussed.

HISTOLOGICAL DIAGNOSIS

This can be performed on a biopsy or lymph node needle aspirate. Lymphoid hyperplasia is present without lymphocytic infiltration, with granulomatous lesions containing giant cells and necrotic areas bordered by lines of epithelioid cells. The bacteria may be seen within the lesions using Warthin-Starry silver staining.

DIRECT DIAGNOSIS

Bartonella can be isolated in a fresh blood-enriched medium kept in a moist atmosphere under CO2. It grows slowly (2 to 4 weeks) and the colonies are small, adherent, rough and whitish or slightly pigmented in colour. They are usually oxidase and catalase negative. Growth occurs more quickly in subsequent passages.

Bartonella can be grown on cell lines (HeLa, Vero) when isolation is faster. They are identified by immunological labelling and molecular analysis.

Bartonella DNA can be detected by PCR in any of the samples described above. The sensitivity and specificity of the test depend on the target gene region (citrate synthase, htrA gene, etc.).

INDIRECT DIAGNOSIS

Serological diagnosis can only be performed in usual practice for *B. henselae* and possibly *B. Quintana* and mostly uses indirect immunofluorescence. The antigen support consists either of a suspension of bacteria or of infected Vero cells. IgG and IgM antibodies can be titred. ELISA and immunoblot tests have been tried without particular success.

INTERPRETATION

The reference diagnostic method is always isolation of *Bartonella* from a sample. In view of the methodological difficulties and slow results, molecular diagnosis using PCR has become the method of choice, particularly in a lymph node sample in cat scratch disease. Although *B. henselae* is the most commonly seen organism, a sufficiently large set of primers and probes must be available to detect other species *(B. quintana, B. clarridgeiae, etc.)* and to avoid false negative results.

Serological diagnosis relies on demonstrating seroconversion. Detection of IgM antibodies is unreliable. Modest antibody titres may persist in people in frequent contact with cats. Cross-reactions occur with *Chlamydia and Coxiella burnetii*. False negative reactions may be seen in *Bartonella* infections with an antigenic composition very different to that of *B. henselae* or *quintana*. Overall, sensitivity and specificity of serological diagnosis are between 85 and 90%.

TREATMENT

Bartonella are sensitive to antibiotics which enter the cell such as tetracyclines, macrolides and rifampicin. Whilst antibiotic therapy is effective in complicated disease or serious disease in the immunosuppressed it has no impact on the clinical course of lymphadenopathy in cat scratch disease.

There is presently no vaccine available. Conventional hygiene measures should be observed but may be difficult in disadvantaged populations or because of exceptional circumstances (growth of body lice). Avoid regular close contact with cats in young children and vulnerable people.

FOR FURTHER INFORMATION

Houpikian P, Maurin M, Raoult D., *Infections à Bartonella*, Encyclopédie Médico-Chirugicale, Maladies infectieuses, Elsevier ed. (8-037-I-30).

BENZENE

TRANS, TRANS MUCONIC ACID

DEFINITION AND SYNONYMS

Benzene is a colourless volatile liquid with a characteristic smell. It is the head of the monocyclic aromatic hydrocarbon group of chemicals. Together with its higher homologues (toluene, xylene, styrene, etc.), benzene is used as a solvent or synthesis intermediary. In view of its toxicity, its use is now highly regulated and occupational exposure is subject to laboratory monitoring by occupational physicians. Chronic poisoning, known as benzolism, has been recognised since 1948 as an occupational illness.

INTRODUCTION

The main route of absorption of benzene is by inhalation, although it is also extremely well absorbed by the skin or gastro-intestinal tract after accidental exposure. Approximately 30 to 60% of inhaled benzene passes into the systemic circulation. Because of its highly lipophilic nature, benzene is distributed mostly in fat-rich tissues: fatty tissue, central nervous system and bone marrow. It is partially removed in the unchanged form through the lungs (10 to 50%) and in urine (< 1%) and an additional proportion is metabolised by cytochrome P450 mono-oxygenases. The main route of oxidation is hepatic and leads to the production of epoxy-benzene (the metabolite probably responsible for benzene myelotoxicity), which itself is converted into phenol which is removed in the form of sulpho or glucurono-conjugated derivatives (33% of inhaled benzene).

A minority part is converted into trans, transmuconic acid (2% of inhaled benzene), the specific benzene derivative excreted in urine and used as a marker of exposure and into S-phenylmercapturic acid (< 1%). Some substances stimulate the metabolism of benzene (phenobarbital, steroids, trichloroethylene and ethanol). Trans, trans muconic acid (sometimes called muconic acid) is rapidly excreted with kinetics similar to those or urinary phenols (half-life approximately 6 hours with total excretion in 48 hours). It should be noted that sorbic acid which is used as a food additive and preservative for cosmetics and pharmaceutical agents, is itself metabolised into trans, trans muconic acid and may interfere with the assay, increasing trans, trans muconic acid concentrations: an average amount of 25 mg of sorbic acid results in a 0.04 mg/d increase in trans, trans muconic acid concentrations.

The features of acute toxicity are mostly due to the irritant and central nervous system depressant properties of benzene: inhalation pneumonia can also be seen when it is inhaled. Chronic toxicity (benzolism) has varying effects: irritative dermatitis, haematological disorders occurring after several months to years of exposure (thrombocytopaenia, leukopaenia and then anaemia as a result of bone marrow aplasia), mutagenicity, oncogenicity and reproductive disorders.

INDICATIONS FOR MEASUREMENT

The indications for measurement of benzene and particularly its urinary metabolites (mostly trans, trans muconic acid and urinary phenols) are in the prevention and monitoring of occupationally exposed workers: the benzene exposure limit has been set by the European Union at 1 ppm i.e. 3.25 mg/m3 (over 8 hours– directive 1999/38/EC). France has adopted the European value since June 2003. The indicative limit values in the United States are 0.5 ppm (TLV-TWA) and 2.5 ppm (TLV-STEL). The general population is exposed through smoking and inhaling combustion fumes.

Measurement of total urinary phenols (free and conjugated) immediately after the work shift is a good indicator for extensive exposure (> 10 ppm). Measurement of trans, trans muconic acid in urine is a good laboratory indicator of exposure when the sample is taken at the end of the shift, in the hour following the end of exposure. A close correlation is seen between air benzene concentrations and the concentrations of urinary trans, trans muconic acid at the end of a shift. Concentrations of trans, trans muconic acid following exposure of 5 or 1 ppm are approximately 5 and 1 mg/L respectively. Measurement of trans, trans muconic acid has the advantage of being sensitive and more specific than measurement of urinary phenols. It is a marker of choice for occupational exposure to average concentrations of at least 0.25 ppm of benzene but it lacks sensitivity below this threshold.

INFORMATION

SAMPLE

Samples for blood benzene measurement are taken into EDTA whole blood. The samples must be taken at the end of exposure week at the start of the shift of the week or 16 hours after the end of a shift. Urine samples to measure metabolites must be obtained within an hour following end of exposure.

A collection device, the URIPREL, has been developed for urinary trans, trans muconic acid. This consists of a syringe and a cartridge and gets around the restrictions of bottling and cold chain. If facilitates transport (no liquid), at the same time ensures that the trapped metabolites are preserved.

QUESTIONS FOR THE PATIENT

Does the patient smoke?

Is the patient exposed to benzene in his/her job?

SAMPLE STORAGE AND TRANSPORT

Samples for assay of urinary metabolites can be obtained using the URIPREL device *(see "Sample" section)*.

ASSAY METHODS

Assays available for benzene are mostly head space gas chromatography or gas chromatography coupled to mass spectrometry. The assay methods for urinary metabolites use high performance liquid chromatography or spectrophotometric methods.

NORMAL EXPECTED VALUES

Benzene concentrations in blood in the general population are < 500 ng/l of blood in smokers (< 250 ng/l in non-smokers). There is no guideline value in France or in the United States for people who are occupationally exposed. There are no official data on reference or guideline values for urinary benzene.

The reference values for urinary phenols in France in the general population are < 30 mg/g of creatinine. There are no guideline values for people exposed occupationally in France or in the United States.

The reference values for urinary trans, trans muconic acid in the general population in France are < 0.5 mg/g of creatinine in smokers and < 0.05 mg/g of creatinine in non smokers. The guideline value for people exposed occupationally in France is < 5 mg/g of creatinine at the end of shift. It should be noted that this guideline value was produced in 1997 when the benzene exposure limit was 5 ppm, whilst it is now 1 ppm. A guideline value of 1 mg/g of creatinine can therefore be taken to correspond to 1 ppm of benzene in air.

PATHOPHYSIOLOGICAL VARIATIONS

The maximum permitted concentration of benzene in blood during exposure is 20 µg/l for a mean exposure level of less than 1 ppm.

FOR FURTHER INFORMATION

- Fiches Biotox, *benzene sanguin – benzene urinaire – acide trans, trans muconique – phenols urinaires,* www.inrs.fr
- Fiche toxicologique n° 49, *benzene*, www.inrs.fr

BENZODIAZEPINES

DEFINITION

The benzodiazepines (BZD) are a relatively structurally homogeneous family, all of the molecules being derived from chlordiazepoxide, the head of the class, first marketed in 1961. They act principally by binding to GABAergic receptors and have anxiolytic, sedative, anticonvulsant and myorelaxant properties which differ between the compounds (more than 20 are currently commercially available) and the doses prescribed.

The benzodiazepines are the most widely-prescribed psychotropic medicine in western countries, particularly in France and the prevalence of chronic BZD anxiolytic and hypnotic use (long term daily doses) is currently estimated to be approximately 6% and occasional use (at least once per year) is estimated to be 25-30% in the general population. In view of their widespread use they are very often tested for in the context of acute, accidental or intentional (suicidal) poisoning. Although they are rarely life-threatening, clinicians expect laboratories to provide a reliable answer to confirm or exclude poisoning. Different screening methods suitable for an emergency context are available in blood, urine or gastric fluid. Precise identification and quantification of the substance in question requires chromatographic techniques which are reserved for a few specialised laboratories.

PHARMACOKINETICS

Following an oral dose, the BZD are generally absorbed rapidly and almost entirely. The plasma peak is obtained after 1 to 6 hours and they have a widespread tissue distribution. In the circulation more than 85% of the drug is bound to plasma proteins independently of concentration. They are metabolised by the liver through demethylation and hydroxylation reactions to form primary active metabolites, some of which have a longer half-life than the parent molecule and then by conjugation (into inactive metabolites). A very small proportion (< 3%) is excreted unchanged in urine.

It is conventional to distinguish three categories of BZD based on their duration of action and half life of elimination (including the active metabolites):

– Short half-life compounds (< 10 hours) such as midazolam, temazepam, triazolam, clotiazepam, etc.

– Intermediate half-life compounds (10-24 hours) such as alprazolam, bromazepam, clobazam, flunitrazepam etc.

– Long half-life compounds (>24 hours) such as chlordiazepoxyde, clonazepam, diazepam, ethylloflazepate, nordiazepam, etc.

In addition, their pharmacokinetics varies with age (delayed gastro-intestinal absorption, reduced metabolism and excretion), the presence of concomitant diseases (renal or hepatic insufficiency), concomitant use of alcohol (increased absorption of diazepam), food (delayed absorption) and drugs which induce (phenobarbital, phenytoin, and rifampicin) or inhibit (cimetidine) cytochrome P450.

(See table below).

INDICATION FOR MEASUREMENT

– Aid to the diagnosis of acute (intentional or accidental) poisoning and rapid screening methods are available as a qualitative guide together with quantitative methods to identify the substance in question and the severity of poisoning.

The clinical symptoms of acute isolated benzodiazepine poisoning are mostly behavioural disorders with agitation, aggression, disinhibition followed by altered consciousness, such as drowsiness or more rarely hypotonic coma. Respiratory failure is occasionally seen particularly in children or the elderly. Circulatory disorders develop more rarely. Outcome is usually good although symptoms may be far more severe and life-threatening if massive doses are taken, in multiple poisonings (combined with alcohol or other central nervous system depressants) or if co-morbidities are present (hepatic, renal, respiratory insufficiency, malnourished patients, etc).

– Anti-convulsant treatment monitoring (clonazepam, diazepam).

– Testing for BZD dependency/drug addiction.

The BZD have high drug addictive potential as they cause physical and psychological dependency plus tolerance. Taken long-term they may cause a withdrawal syndrome with anxiety, rebound insomnia, headache, muscle spasm, tremor, anorexia and nausea, when stopped suddenly, when doses are reduced or the substance is changed. The severity of withdrawal is proportional to the initial dose and length of treatment.

– Testing for BZD use in specific medico-legal situations:

In some people, the BZD may cause "paradoxical aggressive reactions" or even genuine aggressive behaviour (violence, sexual aggression etc.) which the person does not remember (amnesia effect). BZD testing may be used in situations of "chemical submission" (rape victim, for example, after unknowingly taking flunitrazepam in an alcoholic drink). BZD testing is then useful to assess individual responsibility in legal proceedings.

INFORMATION

■ SAMPLE

Serum or plasma taken into EDTA or heparin: Avoid tubes with a gel separator. Blood sample to be taken when clinical symptoms of overdose are present, as early as possible, particularly in a legal context when testing for personal responsibility is required. In treatment monitoring, the sample should be taken to test for trough concentrations immediately before the next dose.

- Random Urine: BZD testing may be combined with testing for other drugs (amphetamines, cocaine, opiates, cannabis, etc.). Excretion in urine lasts for several days (varies depending on the compound).

- Gastric fluid.

■ QUESTIONS FOR THE PATIENT OR THOSE WITH THE PATIENT

What is the context in which testing is being requested (suspected acute unintentional or suicidal poisoning, testing for chemical submission, etc.)? If possible, amount taken, date and time of the dose, substances taken at the same time?

Alcohol and other central nervous system depressants (sedative antidepressants, opiates, neuroleptics, etc.), increased toxicity.

SAMPLE STORAGE AND TRANSPORT

The separated plasma/serum and urine can be stored for several days at +4°C and for several months at –20°C.

Transport at +4°C or if sample is already frozen then transport in the frozen state.

ASSAY METHODS

Screening methods for the BZD family in various biological fluids (plasma, serum, gastric fluid, urine) immunological methods: RIA, EMIT, FPIA, LIMS method, urine tests on solid supports. All of these methods are qualitative and have detection limits of between 50 and 300 ng/ml (in nordiazepam or oxazepam equivalent).

Quantitative measurements of a specific compound for monitoring of treatment: high performance liquid chromatography, gas phase chromatography with electron capture detector, nitrogen phosphorous detector or mass spectrometry.

INTERPRETATION

The BZD are the leading class of medical drugs involved in acute poisoning in adults and are therefore tested for very commonly in emergency toxicology. The samples of choice are blood and urine and the BZD screening methods are available to all laboratories, particularly EMIT and FPIA techniques which are rapid, reliable and automated although have one major disadvantage, their lack of sensitivity (false negatives). The EMIT®d.a.u techniques, (syva) for example, have a limit of detection of 200 ng/ml. Whilst a single tablet of diazepam (5 mg) is sufficient to make this test positive, more than 50 tablets of triazolam 0.125 mg need to be taken to produce the same result.

Several compounds can therefore only be detected at high (toxic) concentrations or may even be impossible to detect. In a medico-legal or monitoring of treatment context a more sensitive chromatographic technique enabling identification and quantification of the compounds is required.

The treatment of acute poisoning is with a specific antidote, flumazenil combined with symptomatic management such as gastric lavage (not systematically), activated charcoal, glucose and electrolytes.

INN	PROPERTIES	URINARY METABOLITES	PEAK	HALF-LIFE	THERAPEUTIC RANGE
ALPRAZOLAM	Anxiolytic	OHAlprazolam	1-2 h	12-15 h	
BROMAZEPAM	Anxiolytic	GRC, 3OHBromazepam, 2amino-5brono-3hydroxy-benzoylpyridine	1-4 h	20 h	
CHLORDIAZEPOXIDE	Anxiolytic	Desmethyldiazepam (DMdiazepam), Oxazepam, Demoxepam	2-4 h	24 h	
CLOBAZAM	Anxiolytic	Clozabam, OHclobazam, DMclobazam	2 h	20 h	0.1 to 0.4 mg/l
CLONAZEPAM	Anti-convulsant	Clonazepam: 50% in urine, Aminoclonazepam	3-12 h	32-38 h	30 to 60 µg/l
CLORAZEPATE DIP.	Anxiolytic Hypnotic	DMdiazepam, Oxazepam, Clorazepate	1 h	40 h	
CLOTIAZEPAM	Anxiolytic	GRC, N-desmethylclotiazepam	30-90 min	4 h	
DIAZEPAM	Anti-convulsant Anxiolytic	DMDiazepam, Oxazepam	30-90 min	32 h	100 to 400 µg/l
ESTAZOLAM	Hypnotic		2 h	17 h	
FLUNITRAZEPAM	Hypnotic	DMflu, 7aminoflunitrazepam	2 h	19 h	
LORMETAZEPAM	Hypnotic	DMlormetazepam	2-3 h	10 h	
LOFLAZEPATE	Anxiolytic		1,5 h	77 h	
LOPRAZOLAM	Hypnotic	Glucuronide conjugated derivative (GRC)	1 h	8 h	
LORAZEPAM	Anxiolytic		1 h	12 h	
MIDAZOLAM	Sedative-anaesthetic		0,1-1 h	1-4 h	
NITRAZEPAM	Hypnotic	7amino, 7acetamidonitrazepam	2 h	25 h	
NORDIAZEPAM	Anxiolytic	Oxazepam	1,5 h	65 h	
OXAZEPAM	Anxiolytic	DMdiazepam, Oxazepam	2 h	8 h	
PRAZEPAM	Anxiolytic	GRC, Oxazepam	4-6 h	65 h	
TEMAZEPAM	Hypnotic	Oxazepam	1 h	5-8 h	
TETRAZEPAM	Myorelaxant	GRC	1 h	1-4 h	
TRIAZOLAM	Hypnotic	OH-derivatives	2,4 h	10-25 h	

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BETA 2-MICROGLOBULIN

DEFINITION

La β2-microglobulin (2M) is an unglycosylated low molecular mass (12 kDa) protein synthesised in all cells in the body, particularly in lymphocytes and tumour cells when these are present. Because of its small size it passes easily into the extravascular space (notably the CSF) and is completely filtered by the renal glomerulus then 99.9% reabsorbed by the proximal convoluted tubule. It is then degraded in renal tubular cells. It has a short plasma half life of approximately 60 minutes.

INTRODUCTION

β2M on the surface of mononucleated cells in the body is bound to major histocompatibility complex (MHC) or human leukocyte antigen (HLA) molecules in human beings, in which it forms the light chain. The expression of HLA A, B and C depends on expression of β2M. It plays an important role in cellular immunity being particularly involved in combating bacterial and viral infections, in prevention of the development of tumour cells and in graft rejection mechanisms.

In nephrology, β2M is a marker of tubular damage. Defective absorption of small proteins (of molecular mass < 40 kDa) which are normally reabsorbed and catabolised by proximal tubular cells occurs in the tubulopathies. These proteins are then found in urine. This applies particularly to β2-microglobulin, alpha1-microglobulin, Retinol Binding Protein (RBP) and free light chains. Albumin is also normally reabsorbed and catabolised in the tubule and only constitutes 10 to 20% of the proteinuria (it is the majority protein in glomerular proteinuria). In common practice, however, urine β2M measurement is not indicated in the laboratory investigation of tubulopathy as it is unstable at acid pH.

INDICATIONS FOR MEASUREMENT

– ***In oncology:*** β2M is a marker of lymphoproliferative haematological diseases such as, multiple myeloma (an initial prognostic marker and marker in monitoring treatment), B lymphomas, and chronic lymphoid leukaemia (CLL). It has also been proposed as a marker to monitor some gastro-intestinal cancers in combination with other tumour markers.

– ***In patients infected with the human immunodeficiency virus (HIV):*** It is a marker of disease activity and is used to monitor treatment.

– ***In nephrology:*** It is useful in the investigation and monitoring of renal function. Compared to serum creatinine its plasma concentration has the advantage of being independent of muscle mass or sex.

– Monitoring the effectiveness of dialysis (pre and post-dialysis measurements)

- Marker of toxicity following exposure to potentially nephrotoxic substances such as aminoglycoside or cyclosporin treatment etc. or exposure to certain heavy metals (cadmium or mercury).

- Monitoring in renal transplant patients (screening for rejection).

– ***Secondary indications:*** β2M is also occasionally used to monitor some auto-immune diseases such as rheumatoid arthritis or systemic lumpus erythematosus.

INFORMATION

SAMPLE

Blood sample: Serum or plasma collected into heparin or EDTA (depending on assay method). A fasting sample is not required.

Urine sample: 24-hour urine collection or a random urine sample. If the urine is acid, the pH must be adjusted to between 7 and 9 with 1 N NaOH as soon as possible after the sample is taken.

Others: Cerebrospinal fluid (CSF), dialysate.

QUESTIONS FOR THE PATIENT

In the context of cancer, type of tumour and current treatment: Chemotherapy, radiotherapy, surgery (methods and dates of treatment)?

Are you taking medical treatment? Nephrotoxic drugs increase serum β2-microglobulin concentrations: aminoglycosides, vancomycin, ciclosporin, amphotericin B and cisplatin.

SAMPLE STORAGE AND TRANSPORT

Serum or plasma can be stored for 8 days at +4°C. If the assay is performed later, the sample should preferably be frozen within 24 hours. Avoid successive freeze/thaw cycles.

Urine: Store alkalinised urine at +4°C (a fall in concentration is seen when acid urine is stored). β2M should preferably be measured in fresh urine. The samples can be stored for up to 48 hours at between 2 and 8°C and for several months at –20°C.

ASSAY METHODS

Immunological assays: Chemiluminescence, electro-chemiluminescence, nephelemetry or immunoassay with a marker.

NORMAL EXPECTED VALUES

These vary depending on the method. As an indication, in adults (by immunonephelemetry):

In blood: < 2.5 mg/l

In urine: < 0.1 mg/l

In CSF: < 2.3 mg/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

- **Depending on age:** Serum concentrations are high in newborn children (approximately 3 mg/l) and then rapidly fall reaching average values of < 1.5 mg/l at puberty. They then rise again very gradually with age, by an average of 0.12 mg/l every 10 years. Urine β2M concentration is approximately 0.3 mg/l at birth, rising to 1 mg/d on D5 and then falling to values of < 500 mg/l at 3 months.

- **In pregnant women:** Serum β2M concentrations rise up to the 25th week of pregnancy and then fall until term. Very high values are suggestive of pre-eclampsia.
- **Intensive physical exercise** is believed to increase urine β2M concentrations.

PATHOLOGICAL VARIATIONS

- **Serum β2M:**

Serum β-2-microglobulin concentration rises as a result either of increased synthesis or from a fall in its glomerular filtration.

Increased synthesis:

– In B lymphoproliferative syndromes, B lymphoma, myeloma, Waldenström's disease and CLL, the rise in serum β2M concentration reflects the extent of the tumour mass and as such as an initial prognostic indicator. It is an aid to monitoring treatment during the course of the disease.

–Serum β2M raises moderately in all symptomatic HIV infected patients although remains normal in asymptomatic patients. It is used in monitoring treatment.

– In some non-specific autoimmune diseases (rheumatoid arthritis, lupus, Sjögren's syndrome, etc.) its serum concentration reflects disease activity and it is an aid in monitoring treatment and a fall in the value reflects effective treatment.

– Other circumstances: Serum β2M raises in patients with neoplasia (rectal adenocarcinoma, breast cancer, etc.) in acute and chronic viral hepatitis (associated with reactive lymphocyte infiltration), in primary biliary cirrhosis and in chronic inflammatory diseases (Crohn's disease, sarcoidosis, etc).

Fall in glomerular filtration:

– Renal insufficiency, whether physiological (newborn baby, the elderly) or pathological, causes a rise in serum β2M concentrations.

– Following renal transplantation: Serum β2-microglobulin usually falls rapidly (concentrations returning to normal 4 days after transplantation is a good prognostic indicator). A persisting high value on day 8 suggests possible rejection and generally results in immunosuppressant therapy being adjusted.

– Raised β2-microglobulin is a good marker of the renal toxicity of some drugs.

- **urine β2M**

– Urinary β2M concentration rises in tubulopathies: This rise is seen particularly in renal tubular necrosis, poisoning with cadmium dust or with nephrotoxic drugs which damage the functional reabsorption capacity of the renal tubule (β2M is then excreted in urine and serum concentrations remain normal).

– Following renal transplantation: Urinary β2M should return to normal within a week after transplantation. Persistent high values suggest rejection. After normal urine output is restored a return of urinary β2M excretion to normal indicates good tubular function.

– Increased urinary β2M concentration can also reflect so-called overload proteinuria, in situations where large amounts are produced (in some neoplastic or immunological diseases); its concentration exceeds the maximum reabsorption threshold (Tm).

- **β2M in CSF**

A rise in CSF β2M concentration indicates intrathecal synthesis. Measurement of β2M in CSF can be used to monitor some neurological or rheumatological conditions (inflammatory or auto-immune).

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BILE ACIDS

DEFINITION

The term "bile acids" covers a number of different steroid carboxylic acids that are synthesised by liver cells from cholesterol, via di- and tri-hydroxycoprostanoic acid intermediates. Distinction is made between the primary bile acids that are produced inside peroxisomes in liver cells, and secondary bile acids which are generated in the digestive tube by the action of intestinal bacteria. Bile salts are sodium or potassium salts of these acids.

Bile acids	Name	Comments
Primary	cholic acid chenodeoxycholic acid	Synthesised in hepatic epithelial cells Storage: gallbladder
Secondary	deoxycholic acid lithoxycholic acid	Generated by bacteria from primary bile acids Storage: gallbladder
	ursodeoxycholic acid	Synthesised in hepatic epithelial cells from lithocholic acid or from an exogenous compound administered for medicinal purposes Storage: gallbladder

INTRODUCTION

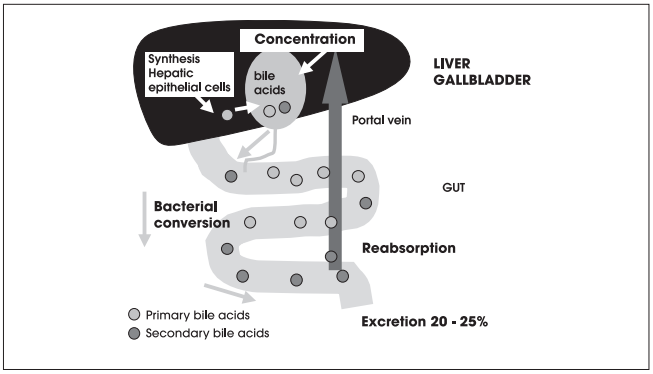
ENTEROHEPATIC BILE ACID CIRCULATION

Hepatic bile acid production depends on two main enzymes, namely cholesterol-7hydroxylase and sterol 27 hydroxylase. The resultant bile acids are conjugated to taurine or glycine and then secreted into the gallbladder. The rate of synthesis is of the order of 1.2 mmol/day.

Primary bile acids are stored in the gallbladder and released into the gut when digestion begins. A fraction is subsequently reabsorbed, some are excreted (with approximately 20-25% lost in the faeces) and some are metabolised by the gut flora to generate secondary bile acids.

The overall pool of bile acids (both primary and secondary) is of between 2 and 5 grams.

The amount of bile acids in the blood depends on the balance between the rates of intestinal reabsorption and hepatic clearance.



ROLE OF BILE ACIDS

Bile acids are active in the liver, the bile and the gut.

In the liver, these by-products of cholesterol catabolism are important constituents of bile.

In the bile, they help solubilise cholesterol and phospholipides (80% bile acids, 15% lecithin, 5% cholesterol), thereby preventing the formation of gallstones (80% of which are made of cholesterol with 20% made of pigments). In normal bile, there is at least fifteen times as much bile acid and phospholipid as cholesterol and any reduction in this ratio will promote calculus formation. In the gallbladder, bile acids are concentrated to up to 1,000 times their concentration in the serum. Any problem with bile storage will be reflected in an increased concentration of bile acids in the blood and, at blood concentrations of over 50 µmol/l, in the urine. A high concentration of bile acids in the blood can cause itching. Bile acids also have a choleretic effect which stimulates bile production.

Finally, in the gut, bile acids are important in lipid digestion and absorption. Their hydroxylated steroid structure enables them to form micelles with dietary fats, thereby facilitating triglyceride digestion by pancreatic lipase-colipase.

INDICATIONS FOR MEASUREMENT

– Liver function testing:
  – marker for cholestasis (a high bile acid concentration in the blood and/or urine is typical of early-stage disease, whether the cause is intra- or extra-hepatic).
  – investigation of jaundice
  – investigation of liver failure.

– Investigation of a porto-systemic shunt, either a congenital vascular abnormality or following surgery on a patient with cirrhosis.

– Investigation of gallstones following biliary colic or an intercurrent complication (acute pancreatitis).

– Investigation of unexplained pruritus.

– Diagnosis and monitoring of cholestasis in pregnant women (bile acids being toxic for the baby's central nervous system).

INFORMATION

SAMPLE

Serum: Please ensure the sample is not haemolysed.

QUESTIONS FOR THE PATIENT

Have you been itching?

Do you have chronic diarrhoea?

Parenteral alimentation? Pregnancy? Liver-toxic medications? Oestrogen? Fibrate (clofibrate)? Crohn's disease?

SAMPLE STORAGE AND TRANSPORT

Samples can be stored at between +2 °C and +8 °C for 24 hours. Bile acids are stable for two years in samples conserved at – 20 °C.

ASSAY METHODS

The assay method will depend on the sample type and which bile acids are being tested for.

Assay method	Bile acids being tested	Sample type
Enzymatic	Total, unconjugated bile acids	Serum
High-performance liquid chromatography	Conjugated bile acids (to glycine or taurine) Ursodeoxycholic acid	Serum and bile
Gas phase chromatography coupled to mass spectrometry	All bile acids Ursodeoxycholic acid	Serum, bile, faeces and urine

The enzymatic method only detects unconjugated, trihydroxylated bile acids. It is based on a bacterial enzyme, 3-alpha hydroxysteroid dehydrogenase with spectrophotometric determination of the NADH formed (via the conversion of nitro blue tetrazolium into formazan with read-out at 540 nm).

High-performance liquid chromatography (HPLC) is the best method for resolving different conjugated bile acids. Various detection systems can be used, including electrochemistry, UV absorption, enzymatic and fluorescence-based systems. The limited sensitivity of detection restricts the power of this method for assaying free bile acids in the blood.

Gas phase chromatography coupled to mass spectrometry (GPC-MS) is the reference method because it detects most bile acids: both free and conjugated bile acids as well as the precursors of primary bile acids. It is particularly applicable to assaying free bile acids in faeces. It is used to diagnose constitutional and acquired abnormalities in bile acid metabolism.

NORMAL EXPECTED VALUES

The normal blood bile acid concentration is below 6 µmol/l.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

The blood bile acid concentration does not follow any circadian rhythm but it rises after eating.

Pregnancy and total parenteral alimentation are both associated with elevated blood bile acid levels. Gestational cholestasis is associated with a rise in ALT coupled with normal gamma-GT.

■ PATHOLOGICAL VARIATIONS

An increase in the blood concentration of bile acids is an early marker for cholestasis.

The aetiology may be extra- or intra-hepatic. Extrahepatic causes correspond to physical blockage of bile release, due either to malformation of the hepatic ducts or as a result of compression by a mass such as a calculus, a tumour or pancreatitis. Intrahepatic causes result from impaired bile acid metabolism in the liver cells due to genetic abnormalities, hepatic infiltration or toxins with an affinity for liver tissue. Aetiology tends to vary with age.

	CHILDREN Disease	*Bile acids*	ADULTS Disease	*Bile acids*
extrahepatic	• Biliary atresia • Biliary occlusion (lithiasis/bile sludge)	+ + +	• Lithiasis • Pancreatitis • Biliary/pancreatic tumours • Gestational cholestasis	+ + **to** + + +
intrahepatic	• Genetic diseases: - Alagille syndrome - alpha1-antitrypsin deficiency - cystic fibrosis - familial cholestasis (Byler's disease) • Sclerosing cholangitis • Neonatal infectious hepatitis	+ **to** + + +	• Primary biliary cirrhosis • Sclerosing cholangitis • Sarcoidosis • Liver toxins • Liver failure	+ **to** + + +

In children, extrahepatic cholestasis is associated with a marked rise in circulating bile acids and this constitutes a surgical emergency. Intrahepatic cholestasis is associated with other symptoms and laboratory findings, e.g. Alagille syndrome is associated with hypercholesterolaemia (up to 30 mmol/l). In primary biliary cirrhosis, the cholestasis is accompanied by the presence of anti-mitochondrial antibodies and the Increase in bile acid levels are variable. In adults, hepatic infiltration and impaired hepatocyte function underlie many cases of cholestasis (induced by medications and toxins, or secondary to sarcoidosis, hepatitis and alcoholic cirrhosis, among other causes); in these cases, conjugated bile acids are found in the urine.

Assaying bile acid precursors is important in the diagnosis of hereditary peroxisomal diseases: cerebrohepatorenal syndrome, neonatal adrenoleukodystrophy, infantile Refsum's disease, and certain isolated enzyme deficiencies, namely in enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase, peroxisomal thiolase or trihydroxy coprostanoyl CoA oxidase: these conditions are characterised by a combination of neurological signs and facial deformities, and are associated with a build-up in the blood of very long-chain fatty acids which are normally broken down in peroxisomes.

FOR FURTHER INFORMATION

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BILHARZIA

DEFINITION

Bilharziasis or Schistosomiasis is a tropical parasitic disease due to the growth of worms called schistosomes or bilharzia in the body.

These flat worms or platyhelminthes belong to the Trematodes class and the *Schistosoma* genus.

Five species are pathogenic to human beings:

– *Schistosoma haematobium* is responsible for genitor-urinary bilharziasis.

– *Schistosoma mansoni* is responsible for hepato-intestinal bilharziasis.

– *Schistosoma intercalatum* is responsible for rectal bilharziasis.

– *Schistosoma japonicum* and *Shistosoma mekongi* are responsible for arterio-venous bilharziasis.

The morphology of the worms is very similar between species:

The adult measures between 10 and 20 mm and has 2 suction pads. The sexes differ and the male shelters the female in the gynaecophoric canal.

The egg measures 70 to 200 microns long depending on the species and has a characteristic spur. It is not embryonated when laid; this occurs in the external environment.

Synonyms: Bilharziasis = Schistosomiasis
Schistosomes = Bilharzia

INTRODUCTION

EPIDEMIOLOGY

The disease is found very widely throughout the world, mostly in tropical countries. It affects more than 200 million people and numbers are currently increasing in some regions.

– *Schistosoma haematobium* affects approximately 120 million people and occurs in Africa, Madagascar and the near and Middle East.

– *Schistosoma mansoni* affects all inter-tropical zones in the African and American Continent. It affects approximately 60 million people.

– *Schistosoma intercalatum* affects Central Africa.

– *Schistosoma japonicum* occurs in tropical areas in the Far East with 2 major locations, China and the Philippines.

– *Schistosoma mekongi* is seen in the Mekong basin (Cambodia, Laos and Thailand).

GROWTH CYCLE

This is identical for all species and human beings are always the final host. The only difference is in the species of intermediary host which is always a freshwater gastropod mollusc, Bulinus sp., snails, Onchomelania genus, etc.

Human beings are infested by contact with fresh water (lakes, rivers, swamps, water channels) as the parasite actively enters through the skin, reaching the blood vessels where it grows.

The adult worms live in pairs inside the venous circulatory system of infested victims, where the female lays large numbers of eggs in the capillaries. These are released when they rupture and are excreted in urine *(S. haematobium)* or in faeces *(S. mansoni and S. japonicum)*.

The excreted eggs are embryonated. The egg breaks open in water and releases a ciliated larva or ***miracidium*** which swims until it meets and intermediary host, a freshwater mollusc. If conditions are favourable (water 20-25°C, high light intensity), the larva converts and multiplies into several successive forms, firstly ***sporocysts*** and then ***furcocercaria*** before escaping from the mollusc. The cercaria swim freely in water and human beings are infected when bathing or walking bare foot on flooded ground. The cercaria enters through the skin, travelling through the venous and lymphatic system to the right side of the heart. They enter the lungs and then liver, becoming adults in approximately 2 months. The adults can live for a very long time, up to 20 years, in human beings. After mating the females become established in their preferred venous system, the perivesical plexus for *Schistosoma haematobium*, the pericolonic venus plexus for *S. mansoni*, the perirectal venous plexus for S. intercalatum, and the superior mesenteric vein for *S. japonicum* and *S mékongi* for years.

Only once established do they produce huge numbers of eggs.

CLINICAL FEATURES

The clinical features of bilharziases can be divided into 3 phases:

– The initial phase or cercarian dermatitis dermatitis is due to penetration of the furcocercaria and is characterised by allergic cutaneous erythema, the severity of which varies depending on the species and which occurs within an hour of infestation. It may be very mild or may even be unnoticed.

– The invasive or toxaemic phase is due to the migration of the schistosomules in the body and their conversion into adult worms, which begins within 3 weeks after infestation. This involves allergic cutaneous reactions (pruritus, episodes of urticaria) associated with diarrhoea and more systemic signs such as fever, asthenia or headache.

– The established disease state occurs a few weeks to years after infestation and represents the period when the females are laying eggs. Symptoms vary depending on the species as they depend on the territories where the eggs are laid.

Urogenital bilharziasis

Is caused by eggs in the wall of the bladder, ureter and genital organs. It produces a combination of haematuria and signs of cystitis and/or renal colic and may have complications affecting the whole urinary tract together with damage to the genital organs, which may result in sterility.

Intestinal bilharziasis

Is due to the presence of periovular granulomas in the intestinal wall or around the intrahepatic portal vessels. It is a disease affecting the left colon and rectum and is dominated by gastro-intestinal signs (abdominal pain, occasionally bloody, diarrhoea, vomiting, etc.) hepatic and splenic signs (hepatomegaly, splenomegaly) and pulmonary fibrosis.

Arterio-venous bilharziasis

Is also due to the presence of periovular granulomas in the right colon and small intestine.

This is dominated by early hepatic and splenic disease which progresses rapidly to fibrosis and cirrhosis of the liver but also has cardiac and pulmonary complications.

INDICATIONS FOR MEASUREMENT

Diagnosis of parasitosis in a person who has stayed in an endemic area and has suggestive clinical signs.

Systemic parasitic assessment.

Discovery of haematuria (for *S. haematobium*) or significantly raised eosinophil count..

Differential diagnosis between another helminthiasis.

INFORMATION

SAMPLE

Urine: to test for *S. haematobium* eggs. Urine must be collected under conditions promoting detachment of eggs adhered to the bladder wall. To do this the urine is collected after asking the patient to take physical exercise (such as walking upstairs).

Faeces to test for eggs from any species of shistosomes, excreted from the colonic wall.

Rectal mucosal biopsies can be used to find eggs from any of the species and are only performed if a parisitology investigation is negative based on a high index of clinical suspicion.

Whole blood (EDTA) for a full blood count to obtain an eosinophil count.

Serum (Dry tube) for serological testing.

QUESTIONS FOR THE PATIENT

Period in a high risk country, in contact with fresh water?

Clinical symptoms?

Raised eosinophil count?

Current treatment?

SAMPLE STORAGE AND TRANSPORT

Faecal samples should be produced in, or very near to the laboratory premises, failing which they should be transported very promptly.

Serum is stored at +4°C.

EDTA is stored at +4°C (< 6h).

DIAGNOSTIC METHODS

DIRECT PARASITOLOGY DIAGNOSIS

This relies above all on finding eggs, which is only possible during the established phase of the disease. It includes a study of egg viability.

– Testing for eggs in urine is performed after gentle centrifugation (2000 rpm) or filtration.

– Testing for eggs in stools involves 2 concentration techniques (MIF, Ritchie…) and is performed 3 times at intervals of 3 or 4 days.

– Testing for eggs in rectal biopsies is performed simply after compression between slide and slide cover or after lightening with chloral gum.

– Testing for eggs in various histological biopsies uses a staining technique such as Ziehl or Methyl green.

Features of eggs:

Shistosoma haematobium: Ovoid shape with a rounded pole and terminal spur on the other pole.

Schistosoma mansoni: Large in size, ovoid in shape with one rounded pole and one more conical pole. The spur is distinct and on the side with a wide base and thick contour.

Schistosoma intercalatum: Similar to *Schistosoma haematobium* but larger. Fusiform at the 2 ends with a terminal spur.

Shistosoma japonicum and mekongi have similar morphology and are smaller than the others. They have a rudimentary side spur which is occasionally difficult to find.

INDIRECT DIAGNOSIS

Detection of antibodies

The great majority of serology methods use antigens extracted from *S. mansoni*.

The complement fixation reaction uses a dilapidated extract of adult *S. mansoni*.

Indirect immunofluorescence (IIF) on frozen sections of parasite infested rodent liver or adult shistososmes included in an organ offers good sensitivity and group specificity.

Indirect haemagluttination (IHA) uses a soluble S. mansoni antigen. It is sensitive (75 to 88%) depending on the stage of the disease and species in question.

ELISA offers good specificity and good sensitivity.

Immunoelectrophoresis (IEP) can reveal specific precipitation arcs for the genus or species (arc 4 for the Schistosoma genus, arc 8 for S. mansoni). It is used as a confirmatory technique for a positive screening reaction.

Western blot, the recently commercially available confirmatory technique has a specificity of 100% and sensitivity of over 95%. A positive diagnosis is based on the presence of at least 3 bands in the 65-120 kDa area except for the 100 kDa band.

The Vogel and Minning reactions and Olivier Gonzales *circum ovale* reaction use live cell antigens (furcocercaria or live eggs) and are no longer used except in endemic areas.

Detection of specific circulating antigens

This is performed in blood and urine using monoclonal antibodies. An antigen titre is determined which is related to parasite load. This is very useful in monitoring the effectiveness of treatment and in testing for cure.

NON-SPECIFIC DIAGNOSIS

– A raised eosinophil count provides only a guide, as it is an inconstant finding and is not specific to the bilharziases. Eosinophils are raised in the invasion phase and fall in the established disease phase. They rise rapidly in response to treatment (rebound effect) and are a simple means of monitoring the effectiveness of treatment.

– Hyperleukocytosis.

INTERPRETATION

Unequivocal diagnosis relies above all on finding parasite eggs although this is not always easy in mild infections and cannot be performed late (in the established disease phase).

The diagnosis may be suspected based on the patient's geographical origin being consistent with symptoms although clinical findings are not always suggestive.

The serological diagnosis can be made from the invasion phase onwards and is also a useful test when eggs have not been found and in monitoring the effectiveness of treatment (antibody concentrations rise as soon as treatment is started and then fall). Serological reactions are occasionally inaccurate and in very recent or very old bilharziases and titres are often low (immune escape phenomenon due to adaptation of the parasite to the host). Cross-reactions with other helminthiases occur.

For this reason a confirmation test must be used and laboratory results always interpreted in the clinical and epidemiological context.

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BISMUTH

DEFINITION

Bismuth (Bi) is a light coloured crystalline, fragile metal, the least toxic of the heavy metals. It is the poorest heat conducting metal after mercury and has a very high electrical resistance. It is an extraction sub-product of lead, copper, tin, silver and gold.

It is used:

– In electricity in the manufacture of fuse wires.

– In the manufacture of fire extinguishers.

– In shotgun pellets, lead, bismuth and antimony alloy.

– In the glassware and ceramics industry or as a paint colouring agent used as a white pigment.

– In pharmacy in dressings for burns, anti-ulcer local gastric treatments, treatments for diarrhoea and gastrointestinal disorders.

– In cosmetics, in lipsticks for its lustrous shining appearance.

METABOLISM

Bismuth is only very partially absorbed by the respiratory and gastro-intestinal trace. It is significantly absorbed through the skin and is stored in the kidneys and bone. Its excretion is mostly in faeces as insoluble derivatives (aluminate, phosphate, silicate, etc). 90% of soluble derivatives are excreted rapidly in urine.

MECHANISM OF ACTION

Bismuth is used above all for is anti-ulcer activity protecting the gastric and duodenal mucosa from acid secretions. It is believed to have bactericidal activity on *Helicobacter pylori*. Its sale, however, is prohibited in France.

CLINICAL SIGNS OF POISONING

Until the 1970s, bismuth was widely used in various gastro-intestinal indications. Around 1974, cases of encephalopathy clearly linked to oral bismuth were described. These encephalopathies involved a prodromal phase with asthenia, memory loss, insomnia and headaches, followed in the acute phase by neurological disorders, dysarthria, ataxia, walking disorders, myoclonus, tremor, agitation, confusion, hallucinations and seizures.

The incidence and severity of these cases of poisoning led the French Ministry of Health to withdraw the product from the market. No occupational risk has been described in workers exposed to bismuth.

In other countries at present, bismuth salts are used mostly in gastro-intestinal ulcers because of their action on *Helicobacter pylori*.

INDICATIONS FOR MEASUREMENT

Laboratory monitoring (measurement of blood and urine bismuth) in occupationally-exposed workers is of limited use.

Blood or urine bismuth measurement may be considered to identify accidental or non-accidental poisoning with bismuth treatment.

INFORMATION

■ SAMPLE

5 ml of heparinised whole blood.

20 ml of unacidified urine sample.

■ SAMPLE STORAGE AND TRANSPORT

Blood or urine can be stored and transported to the laboratory at ambient temperature or between + 2 and + 8°C.

ASSAY METHODS

Electrothermal atomisation, atomic absorption spectrophotometry (graphite furnace).

Induction coupled plasma-mass spectrometry (ICP-MS).

REFERENCE VALUES

Reference value in the general population:

– Whole blood bismuth < 0.01 µg/l.

– Urine bismuth < 2 µg/l.

FOR FURTHER INFORMATION

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BLADDER TUMOUR ANTIGEN

DEFINITION - INTRODUCTION

Bladder Tumour Antigen (BTA) is a protein expressed by several malignant bladder cell lines. It was isolated from the urine of people suffering from bladder cancer and has not been found in the urine of healthy individuals. It is a structural analogue of human haemolytic complement factor H (CFH). Like factor H, BTA is able to reduce the activity of the alternative complement pathway. This HCF-like activity could enable cancer cells to escape lysis by inhibiting complement activity.

In order of incidence, bladder cancer is the 5th most common cancer in men and the 9th most common in women. BTA is currently considered to be a marker for bladder cancer.

INDICATIONS FOR MEASUREMENT

Monitoring bladder cancer for early detection of recurrence.

Aid to the diagnosis and selective screening tool for bladder cancer in at risk people; smokers over 50 years old; dye, rubber, metal, petrochemical and leather industry workers; and people with a familial predisposition. BTA measurement is used in this situation in addition to urine cytology and cystoscopy which is the only process able to confirm the diagnosis.

INFORMATION

■ SAMPLE

Urine sample: Random urine sample collected without addition of preservative or fixing agent.

■ QUESTIONS FOR THE PATIENT

Current treatment: Chemotherapy, radiotherapy or surgery (types and date of treatment)? Have you had a recent cystoscopy examination? Trauma secondary to this type of examination causes an increase in urinary BTA for the following two weeks. This time period should therefore be observed before obtaining a sample for measurement. Similarly, a urine sample for BTA measurement must be obtained distant to any endoscopic or catheterisation procedure.

■ SAMPLE STORAGE AND TRANSPORT

Store for 1 week at +4°C, beyond that, freeze the sample at –20°C.

ASSAY METHODS

Sandwich immunoenzymologic method.

NORMAL EXPECTED VALUES

From a study on 212 apparently healthy people including smokers and non-smokers, 97% of people had a urinary BTA concentration < 14 U/ml and no patients suffering from non-urogenital disease had a urinary BTA > 14 U/ml. The value of 14 U/ml has therefore been selected as the threshold.

PATHOLOGICAL VARIATIONS

Increased urinary BTA concentrations in bladder cancer

The sensitivity of BTA at the 14 U/ml threshold in patients suffering from bladder cancer varies between 40 and 75% depending on the study. It increases with the stage of the disease; therefore urinary BTA levels are higher with more advanced stages of the disease.

A comparison with urine cytology has shown BTA to be more sensitive (the sensitivity of cytology is approximately 38%), regardless of the tumour grade or stage. This is also true for the detection of recurrence. A combination of BTA with urine cytology increases the sensitivity for diagnosing tumours and relapse.

As increased urine BTA concentrations correlate with tumour severity they are a prognostic indicator for the disease.

Increased urine BTA has shown to be useful for early detection of recurrence in the follow up of bladder cancers.

Increase in other diseases

BTA is not a specific indicator for bladder cancer. Raised urine BTA concentrations may be seen in various circumstances including infections (acute or chronic cystitis, acute pyelonephritis), inflammation (orchi-epididymitis) or trauma to the urinary tract, benign prostatic hyperplasia and renal or ureteric stones. False positive results have also been described in patients with haematuria and those who have been given prophylactic endovesical chemo- or immunotherapy after transurethral resection.

FOR FURTHER INFORMATION

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BNP AND NT-proBNP

DEFINITION

Brain natriuretic peptide (BNP) or type B naturetic peptide, is a peptide which was initially isolated from pig brain (hence its name Brain), the major physiological effect of which is natriuresis. It is synthesised mostly by left ventricular myocytes (the majority) and the right side of the heart, in response to volume overload or an increase in ventricular pressure (load conditions). It is synthesised at the time of use and is not stored.

BNP exists in a reserve form, preproBNP (134 AA) which is cleaved in equimolecular amounts into pro-BNP (108 amino acids) which itself is cleaved into biologically inactive NT-proBNP (N-Terminal proBNP) (amino acids 1-76) and into active BNP (AA 77-108) which are released into the circulation. Measurements for one or other of these molecules are based on this equimolar production of BNP and NT-proBNP.

In reality, testing for BNP in patients with heart failure using gel filtration techniques showed that the majority of circulating forms were of high molecular mass, mostly glycosylated proBNP. ProBNP has very weak biological activity (BNP is 6 to 7 times more active) and can undergo several post-translational modifications including o-glycosylation and proteolysis of the N and C-terminal parts.

Circulating plasma forms of BNP

– BNP1-32: Biologically active form

– N-terminal peptides: NT-proBNP

– Native proBNP1-108

– Post-translational modifications (O-glycosylation)

– Truncated forms N- and C-terminals of NT-proBNP

– Truncated forms of BNP: BNP3-32 (-ser, -prol) and BNP7-32

INTRODUCTION

BNP binds to type-A receptors on the vascular endothelium, activating cGMP to produce its biological effects. It has a very short half life in the circulation of approximately 20 minutes and is rapidly degraded via clearance receptors (type-C) or neutral endopeptidases in the endothelium. NT-proBNP is removed in all tissues with high blood flow and has a half life of 1 to 2 hour.

The main physiological role of BNP is to counter the increase in cardiac pressures by peripheral vasodilatation, increasing glomerular filtration (diuretic effect), reducing sodium reabsorption from the renal tubules (natriuretic effect) and inhibition of the Renin-Angiotensine-Aldostérone system. Its effects therefore compensate partly for the volume overload in heart failure (HF).

Heart failure is due to a failure of the ventricles, either because of a contractile disorder of the heart (systolic function: ejection) or a filling disorder (diastolic function: pressure). Systolic dysfunction is an inability of the heart to provide the necessary blood flow to cover organ oxygen and metabolite requirements. This results in low cerebral blood flow (confusion), muscle fatigue and renal dysfunction (oliguria). Diastolic dysfunction causes an increase in proximal pressures (poor blood return to the heart), dyspnoea or even acute pulmonary oedema. The mechanism of heart failure can be studied by echocardiography. BNP is also a diagnostic aid, the interpretation of which depends on a clinical assessment to establish pre-test whether the dyspnoea is (or is not) likely to be of cardiac origin.

BNP is the first overall global laboratory marker of heart failure which is both diagnostic, prognostic and used in monitoring treatment. NT-Pro BNP and BNP have the same clinical relevance.

INDICATIONS FOR MEASUREMENT

HEART FAILURE (HF.)

– Diagnosis of HF: Triage of patients presenting to the emergency services with acute dyspnoea which may be of cardiac, pulmonary or mixed origin.

– Risk stratification for discharge from hospital.

– Prognosis of HF.

– Monitoring treatment.

ACUTE CORONARY SYNDROMES

– Risk stratification in patients with an extensive myocardial infarction.

INFORMATION

SAMPLE

BNP: EDTA tubes, plastic only.

NT-proBNP: Dry tubes (serum) or with anticoagulants (heparin or EDTA).

No special sampling precautions (no circadian rhythm or postural changes).

QUESTIONS FOR THE PATIENT

Suspected disease?

Current treatment? Nesiretide (rhBNP) increases plasma BNP concentrations but does not change NT-proBNP concentrations. Other treatments for heart failure may lower BNP and NT-proBNP (a marker of the effectiveness of treatment)

SAMPLE STORAGE AND TRANSPORT

BNP: Stable for a maximum of 4 h at ambient temperature; 48 h at + 4 °C. It is recommended that the sample be centrifuged promptly and plasma frozen within 4h of sampling if the measurement is to be performed later.

NT proBNP: Stable for 3 days at ambient temperature; 6 days at + 4°C.

Storage of frozen plasma: 2 months at– 20° C, 6 months at– 80° C.

ASSAY METHODS

BNP

– The reference method is radio immunological although this is a long process and requires a suitable laboratory;

– Rapid immunofluorimetric assay (BNP result obtained in 15 min with a small portable instrument);

– Recent automated immunoanalyser techniques (2004) with several antibody couples used.

■ **NT-proBNP**

– Automated immunoanalysis with a single antibody couple is used (2008-2009: Roche, Siemens, bioMérieux monoclonals).

Overall, all of the analytical methods correlate well although some values obtained are extremely different. It is still difficult to standardise measurements because of the many circulating forms of BNP. As a result, the same kit needs to be used to monitor the same patient.

NORMAL EXPECTED VALUES

These depend on the method used, increase with age and depend on sex (women > men), body mass index (plasma BNP concentrations are lower in the obese) and glomerular filtration rate.

As an indication:

N.B.: 1 ng/l = 0.29 pmol/l.

BNP (ng/l)	<45 years	45-54 years	55-64 years	65-74 years	>75 years
Men	24	39	72	63	78
Women	47	72	81	95	179
NT-proBNP (ng/l)		**<50 years**	**50-65 years**		
Men		84	194		
Women		155	222		

NT-proBNP in children:

	Median NT-proBNP (ng/l)	Reference interval 95 % (ng/l)
Boys	38.7	5-742
Girls	70.8	
Girls 0-10 years	173.8	21-1122
Girls 11-13 years	118.5	11-1122
Girls 14-18 years	61.1	6-1122

PATHOLOGICAL VALUES

■ HEART FAILURE

BNP and NT-proBNP are very good diagnostic tools when the origin of the dyspnoea is uncertain and low concentrations are valuable to exclude HF and high concentrations to confirm it (*cf* table). In reality, the clinician must establish the pre-test probability of HF to best use the BNP or NT-proBNP values.

Thresholds to consider for BNP and NT-proBNP to exclude or confirm acute dyspnoea of cardiac origin (from the BNP and PRIDE studies). NPV: negative predictive value/ PPV: positive predictive value.

	High likelihood HF is absent (NPV > 98 %)	Grey area	High likelihood HF is present (PPV > 90 %)
BNP (ng/l)	< 100	100 - 400	> 400
NT-proBNP (ng/l)	< 300	Depending on age 300-450 < 50 years 300-900 > 50-75 years 300-1800 > 75 years	Depending on age > 450 < 50 years > 900 > 50-75 years > 1800 > 75 years

BNP/ NT-proBNP can therefore be used to support (or exclude) a diagnosis of HF but not to explain its mechanism (systolic or diastolic). It relates to all types of HF, both those where the problem is primarily the heart but also those due to a lung disorder dilating the heart and increasing its pressures. Increased pressures in the right side of the heart for example in pulmonary embolism (PE) leads to BNP/ NT-proBNP secretion. In this situation the rise in BNP/ NT-proBNP reflects high risk PE.

In general terms, dyspnoea associated with lung disease does not produce a rise in BNP/ NT-proBNP except in PE with decompensation, when it is so severe that it causes hypoxia.

■ **Causes of increased BNP/ NT-proBNP**

– Acute and chronic heart failure

– Left or right ventricular dysfunction or dysfunction without acute heart failure

– Septic shock

– Acute coronary syndrome

– Acute respiratory distress syndrome

– Pulmonary embolism

– Renal failure

– Cirrhosis of the liver

– Hyperthyroidism

– Atrial fibrillation.

■ **BNP/NT-proBNP concentrations below expected values**

– Obesity

– Acute "flash" pulmonary oedema (synthesis but not storage of BNP).

■ PROGNOSIS

When a heart failure patient is discharged from hospital, plasma BNP/ NT-proBNP concentrations are of prognostic value and correlate with the likelihood of the patient being rehospitalised within 6 months.

■ MONITORING TREATMENT

In view of the short half-life of BNP (20 minutes), a fall in plasma concentration can be used to confirm effectiveness of treatment.

Outpatients treated successfully have plasma BNP concentrations which are above normal but stable in the region of 200-350 ng/l; these concentrations represent a low risk of re-admission within 30 days (stability to be confirmed by a BNP measurement every 3 months).

BNP is not however always used as clinical findings *a priori* provide sufficient information.

NT-proBNP has also been proposed to optimise treatment monitoring and its serum/plasma concentration falls in response to the positive effect of all treatments for heart failure (converting enzyme inhibitors, angiotensin II receptor antagonists, aldactone, long term beta blockers, rehabilitation and bi-ventricular re-adaptation and pacing). A transient rise in NT-proBNP occurs when beta- blocker therapy is started although this does not strictly reflect lack of effect of the treatment.

Should BNP be given to patients with heart failure?

A recombinant form of BNP is available for the treatment of heart failure (nesiritide). This has vasodilating, diuretic and natriuretic effects. Its indications are still unclear and it is currently used in severe salt and water retention which is resistant to diuretics. Treatment is monitored by NT-proBNP measurements.

BNP AND MYOCARDIAL INFARCTION

Some people have proposed to use BNP in risk stratifications of patients who have had an extensive myocardial infarction. It is certainly a powerful prognostic marker although there is no clearly defined cut off. It would appear to be particularly useful when the troponin is negative. If raised, invasive treatment appears to be useful.

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BONE ALKALINE PHOSPHATASE

DEFINITION

Bone specific, serum alkaline phosphatase (ALP) (SAP or BSALP or *Bone Specific Alkaline Phosphatase*) is a marker of bone formation. The role of the protein has not been fully determined, although it is probably involved in bone mineralisation. It is an 80 kDa glycoprotein tetramer, present on the surface of osteoblasts and released into the bone matrix as a dimer, following hydrolysis. Serum concentrations therefore reflect osteogenesis activity. Physiologically, bone and hepatic ALP isoforms represent approximately 50% of serum ALP activity. Unlike osteocalcin they are not removed by the kidneys and have a half-life of approximately 2 days.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

– Primary indication is for monitoring the effectiveness of treatment in Paget's disease, through a fall in BSALP concentrations.

– Biochemical marker of bone formation used in osteoporosis (an aid to treatment decisions).

– Useful marker to monitor bone forming treatments (teriparatide (rh-PTH); 25 hydroxy and 1,25 dihydroxy vitamin D) and anti-resorption treatments (oestrogen, bisphosphonates and raloxifen). Effectiveness can be estimated by comparing values before/after treatment for 6 months (expected fall by 20 to 40%).

– Marker used in nephrology and in dialysis (assessment of renal osteodystrophy) as it is not removed by the kidney.

INFORMATION

SAMPLE

1 ml of non-haemolysed serum frozen within 4 hours of sampling.

The sample should be taken preferably in the morning between 08 and 09 hours, with the patient fasting.

QUESTIONS FOR THE PATIENT

Current diseases (hepatic, renal, etc.), reason for measurement and treatment?

SAMPLE STORAGE AND TRANSPORT

Frozen at – 20°C.

ASSAY METHODS

Immunoassays with a monoclonal antibody in a radiolabelled suspension and another solid phase antibody bound to a plastic bead.

The method is not entirely specific vis-à-vis hepatic isoenzymes, although significant interference only occurs when these are markedly raised.

Automated IRMA and ELISA methods are available.

NORMAL EXPECTED VALUES

For reference, in adults (with physiological bone remodelling):

– Men < 22 µg/l,

– Women (with menstrual cycles) < 12 µg/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Circadian rhythm.

Age (increases with age in both sexes).

Sex.

Bone growth.

Physical activity.

Vitamin D, calcium and phosphate intake.

PATHOLOGICAL VARIATIONS

Rises

Primary and secondary hyperparathyroidism.

Bone metastases and osteosarcoma.

Paget's disease

Formation phase of a bone callus.

Falls

Hypoparathyroidism.

Cushing's disease.

Treatments inhibiting bone remodelling (bisphosphonates).

FOR FURTHER INFORMATION

■ Cormier C., Kindermans C. et Souberbielle J.C., *Marqueurs du remodelage osseux: données récentes sur leur intérêt et leurs limites dans l'exploration des ostéoporoses*, Ann Biol Clin, 2001; 60: 343-346.

■ Garnero P, Bianchi F, Carlier MC, et *al. Les marqueurs biologiques du remodelage osseux : variations pré-analytiques et recommandations pour leur utilisation*. Ann Biol Clin 2000; 58:683-700.

BROMINE

DEFINITION

Bromine (Br) is a brownish coloured liquid at room temperature, the name of which comes from the Greek "bromos" (foul smell) because of its sharp smell. It belongs to the halogen family. It is an extremely volatile liquid releasing rust coloured toxic suffocating vapours. Bromine is widely found in nature particularly in seawater (potassium, sodium and magnesium bromide).

Salts of hydrobromic acid or bromides are used in industry (halogen lamps, extinguishers, paint industry, cosmetics, photography, petroleum industry, etc), in agriculture (pesticides) and in the treatment of swimming pool water. Bromides were also used therapeutically for their sedative and anti-convulsant properties and are still used in a few specialities.

METABOLISM

Bromine vapour enters the body through the respiratory tract. Bromides penetrate rapidly through the gastro-intestinal tract and then are distributed in the extra-cellular fluids and red blood cells. They cross the meningeal barrier and placenta. After application to skin, they enter the body quickly and become distributed in adipose tissue, liver and lungs. It is excreted mostly in urine, very slowly, with a plasma half life of 12 days. In the kidney it undergoes glomerular filtration followed by tubular resorbtion in competition with the chloride ion.

MECHANISM OF ACTION

In the central nervous system, bromides appear to act competitively with chlorides on sodium channels, altering membrane polarisation. They reduce the release of noradrenaline and serotonin, hence their anticonvulsant activity.

CLINICAL SIGNS OF POISONING

■ ACUTE POISONING

Muco-cutaneous inflammatory reaction (erythema, pustules, ulcers).

Ocular irritation and photophobia.

Cough, dyspnoea, bronchopneumonia which may progress to fatal acute respiratory distress syndrome (ARDS) with massive exposure.

■ CHRONIC POISONING

Mostly skin reactions (facial oedema, acne and yellow discolouration of the hair and nails), keratitis and conjunctivitis.

Neurobehavioral disorders after chronic ingestion of medicines containing bromine salts but not from occupational exposure.

INDICATIONS FOR MEASUREMENT

■ MONITORING OF OCCUPATIONAL EXPOSURE

Blood and urine bromide measurements at end of shift are useful in the diagnosis of accidental poisoning and also for monitoring exposed workers.

■ TREATMENT FOLLOW-UP

Very rarely in human beings but particularly in dogs in which bromine salts are still widely prescribed.

INFORMATION

■ SAMPLE

Plasma.

Unacidified urine sample at the end of a shift to monitor exposure.

■ QUESTIONS FOR THE PATIENT

Establish smoking habit as smoking can increase bromide concentrations in the body.

■ SAMPLE STORAGE AND TRANSPORT

Plasma and urine samples can be stored and transported to the laboratory at between + 2 and + 8°C.

ASSAY METHODS

Direct potentiometry

Induction coupled plasma-mass spectrometry (ICP-MS).

REFERENCE VALUES

Reference value for treatment monitoring

Human beings: 100 to 500 mg/l.

Dogs: 800 to 2000 mg/l.

Reference values in the general population (unexposed people)

Plasma < 5 mg/l.

Urine < 10 mg/l.

FOR FURTHER INFORMATION

■ Lauwerys. R., *Toxicologie industrielle et intoxications professionnelles, Brome*, 3rd edition, Masson.

■ Fiche du brome: guide BIOTOX 2002, INRS.

BRUCELLA

DEFINITION

The *Brucella genus* has only one species: *Brucella melitensis*, which is divided into several sub-species: *Brucella melitensis subsp melitensis, subsp. abortus, suis, ovis, canis* and *neotomae*. These sub-species are themselves sub-divided into biovars. *Brucella* is a small Gram negative coccobacillus found either in isolation or in groups (pairs or short chains). It is aerobic, non spore forming and uncapsulated. Brucellosis is an infectious disease common to human beings and some animals.

In France, it is a notifiable occupational disease (mandatory declaration).

Synonym: Brucellosis = Malta Fever = Undulant fever.

INTRODUCTION AND INDICATIONS

EPIDEMIOLOGY

Although it has reduced greatly in developed countries, brucellosis still affects many areas around the Mediterranean basin, Middle East and South America. Human brucellosis occurs where animal brucellosis is present.

ANIMAL BRUCELLOSIS

This affects cattle, poultry, pigs, sheep and goats and presents with reproductive disorders (the bacterium grows in the placenta and foetus, resulting in spontaneous miscarriage).

HUMAN BRUCELLOSIS

Transmission occurs accidentally by contact with infected animals in farm workers, veterinary practitioners, shepherds and abattoir workers. Food contamination (unpasteurised milk, unpasteurised cow's, ewe's and particularly goat's cheeses) also occurs.

CLINICAL FEATURES

Brucellosis has a wide range of symptoms.

The different phases of the disease represent the different stages in its pathophysiology:

– acute brucellosis,

– subacute or localised brucellosis,

– Chronic brucellosis.

- **Acute brucellosis:** Muco-cutaneous or gastro-intestinal infection is followed by a primary invasion phase which is usually silent (this is the primary infection or septicaemia phase). If the primary infection is symptomatic it mostly presents with cyclical fever (over 10 to 15 days) typically associated with profound night sweats, arthralgia and asthenia.
- **Sub-acute brucellosis:** This is the spreading phase of the disease to secondary sites (the bacterium is found in blood and suppurative lesions). It develops either immediately or a very long-term after primary infection and is characterised by osteoarticular problems with bone abscesses and also visceral disease (genital organs, hepatic disease) or meningeal disease (neurobrucellosis).
- **Chronic brucellosis:** This is an inflammatory reaction due to the persistence of the organism in body cells without antibiotic treatment.

  And is characterised by severe physical and psychological asthenia with or without spiked fever.

INFORMATION

SAMPLE

Blood culture.

Serum (Dry tube): Blood taken as early as possible, as soon as the clinical signs appear. Consider resampling 2 to 3 weeks later for serological diagnosis.

Other possible samples: Pus from various foci, lymph nodes, CSF.

QUESTIONS FOR THE PATIENT

Clinical signs?

Occupational contact?

Dietary habits (drinking unpasteurised milk or eating unpasteurised cheeses)?

SAMPLE STORAGE AND TRANSPORT

Bacteriology samples are transported to the laboratory at ambient temperature; serum at + 4°C.

ASSAY METHODS

The laboratory diagnosis is particularly useful in view of the relative non-specificity of clinical symptoms

DIRECT DIAGNOSIS

- **Culture:**

  – Blood cultures are usually positive in the acute and even subacute phases of the disease.

  – Culture from biopsies or pus shows transparent colonies which are convex, obligate aerobic, catalase + and oxidase +. Culture is slow and the organisms are very difficult to grow which limits the use of this method.
- **PCR:** Techniques have recently been developed but are not widely available.

INDIRECT DIAGNOSIS

- **This is the most commonly used diagnosis.** There are many techniques of differing value and not all use the same class of antibodies. IgM can be detected from the 10th day after the first clinical signs of the disease, following which IgG's develop and rise in concentration. IgG and IgM are found in the acute phase of the disease. IgM then disappears and can no longer be found in the chronic phase whilst IgG is still present. There are many cross reactions particularly with other bacteria such as *Francisella tularensis, Yersinia enterocolitica* serotype 09 and *Vibrio cholerae*.
- **Wright's serodiagnosis (WSA):** This is the reference method currently recommended by the WHO as it has been standardised. It is a tube agglutination technique which reveals the presence of mostly IgM antibodies and is therefore used in the diagnosis of acute brucellosis.

A titre of ≥ 80 or 120 IU (after incubating for 24 hours at 37°C) is considered to be significant. False positives occur as do false negatives due to the presence of blocking antibodies (IgG or IgA) or through the zone effect. For this reason, testing for blocking antibodies is essential when the result is negative as is agglutination with all serum dilutions to avoid the zone effect.

- **Bengal rose reaction or buffered antigen test (BAT):** This is a slide agglutination method using an antigen stained with Bengal rose.

  This is rapid, specific and sensitive and reveals IgG antibodies in serum. It should be followed by a WSA for quantification if the result is positive. This method is used above all for the diagnosis of acute and subacute brucellosis but also for chronic brucellosis (it remains positive for an extremely long time).

- **Complement fixation reactions (CFR):** This reveals the presence of IgG, becomes positive later than the agglutination reactions and remains so for longer. As such, it is used in the diagnosis of subacute and chronic brucellosis.

- **Indirect immunofluorescence (IIF):** This reveals and can titre IgM and IgG. It is a highly sensitive, specific method useful in the diagnosis of chronic forms of the disease (it remains positive for at least 18 months).

- **Immuno-enzymatic method (ELISA):** This is a promising technique for the diagnosis for brucellosis particularly in epidemiological investigations. It has the same advantages as the IIF method. In view of the wide range of antigens used its commercial availability is limited.

- **Melitin intradermal reaction:** This reveals delayed hypersensitivity to brucella antigens and is therefore use in chronic forms of the disease as it can establish distant contact with brucella. Despite its use, the melitin intradermal reaction is almost no longer used because of a lack of antigens.

- **Summary:** Testing for antibodies using the Bengal rose reaction and WSA remain valuable as screening techniques. Testing for antibodies using IIF or ELISA methods are more specific and provides more secure information about the active nature of the infection. Extreme hypersensitivity to brucella antigens is used to confirm a chronic form of the disease.

	Acute brucellosis	Sub-acute brucellosis	Chronic brucellosis
Blood culture	+++	+/-	-
Bengal rose	+	+++	+/-
Wright's serodiagnosis	+	+++	-
IIF /ELISA	+	+++	+

Laboratory diagnosis of brucellosis

TREATMENT

Brucellosis is treated with a combination of 2 antibiotics: Tetracycline (doxycycline) and rifampicin, for 6 to 8 weeks. Antibiotic therapy is no longer effective in chronic forms of the disease.

Prevention involves food and occupational hygiene precautions and relies on eradicating animal brucellosis by culling affected herds and animal vaccination etc.

There is no human vaccine.

FOR FURTHER INFORMATION

- Charadon S., Ramuz M., *Brucella*, Encycl Med Biol (Elsevier Paris) 2003.

BUPRENORPHINE

DEFINITION

Buprenorphine is a semi-synthetic opioid derived from thebaine, an opium alkaloid. It is used therapeutically as an analgesic, as sublingual tablets or injectable solution, and is indicated for use in the treatment of severe pain, particularly post-operative or pain associated with malignancy. It is also used as a substitution treatment for major opiate drug dependency as part of the overall medical, social and psychological management. Administration of substitution drugs under medical supervision is usually accompanied by urine screening to ensure that no illegal drugs have been taken. This screening is not compulsory in France for the prescription of buprenorphine. It is however, compulsory for methadone substitution, which also requires regular toxicology monitoring. For equivalent efficacy, buprenorphine has the advantage of causing a far less severe withdrawal syndrome, whereas long term use of methadone leads to prolonged morphine dependency. The French Centre for Drugs and Drug Addiction estimated in 2001 that between 150 and 180,000 people were dependent on opiates or cocaine, more than 70000 of whom were being treated with buprenorphine (compared to a few thousand with methadone).

Buprenorphine is a morphine agonist-antagonist which binds to the cerebral mu and kappa receptors. Its effect in opioid substitution treatment is attributed to its slowly reversible binding to mu receptors which appears to produce a prolonged reduction in drug craving in addicts.

The partial agonist effect of buprenorphine provides it with considerable safety of use, limiting particularly the cardio-respiratory, depressant effects.

PHARMACOKINETICS

Sublingual absorption	15 to 55%
Plasma peak (Tmax)	90 minutes
Plasma half-life	2 to 5 hours, but long duration of action because of its lipophilic nature and therefore its high cerebral concentrations and extensive tissue binding
Metabolism	By N-dealkylation by CYP3A4* and glucuronide conjugation of the parent molecule and dealkylated derivative
Excretion	80% in faeces by biliary excretion of the glucuronide conjugated metabolites, 20% in urine

** Note, therapeutic interactions: potent CYP3A4 inhibitors such as ketoconazole, itraconazole, ritonavir, nelfinavir and indinavir result in an increase in peak concentration (Cmax) and area under the curve (AUC) of buprenorphine (and therefore, dose of which may need to be reduced). Interactions between buprenorphine and CYP3A4 inducers (phenobarbital, carbamazepine, phenytoïn and rifampicine) have not been studied although patients should be monitored in the event of concomitant administration.*

INDICATIONS FOR MEASUREMENT

Confirmation of buprenorphine poisoning: The main causes of which are intravenous misuse (after crushing the tablets into powder and filtering the fluid to be injected) and possibly its association with other central nervous system depressants. In addition, some people use buprenorphine to reduce the side effects of large doses of psychostimulants such as LSD, ecstasy or amphetamines.

The clinical features of poisoning are generally those of the opiates (nausea, vomiting, respiratory difficulties, dizziness, confusion, drowsiness) with, in particular, excessive sweating, mydriasis and hallucinations. Poisoning may be fatal.

INFORMATION

SAMPLE

Urine: urine sample.

Serum: Do not use a tube with gel separator.

QUESTIONS FOR THE PATIENT

Any request for measurement of medical drugs must include the reasons for requesting the test (investigation for efficacy or toxicity), sampling time, treatment start date and/or any change in dosage, dosage information (amount administered, frequency, route of administration) and the age, height and weight of the person wherever possible.

SAMPLE STORAGE AND TRANSPORT

If the analysis is to be delayed, freeze the sample (urine or serum) within 4h of sampling.

ASSAY METHODS

Screening: Immunochemical or immunoenzymatic.

Specific confirmation methods: Gas phase chromatography – mass spectrometry, HPLC, mass spectrometry or HPLC-UV.

NORMAL EXPECTED VALUES

As an indication:

In urine by immunoenzyme assay:

Concentration < 1 µg/l: No excretion of buprenorphine in urine.

Concentration from 1 to 2.5 µg/l: Equivocal result.

Concentration > 2.5 µg/l: Buprenorphine present in urine.

In serum:

For analgesic treatment, serum buprenorphine concentrations are usually 0.5 µg/l.

In substitution treatment, serum buprenorphine concentrations are usually < 10 µg/l.

INTERPRETATION

Urine concentrations in patients on buprenorphine treatment may be extremely high up to 1000 µg/l and above.

The terminal elimination phase is long (20 to 25 hours) and serum measurement is required in poisoning.

FOR FURTHER INFORMATION

- Pepin G., Cheze M., *Opiacés et produits de substitution*, Encycl Med Biol, Elsevier, Paris 2003.
- Pepin G., Dubourvieux N., Cheze M., *Opiacés et opioïdes: usages, effets, dépistage, dosage*, Revue française des laboratoires 2000; 322:41-5.
- Pepin G., *Opiacés et opioïdes. In: Toxicologie et pharmacologie médicolégales*, coordinateur P. Kintz, Collection Option/Bio, Elsevier Ed, Paris 1998 : 366-72.
- Dictionnaire Vidal®.

C-PEPTIDE

DEFINITION

C-peptide (or *Connective peptide* or connecting peptide) is a 31 amino acid molecule (3020 Da), derived from enzymatic cleavage of proinsulin in the pancreatic islet of Langerhans β cell Golgi apparatus. At the same time cleavage produces equimolecular amounts of insulin. Unlike insulin, C-peptide is not metabolised in the liver and is removed mostly by the kidney. Approximately 6% of C-peptide is found intact in urine.

It has a half-life of between 20 and 30 minutes, compared to approximately 4 minutes for insulin. Because of this metabolic difference, insulin and C-peptide concentrations are different in peripheral veins. C-Peptide has long been thought of as having no biological activity, although this concept has since been questioned. During prolonged administration, it improves neuronal conduction and renal function in type 1 diabetic patients with neuropathy, and at physiological doses C-peptide has effects on glomerular filtration in type 1 diabetics.

INDICATIONS FOR MEASUREMENT

Measurement of C-peptide is recommended to assess endogenous insulin secretion and to diagnose hyperinsulinism and insulinoma. Measurement is essential to identify administration of insulin (intentional or otherwise). In insulin administration, insulin concentrations are very high, whereas C-peptide concentrations are very low.

In type 1 diabetics, C-peptide can be used to assess residual insulin secretion, particularly when patients are receiving insulin.

Measurement of C-peptide is indicated when insulin measurement cannot be interpreted because of exogenous insulin or anti-insulin antibodies.

INFORMATION

SAMPLE

C-peptide can be measured in EDTA or heparinised plasma or in serum. The sample type, however, must always be checked against the recommended anti-coagulant for the method used.

The sample is generally taken fasting, unless specifically instructed otherwise by the requesting physician. Blood samples should be centrifuged promptly and then the serum or plasma separated and frozen at –20°C within 4 hours of sampling, until measurement. Unlike insulin, it is important to be aware that haemolysis does not influence C-peptide measurements. Blood glucose is of course always measured simultaneously in order to interpret the result.

For urinary C-peptide measurement, a 24 hour urine collection is obtained. After the urine has been mixed and its volume measured, an aliquot is frozen at –20°C within 4 hours of sampling, until measurement.

NECESSARY INFORMATION

Fasting blood glucose and the sample time must be stated, together with the patient's clinical context (pregnancy, diabetes, obesity, Cushing's syndrome, etc.) and treatment received (insulin and oral anti-diabetic agents). The request should also state whether the test forms part of a dynamic function test, such as a fasting test, oral glucose tolerance test (OGTT), intravenous glucose tolerance test (IVGTT) or glucagon test.

SAMPLE STORAGE AND TRANSPORT

Serum or plasma C-peptide is stable for 2 weeks at -20°C and for several months at –70°C. Stability is increased by adding aprotinine. In addition, multiple freeze-thaw cycles do not influence assay results.

ASSAY METHODS

C-peptide is generally measured by immunological methods with radioactive tracers and more recently, non-isotopic tracers which enable assays to be automated. Most of the antibodies used do not recognise insulin, although proinsulin does have major cross-reactions; interference from the proinsulin, however, may be considered to be negligible in most cases as serum C- peptide concentrations are approximately 50 times higher than those of pro-insulin. Proinsulin interference becomes important in hyperproinsulinism. The most widely used standard is IRP 84/510.

DYNAMIC TESTS

The same stimulation tests are used as those described in the "Insulin" section.

REFERENCE VALUES

SERUM OR PLASMA C PEPTIDE

Fasting values in healthy adults range between 1.1 and 5.0 ng/ml (0.4 – 1.7 nmol/l). Using the IRP 84/510 standard, 1 ng/ml is equivalent to 3.021 nmol/l.

In the oral glucose tolerance test, concentrations around 30 – 60 minutes are generally 4 times higher than baseline. In the glucagon test, C-peptide increases very rapidly by at least 50% of the baseline value from the 6th minute of the test.

URINARY C PEPTIDE

Urinary C-peptide concentrations in a 24 hour urine sample are between 2 and 260 µg/24 hours. Note that these vary from day to day in individual people.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Values are approximately 50 to 60% lower in children under 6 years old than in adults. They subsequently rise regularly until puberty when they reach adult values.

PATHOLOGICAL VARIATIONS

Hyperinsulinism/insulinoma

Insulin and C-peptide concentrations are raised.

Conversely, in hypoglycaemia secondary to insulin injection (intentional or otherwise), C-peptide concentrations are very low, whereas insulin concentrations are high.

- **Assessment of residual pancreatic secretion** during insulin therapy in patients with type 1 diabetes or who have undergone pancreatectomy. If the fasting concentration is very low or at the limit of detection of the method for the stimulation test, then usually the glucagon test is required.

  Measurement of urinary C-peptide is also a good indicator of residual pancreatic secretion.

- **C-peptide measurement can be used to distinguish between the two types of diabetes.** In type 1 diabetes, C-peptide concentrations are very low or undetectable and cannot be stimulated by glucagon, whereas in type 2 diabetes they are equal to or greater than values in controls.

- **Renal insufficiency**

  C-peptide concentrations are raised in renal insufficiency because of defective excretion by the kidney. Fasting concentrations are also difficult to interpret. Secretion, however, can be assessed by measuring the increase in C peptide after stimulation.

FOR FURTHER INFORMATION

- Chevenne D., *C peptide*, EMB (Elsevier) 2003.
- Chevenne D., Trivin F., Porquet D., *Insulin assays and reference values*, Diabetes Metab (Paris) 1999,25: 459-476.
- Porquet D., Chevenne D., *Stratégies d'exploration fonctionnelle et de suivi thérapeutique*, Introduction au chapitre diabétologie du CD-ROM produit par le CNBH, Immuno-analyse & Biologie spécialisée, 2002; 17: 369-374
- Sapin R., Demangeat C., *Aspects analytiques des dosages d'insuline, peptide-C, proinsulines et glucagon*, Médecine Nucléaire – Imagerie fonctionnelle et métabolique, 2001; 25: 73-84.

C-REACTIVE PROTEIN

DEFINITION

C-reactive protein (CRP) is an acute phase protein produced in inflammation. It is synthesised by hepatocytes under the control of cytokines such as interleukin-6, interleukin-1 and a *Tumour Necrosis Factor alpha* and has a plasma half-life of approximately 12 hours. CRP is present in very low levels in healthy people. Blood concentrations can rise extremely quickly to very high levels (up to 1000 times) in response to external stimuli and then return to normal values quickly. These features make it widely used for the clinical diagnosis and monitoring of many infectious and systemic diseases. CRP has recently seen a return in interest as a cardiovascular risk marker since the development of new "high sensitivity" assay techniques (CRPhs).

Synonyms: CRP.

INTRODUCTION

The precise physiological role of CRP is still poorly understood. It appears to play a central role in defence against external antigens by stimulating the synthesis of tissue factors and activating complement. Its large variation in amplitude and short half-life make it a marker of choice for acute inflammatory reactions.

It has also now been clearly established that inflammation plays an integral part of atherosclerosis and it was therefore reasonable to consider whether inflammatory markers such as CRP could predict atherosclerosis or assess the prognosis of atheromatous disease. It has recently been proposed that high sensitivity CRP *(cf. Assay methods)* be included as an additional risk marker in assessing cardiovascular risk, although this role is still debated and is currently restricted to certain patient subgroups.

INDICATIONS FOR MEASUREMENT

CRP: Marker of inflammation and/or infection used for:

– Early diagnosis of bacterial or fungal infections, particularly in neonatology, paediatrics and in at risk patients (neutropaenic or bone marrow transplant patients), detection of infections after surgery etc.

– Monitoring antibiotic therapy.

– Assessing the activity of chronic inflammatory disease (rheumatoid arthritis, Crohn's Disease, etc.).

– Determining inflammatory and nutritional protein profiles.

CRPhs: Measurement is recommended *(American Heart Association/American College of Cardiology, 2003)*:

– In asymptomatic patients with coronary artery disease to identify those at moderate risk (10 to 20% risk of serious cardiovascular event in 10 years) and in patients with unstable angina to provide supportive evidence for preventative measures (risk factor control).

– CRPhs measurement at present is not recommended in other potential indications (as a marker of re-stenosis after angioplasty, systematic screening for cardiovascular risk in the general population etc.).

INFORMATION

SAMPLE

Serum (preferably) or plasma taken in heparin or EDTA; avoid tubes with separator gel (values may be increased by up to 10% with some methods).

Samples should preferably be taken from fasting patients. Cloudy samples should be centrifuged (turbidity interferes with the analysis, producing bias which is directly proportional to triglyceride concentrations).

QUESTIONS FOR THE PATIENT

Are you taking medical treatment? Hormone replacement therapy for the menopause (HRT) increases CRP. Regular HRT users have CRP values which are raised by a factor of approximately 2. Serum CRP remains slightly higher in former HRT users than in women who have never been treated. The influence of oral contraception on CRP is still under debate. Statins cause a fall in CRP (dose-independent effect) as soon as treatment is started (15 to 25% fall after treatment for 6 weeks). Some fibrates also reduce CRP.

SAMPLE STORAGE AND TRANSPORT

Storage of serum or plasma: For 1 week at +4°C and several months at –20°C.

ASSAY METHODS

Depending on the purpose, the following may be used:

– The conventional method, to assess inflammation (measurement range ≥ 5 mg/l).

– The high sensitivity method to screen for cardiovascular risk (measurement range from 0.1 to 20 mg/l).

– 3rd generation methods (wide measurement range from 0.1 to 200 mg/l).

Many methods and analytical combinations are available:

– Liquid immunoprecipitation techniques: Turbidimetry or nephelometry

– ELISA, radial immunodiffusion, latex particle agglutination techniques etc. A quantitative immunoturbidimetric or immunonephelometric method suitable for emergency use is recommended. There is currently no reference method although the most widely used comparison method (used in the American consensus, *Center of Disease Control-American Heart Association*) is the Siemens nephelometric method.

NORMAL EXPECTED VALUES

CRP values in healthy people are usually < 6 mg/l; a threshold of < 3 mg/l has been proposed for CRPhs in cardiovascular risk assessment by the *American Heart Association/American College of Cardiology*, 2003.

Reference material: There is a World Health Organisation reference material (WHO IRP 85/556) and a European reference material (CRM 470), calibrated as 39 mg/l against the WHO material. Both are suitable for conventional CRP measurement. CRM 470 is widely used by companies in France although it would be preferable to have a secondary reference material for ultrasensitive techniques or for the wide measurement range.

PATHOPHYSIOLOGICAL VARIATIONS

BIOLOGICAL VARIATIONS

- **Age:** Serum CRP is slightly higher in the first 2 days after birth in newborn babies, with average values of 5 mg/l at birth, 14 mg/l on D1 and 9.7 mg/l on D2. It increases with age in adults, the increase generally being associated with increased cardiovascular risk. Measurement of CRPhs in elderly patients is difficult as many interfering factors exist (mild transient infections, inflammation, often subclinical, etc.).
- **Sex:** CRP is not significantly different between men and women between 5 and 39 years old. Above this age the possibility that the patient is taking hormone replacement treatment must be considered.
- **Obesity:** CRP is increased in people with a high body mass index (obesity is a known cardiovascular risk factor in adults and children).
- **Smoking:** CRP is increased in smokers. This rise is associated with increased cardiovascular risk.
- **Alcohol:** CRP falls with moderate alcohol consumption and rises with high consumption.
- **Intra-individual variations:** CRP is relatively stable throughout the day although intra-individual variations are found (according to one study in 20 healthy people followed up for 6 months, the dispersion of CRP values was 30 to 63% or approximately 10% after fliers were excluded).

PATHOLOGICAL VARIATIONS

- **In an inflammatory reaction**

Serum CRP concentrations rise in the initial hours (from the 8th hour) after the onset of an inflammatory process and may rise to very high values (x 1 000). Values return to around normal quickly (3 to 5 days) if the inflammatory reaction resolves.

CRP is the first of the inflammatory markers measured in the inflammatory protein profile (CRP, orosomucoid and haptoglobin) to rise in serum concentration. This makes it more of an acute phase marker whereas orosomucoid is more a marker of chronic inflammation. In practice, joint interpretation of these two proteins can be used to monitor the change in inflammation. Initially CRP alone is raised; in the established inflammatory state both are raised and with effective treatment, CRP is the first to fall, followed by orosomucoid indicating recovery.

Inflammatory state	CRP	Orosomucoid	Haptoglobin
Early acute	+++	+	N or +
Acute, established inflammation	+++	+++	+++
Resolving	N or +	+	++
Chronic	N or +	+ or ++	++

From J. Rousseaux, www.med.univ.lille2.fr

- **Interpretation in disease**

Diagnosis of bacterial and fungal infections – differential diagnosis from viral infections

Serum CRP is the most sensitive and earliest diagnostic marker for these infections. Above 200 mg/l patients must be investigated for deep or systemic infection.

It is very widely used in this situation particularly in neonatology and values of > 20 mg/l at 12 hours after birth are highly suggestive of bacterial infection whereas values of < 5 mg/l at 24 hours generally exclude bacterial infection (note however, cases of "normal" CRP have been reported in newborn babies with very severe infection, particularly due to Streptococcus B).

An increase in value of > 40 mg/compared to baseline in neutropaenic or bone marrow transplant patients suggests complicating infection 24 to 48 hours before the development of clinical signs of infection.

In paediatric use, CRP helps to discriminate between viral or bacterial infections (primarily pneumonia and meningitis). The threshold under 6 years old is 20 mg/l (higher values indicating bacterial infection, lower values viral infection); the proposed cut off over 6 years old is between 50 and 75 mg/l.

Monitoring antibiotic therapy

CRP concentrations should fall after effective antibiotic treatment for 24 to 48 hours (a marker of effectiveness). Failure to fall indicates that the treatment is inappropriate and must be changed. In this situation, restoration of normal orosomucoid values, a marker of recovery, is often used jointly as an aid in deciding to stop treatment.

After surgery

Persistently raised CRP concentrations after day 5 or a clear secondary increase in values generally indicate septic complications.

In connective tissue diseases and chronic inflammatory diseases

CRP can be used to assess the activity of diseases such as rheumatoid arthritis, chronic juvenile arthritis and Crohn's Disease. It can also be used to monitor change on treatment. A very large increase in serum CRP (up to 500 to 600 mg/l) in severe inflammatory states indicates tissue necrosis although normal or slightly raised concentrations can also be seen in these situations, possibly because of increased removal.

Main causes of raised serum CRP *(from Bienvenu F., 2003)*

	Large rise (mg/l)	Modest rise (mg/l)
Infections	Bacterial - Septicaemia (100 - 600) - Septic arthritis (80 - 200) - Meningitis (80 – 200) - Pneumonia (80 – 200)	Viral - Meningitis (10 – 30) - Pneumonia (10 – 30) - Influenza (10)
Connective tissue diseases	Rheumatoid arthritis, Chronic juvenile arthritis (30 – 200)	Lupus erythematosus (10 – 40) Scleroderma (10 – 40) Dermatomyositis (10 – 40) Sjögren's syndrome(10 – 40)
Enteropathies	Crohn's Disease (50 - 200)	Ulcerative colitis (10 -50)
Malignant diseases		Leukaemia (10 – 40)
Heart disease	Myocardial infarction (50 – 200)	
Trauma	Surgery, burns (50 – 200)	

Nutritional protein profile

CRP can be used to assess the extent of an inflammatory reaction, the main cause of changes in serum proteins particularly proteins measured to assess nutritional status: Albumin, prealbumin, orosomucoid and *Retinol binding protein* (RBP). It is also used to calculate the PINI, the nutritional index used particularly in pregnancy.

Assessment of nutritional state with the PINI

(Prognostic Inflammatory and Nutritional Index)

PINI = CRP (mg/l) x orosomucoid (mg/l)/albumin (g/l) x prealbumin (mg/l)

(from Ingelbleek Y et al. Int J Vit Nutr Res 1985; 55: 91 101).

PINI<1: No risk PINI>30: Life-threatening risk.

CRPhs: A new cardiovascular risk marker?

CRP has become the most promising cardiovascular disease marker of inflammation over the last ten years. Several prospective studies in large diverse populations (men, women and the elderly) have shown that a rise in the concentration of serum markers of inflammation, particularly CRP, in apparently healthy people, is associated with development of major cardiovascular events (myocardial infarction, cerebrovascular accident or sudden death). When measured using a high sensitivity method (values "of interest" being between 0.1 and 5 mg/l), CRPhs appears to be a good predictive marker for the risk of clinical events.

The *Women's Health Study* conducted in 28000 women over 45 years old followed up for 8 years suggested that CRPhs could be used as a better predictive marker of cardiovascular events than LDL-cholesterol. It should be noted that although CRPhs concentrations correlate with the risk of clinical complications of atherosclerosis, they do not appear to be an indicator for the severity or progression of atherosclerosis as measured by CT scan (calcification score) or ultrasound (intima-medial thickness).

Similarly, serum CRPhs concentrations in patients with coronary artery disease may be low in patients with severe but clinically stable atheromatous disease of the three coronary arteries. On the other hand, an acute coronary event (intracoronary thrombosis producing transmural infarction or even sudden death) is associated with a rise in CRPhs.

Prevalence of CRPhs > 3 mg/l

< 10% in apparently healthy people.
< 20% in patients with stable coronary atheroma.
> 65% in patients with unstable coronary syndrome.
> 90% in patients with unstable syndrome complicated by transmural necrosis (vs < 50% if infarction occurs without prodrome).

Serum CRPhs concentrations also correlate with the development of re-stenosis after coronary angioplasty or recurrent ischaemia after aorto-coronary bypass. It also appears to be associated with a risk of first ischaemic cerebrovascular accident (Framingham cohort) and obstructive lower limb arterial disease *(Physicians' Health Study)*.

In practice, the risk of an asymptomatic person with coronary disease or a patient with unstable angina developing a cardiovascular event is considered to be low if the CRPhs is less than 1 mg/l and high if it is over 3 mg/l. A focus of clinically silent inflammation should be investigated if the CRP is over 10 mg/l.

Several authors have also highlighted the inter-individual variability of the inflammatory response in cardiovascular disease, where the rise in CRP in some patients is small (or zero) and in others it is very large. Because of this, the role of CRP in the assessment of individual cardiovascular risk is still being debated and further studies are required to provide a definitive conclusion.

FOR FURTHER INFORMATION

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C1 INHIBITOR

DEFINITION

C1 inhibitor (C1Inh) is a 105 kDa glycoprotein synthesised by the liver, monocytes, fibroblasts, platelets and placental cells which belong to the serpine family (serine protease inhibitors). This protein inhibits the proteases C1s, C1r, kallikrein, plasmin and factor XII through complex formation. C1Inh controls the classical complement pathway, reducing and delaying auto-activation of C1 by inhibiting C1s and C1r esterase activity.

Quantitative or qualitative C1Inh deficiency is responsible for angio-neurotic oedema (ANO) or angioedema. This is a clinical syndrome involving sudden localised swelling of subcutaneous tissues or mucosal membranes, due to the abrupt highly localised production of high levels of mediators increasing vascular permeability. The main risk is of sudden death due to laryngeal oedema and asphyxia.

INTRODUCTION

C1Inh deficiency causes disturbance of several proteolytic cascades. Any endothelial injury activates an excess of the coagulation contact phase through Hageman factor with production of kallikrein and plasmin, which release large amounts of bradykinin and activate C1s; C1s cleaves C2 and C4 and releases C2b and C2k, which are anaphylatoxins, increasing vascular permeability. Bradykinin, C2b and C2k are responsible for the symptoms of angio-neurotic oedema.

C1Inh deficiencies can be hereditary or acquired.

There are two types of hereditary angio-neurotic oedema (HANO):

– Type I HANO makes up the great majority of cases and is due to defective C1Inh synthesis, occurring as a result of point mutations which are very heterogeneous, autosomal dominant in transmission and located on chromosome 11. Although these are exclusively heterozygote forms average protein concentrations are well below 30% because of inhibition of the healthy allele by the mutated allele and increased catabolism of C1Inh.

– Type II HANO is far rarer than type I and is due to the production of a normal but non-functional protein. It is divided into two subtypes.

Subtype IIa is the most common and the mutation is located on the active site of C1Inh preventing the formation of C1Inh-protease inhibitory complexes. Free 105 kDa molecule circulates in serum.

Subtype IIb occurs as the result of a mutation in the N-terminal part of the protein and converts C1Inh into a simple substrate for the target proteases, with no inhibitory activity. C1Inh is present in serum in the form of a 95 kDa fragment.

There are also two types of acquired angio-neurotic oedema (AANO):

– Type I AANO is due to excessive C1Inh consumption by over-activation of the classical complement pathway (by circulating immune complexes, cryoglobulins, etc.). This is almost always associated with a lymphoproliferative syndrome, cancers, vasculitis or infections etc. The pathophysiological hypothesis in the lymphoproliferative syndromes is synthesis and formation of immune complexes activating C1 by the malignant anti-idiotype Ab clone.

– Type II AANO involves the presence of anti-C1Inh Ab which may be associated with a disease, such as malignancy, infection, lympho-proliferative disorders, etc.

– Drug-induced AANO is also seen with oral contraceptives and angiotensin converting enzyme inhibitors.

INDICATIONS FOR MEASUREMENT

Laboratory investigations should be requested in cases of repeated localised attacks of oedema which may occur following a triggering event such as stress or trauma (even minimal exertion, such as typing, standing for long periods of time, etc,), events in the reproductive cycle (menstruation, pregnancy, etc,), ENT infections or tooth extractions, etc.

Hereditary ANO should be considered by physicians when a C1Inh abnormality is found in a child or even adult and acquired ANO in patients in their sixth or seventh decade of life.

The episodes of subcutaneous oedema vary in severity, frequency and location (hand, face, perineum etc.), and can affect all areas. Oedematous swelling, which may be very severe, develops without any prodrome over a few minutes or hours and is painless, non-itchy, firm, white, elastic and lasts for two to four days. The skin is entirely normal between attacks.

Submucosal involvement often develops after the condition has been present for a few years although may be present from the outset and may not associated with any skin involvement. Laryngeal effects are common and always worrying, causing dysphonia, inspiratory wheezing and at worst, asphyxia requiring intubation or tracheotomy. Gastro-intestinal submucosal oedema results in attacks of abdominal pain which may cause obstruction, gastric signs (nausea, vomiting, etc.); gastro-intestinal signs are absent between attacks and abdominal examination is normal. More rarely the submucosal oedema can affect the urinary tract (with acute urinary retention), or biliary tract. Central nervous system involvement has been suspected from headaches, transient aphasia or dizziness.

INFORMATION

■ SAMPLE

Whole blood collected into citrate, concentration 3.2% (0.109 M) or 3.8% (0.129 M), 1/10 (0.5 ml per 4.5 ml of blood) is essential for functional C1Inh studies.

Two ml of serum is required for C1Inh mass assay which must be performed in parallel with functional studies.

A fasting sample is not required.

Testing for C1Inh protein abnormalities which are characteristic of all forms of ANO can be performed distant to the oedema attacks.

■ QUESTIONS FOR THE PATIENT

Has the patient recently and/or previously had an attack of painless, white, non-itchy, subcutaneous oedema for a few days and if so, in which area(s) of skin (each patient has his/her own areas of predilection)?

Has the patient recently and/or previously suffered from gastro-intestinal disease whether or not associated with subcutaneous oedema involving abdominal colic, diarrhoea, gastric cramps, nausea or vomiting?

Are there circumstances predisposing to episodes of subcutaneous or submucosal oedema: Trauma, event in reproductive cycle?

Has the patient suffered headaches or dizziness?

Do other members of the patient's family suffer from symptoms of subcutaneous or submucosal oedema?

Does the patient have a malignancy (non-Hodgkin's malignant lymphoma, breast or uterine cancer, monoclonal dysglobulinaemia), autoimmune disease or infection?

Is the patient taking medicines: Angiotensin converting inhibitors, oral contraceptives in women?

In cases of known HANO, affected family members, who will benefit from contacting a patient association which will put them in touch with affected people and offer practical information.

■ SAMPLE STORAGE AND TRANSPORT

Laboratory samples must be stored at +4°C and transported at this temperature to the laboratory within 24 hours.

ASSAY METHODS - VALEURS NORMALES

C1Inh mass assay is widely performed by nephelometry. Normal concentrations are 210 to 345 mg/L and are reached by the age of 6 months old. Values are only 66% of normal at birth but rise to 91% from 5 days after birth.

Functional C1Inh assay measures the inhibitory activity of C1Inh on C1s.

Assays of complement activity and different complement fractions may be included in the profile: CH50 (kinetic method), C1q, C2 and C4 (nephelometry method).

Investigations to diagnose the less common forms of ANO can be performed in highly specialised hospital laboratories:

–C1Inh electrophoresis: C1Inh protein is analysed by vertical polyacrylamide gel electrophoresis followed by immunoblot. This distinguishes the native 105 kDa C1Inh from the short 95 kDa forms and forms complexed with C1s.

– Anti-C1Inh antibodies are tested by ELISA.

INTERPRETATION

The general diagnostic approach to ANO involves:

– Measurement of C4, which is a useful first line investigation: A fall may prompt further C1Inh investigations although normal values do not exclude the diagnosis. C4 may be normal particularly outside of attacks.

– Assay of C1Inh inhibitory activity can confirm a diagnosis of ANO as it is invariably low regardless of the type of ANO.

–C2 concentration is only reduced during attacks or if attacks are frequent.

The different types of ANO can be established using several laboratory parameters.

– Type I HANO involves:

- A reduced C1Inh protein, levels generally being 30 to 40% of normal values.
- Reduced functional C1Inh activity.

– Type II HANO involves:

- Normal (or even increased) C1Inh mass measurement.
- Reduced functional C1Inh activity.
- On C1Inh electrophoresis, type IIa reveals C1Inh forms of molecular weight 105 kDa, whereas type IIb is characterised by short, 95 kDa molecules.

The AANO are characterised by several abnormalities:

– Type I AANO is defined by:

- Reduced or normal C1Inh mass measurement.
- Reduced functional C1Inh measurement.
- One characteristic abnormality: Reduced C1q.

– Type II AANO involves:

- Normal or low C1Inh levels.
- Very low C1Inh activity.
- Characteristic presence of anti-C1Inh antibodies.
- C1Inh electrophoresis shows cleaved forms of the protein (95 kDa).

– Drug-induced AANO is characterised by:

- Normal C1Inh levels.
- Very low C1Inh activity.
- Short 95 kDa forms of C1Inh on electrophoresis.

FOR FURTHER INFORMATION

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C4b-*BINDING PROTEIN*

DEFINITION

C4b-Binding Protein (C4b-BP) is a complement system glycoprotein of which has a molecular weight 570 KDa, and is present in plasma at concentrations in the region of 300 µg/ml. It is a multimeric protein formed from six or seven 549 AA α sub-units bound to each other at their C terminal end into a central nucleus by disulphide bridges, with or without a 45KDa β chain bound to the central nucleus by a disulphide bridge, which binds protein S. Each C4b-BP molecule binds several C4b molecules (by the β chains) and a single molecule of protein S (by the β chain). It circulates in plasma in two forms; a high molecular weight form and a lower molecular weight form.

INTRODUCTION

C4b-BP acts as a complement system regulating protein, binding C4b deposited on cell surfaces and thereby preventing the binding of other C3 convertase components, which blocks the complement activation cascade. C4b-BP is an inflammatory protein and in this context values may vary greatly.

C4b-BP can reversibly bind protein S with a dissociation constant in the region of 107 M and as such is involved in the coagulation regulating process. The high molecular weight form which represents approximately 80% of plasma C4b-BP is the only form able to bind protein S. Only free protein S, which in the normal state makes up 40% of total protein S, has anticoagulant activity.

In some forms of protein S deficiency (type III qualitative deficiencies or selective deficiencies, free fraction deficiencies) an abnormality of C4b-BP binding is present. Total protein S (PS) is normal, and both protein S free antigen and anticoagulant activity are reduced.

INDICATIONS FOR MEASUREMENT

C4b-BP measurement is indicated when a suspected disorder of the distribution of PS between free (and active) form and the C4b-BP complex form is suspected.

INFORMATION

■ SAMPLE

Blood is collected into 0.129 M or 0.109 M citrated anticoagulant (0.5 ml of anticoagulant per 4.5 ml of blood), mixed promptly by repeatedly turning over the tube. The sample should be centrifuged for 10 minutes at 2500 g.

Samples are generally taken in the morning between 0700 hrs and 1100 hrs. Patients do not have to be fasting and a light, fat free snack is permitted. Coffee, smoking and alcohol should be avoided in the hour before the sample is taken.

Refer to the "General haemostasis pre-analytical conditions" section for more information. Any suspect samples should be discarded, as should any cloudy or lipaemic samples.

■ QUESTIONS FOR THE PATIENT

Is the patient known to have rheumatoid factor (which interferes with latex measurement methods)?

Does the patient have a severe inflammatory reaction?

Is the patient pregnant?

Is the patient taking estrogen-progestogens (oral contraception causes a fall in C4b BP concentrations)?

■ SAMPLE STORAGE AND TRANSPORT

The samples must be centrifuged within 4 hours of sampling. If the analysis is to be performed later, the samples must be frozen immediately and transported at -20°C. They may be stored for 1 month at –20°C and for 6 months at –70°C. It is recommended that they be thawed in a water bath at + 37°C.

ASSAY METHODS

Different assay methods are available: Radial electroimmunodiffusion (Laurell), nephelometry and Latex immunological assay.

NORMAL EXPECTED VALUES

Normal values for the Liatest® C4b-BP kit are 65 to 140%.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

C4b-BP is absent in the healthy foetus and only develops at the end of pregnancy. Levels are below 18 % at birth. Expected values in premature infants are in the region of 6 %.

C4b-BP levels increase physiologically in pregnancy and are raised *post-partum*. The free fraction of protein S then falls significantly in the second trimester and *post-partum*.

■ PATHOLOGICAL VARIATIONS

Total PS falls less than C4b-BP in liver disease. Inflammatory states are associated with a rise in C4b-BP causing redistribution of PS towards the complexed form.

FOR FURTHER INFORMATION

■ Bezeaud A., *Protéine* S et *C4b-BP*, STV, 1990; 2: 159-60.

CA 15.3

DEFINITION

CA 15.3 is an antigen defined by two different monoclonal antibodies (115 D8 and DF3) which recognise repeated high molecular weigh glycoprotein epitopes belonging to the polymorphic epithelial mucin family.

Synonym: *Carbohydrate antigen* 15.3

INDICATIONS FOR MEASUREMENT

CA 15.3 is the serum marker of choice for breast cancer. Measurement is useful in monitoring treatment and early detection of recurrences or metastases of the cancer. In monitoring for recurrence or metastases, CA 15.3 measurement is the factor used to assess the effectiveness of treatment.

INFORMATION

SAMPLE

Preferably serum (dry tube). Do not use a tube with separator gel (some gels increase serum concentrations of all carbohydrates or mucins).

Plasma collected into EDTA or heparin can be used depending on the method. Refer to the manufacturer's recommendations.

It may also be measured in an aspiration fluid (cyst, ascites, etc.).

A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Current treatment: Chemotherapy, radiotherapy, surgery, etc., types and dates of treatment?

SAMPLE STORAGE AND TRANSPORT

Five days at + 4°C; three months.

ASSAY METHODS

Immunometric "sandwich" method.

NORMAL EXPECTED VALUES

These vary depending on the technique.

As an indication: Usual serum values < 25 U/ml (95th percentile).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Increased in 2 to 7% of healthy people.

Increased in 10% of pregnancies.

PATHOLOGICAL VARIATIONS

Serum CA 15.3 concentration in breast cancer

Screening - diagnosis

CA 15.3 is not a specific marker for breast cancer or breast diseases and because of this cannot be used for screening or early detection of breast cancers. Its sensitivity is also low and only 7.1 to 34% of non-metastatic breast cancers (all grades combined) are associated with serum CA 15.3 concentrations above usual values.

Staging assessment

Measurement of CA 15.3 in the initial assessment provides a reference value and is part of the staging assessment.

CA 15.3 correlates with the stage of the disease and CA 15.3 values are higher in more advanced stages. Initial serum concentrations also correlate with the size of the primary tumour, with lymph node extension and with the number of diseased lymph nodes. Very high pre-treatment values (from 5 to 10 times normal) suggest advanced or even metastatic disease.

Monitoring after initial treatment: Indicator of relapse or metastasis

CA 15.3 is widely recognised as an early marker of relapse or metastases before curative surgery and approximately 70% of patients with their first metastatic progression of the disease have raised serum CA 15.3 concentrations. The sensitivity of the marker to detect relapses or metastases varies depending on the site. Serum CA 15.3 concentrations are higher in bone (above usual values in 68 to 81% of cases), hepatic (75 %) and pulmonary (50 to 70%) sites than in loco-regional recurrences (23%). Increased CA 15.3 concentrations precede the development of clinical and/or radiological signs of the first relapse and/or metastasis by approximately 4 months (from 3 to 14 months depending on the study).

Monitoring treatment for a relapse or metastasis of breast cancer

CA 15.3 correlates with the response to treatment in metastatic cancer. It rises in 80% of cases of tumour progression and stable concentrations are found in 73% of stable patients. Values fall in 66% of patients who respond to treatment. Monthly CA 15.3 measurements can be used in follow up to adjust treatment as soon as possible.

Note however, that whilst the clinical-biological correlation (CA 15.3 concentration/progression of recurrence or metastasis) is good in approximately 80% of cases, the marker cannot be used as the only indicator of effective treatment. Discordances are seen, particularly when the initial CA.15.3 rises after systemic treatment is started ("induction effect", lasting 1 to 3 months) which can sometimes be difficult to distinguish from tumour progression.

Rises in CA 15.3 in other diseases

– Liver disease (acute or chronic hepatitis and cirrhosis): CA 15.3 rarely rises above 70 U/ml.

– Haemodialysis and renal insufficiency.

– Auto-immune endocrine diseases: CA 15.2 concentrations are usually < 100 U/ml.

– Other cancers: Ovarian, hepatic, gastro-intestinal, thyroid and lung adenocarcinomas (occasionally very large rise in CA 15.3).

FOR FURTHER INFORMATION

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CA 19.9

DEFINITION

CA 19.9 is an antigenic determinant carried by a mucin protein in serum. It is a sialoglycoprotein structurally similar to the "Lewis blood group", detected by a monoclonal antibody obtained from a colorectal carcinoma cell line (SW 1116).

CA 19.9 is present in many foetal tissues and in healthy adults, in the pancreas, stomach, biliary tract, liver, salivary glands, bronchi, lungs and prostate gland.

Synonym: GICA *(Gastro-Intestinal Carbohydrate Antigen)*.

INDICATIONS FOR MEASUREMENT

CA 19.9 is a marker for gastro-intestinal cancers, particularly pancreatic carcinoma. Measurement is useful in the differential diagnosis and monitoring of patients suffering from pancreatic cancer. It is also used to monitor colorectal cancer (in combination with CEA) and for post-operative monitoring of gastric cancers (combined with CEA and CA 72.4) and mucinous ovarian cancers.

CA 19.9 can also be measured in aspiration fluids (cysts, ascites, etc.).

INFORMATION

SAMPLE

Preferably serum (empty tube). Do not use a tube with separator gel (some gels may increase the concentrations of these markers).

Plasma collected into EDTA or heparin can be used depending on the method. Refer to the manufacturer's recommendations.

A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Current treatment: Chemotherapy, radiotherapy, surgery, etc., types and dates of treatment.

SAMPLE STORAGE AND TRANSPORT

Five days at + 4°C; three months.

ASSAY METHODS

Immunometric "sandwich" method.

NORMAL EXPECTED VALUES

These vary depending on the technique.

As an indication: Usual serum values < 37 U/ml (95th percentile).

PATHOPHYSIOLOGICAL VARIATIONS

VARIATIONS TO BE AWARE OF

People not carrying Lewis genes (Lewis a or b); 3 to 7% of the population cannot synthesise the CA 19.9 antigen because of a lack of fucosyltransferase. Serum CA 19.9 concentrations are undetectable in these people.

The CA 19.9 molecule may reveal previously hidden epitopes after freezing or when a serum sample is diluted resulting in increased recognition by the antibodies used for measurement, and therefore a rise is seen compared to the initial concentration.

PATHOLOGICAL VARIATIONS

Serum concentrations above usual values are seen in 0.5% of healthy people, 2 to 9% of people with benign disease, 19% of people with cirrhosis, 32% of people with chronic active hepatitis and in many malignant diseases.

Non-specific rises in serum CA 19.9 concentrations

– Benign gastro-intestinal diseases: Pancreatitis, gallstones, acute or chronic hepatitis and liver transplantation. Serum CA 19.9 concentrations are generally moderately raised (< 120 U/ml) although very high values (up to 32000 U/ml) have been reported in bowel duct stones complicated by acute cholangitis.

– Benign lung disease (cystic fibrosis and severe broncho-pulmonary disease).

– Diabetes in the acute decompensation stage.

Increased serum CA 19.9 concentrations in malignant diseases

Gastric and colorectal cancers, cholangiocarcinomas, bronchial, breast and mucinous ovarian cancers. Generally, CA 19.9 may be useful in monitoring these patients and can enable early detection of recurrences or metastases.

Pancreatic cancer

– CA 19.9 cannot be used as the only early diagnostic tool for pancreatic cancer because of its lack of specificity and sensitivity. 15 to 20% of people with pancreatic cancers have normal serum CA 19.9 concentrations at the time of diagnosis.

– CA 19.9 is a prognostic indicator, with serum concentrations correlating with tumour size. Values over 1000 U/ml are highly suggestive of metastatic disease.

– Monitoring after initial treatment: Assessment of residual volume.

– Identification of recurrence or metastases: Increasing serum CA 19.9 concentrations during the month following surgical removal of the tumour suggest the development of a recurrence or metastasis. Combination with CEA can be used to identify approximately 90% of recurrences, 4 to 6 months before clinical confirmation.

Colorectal cancer

– CA 19.9 is raised in 17 to 32% of patients suffering from colorectal cancer. Serum concentrations and the incidence in the rise of the marker correlate with stage of extension. The sensitivity of CA 19.9 in this disease however is less than that of CEA.

– CA 19.9 is a prognostic indicator for survival independent of other prognostic indicators (Dukes classification, site of tumour, age, sex and serum CEA concentration).

– When monitoring treatment, a rise in serum CA 19.9 concentrations after previously returning to normal, suggests recurrence or metastasis of colorectal cancer an average of 3 months before clinical or radiological signs develop (sensitivity approximately 90%, in combination with CEA).

FOR FURTHER INFORMATION

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CA 50

DEFINITION

CA 50 is an antigenic determinant carried by a mucin glycoprotein, present physiologically on the surface of many epithelia and in biological fluids, particularly serum. It is a sialoglycoprotein defined by a monoclonal antibody (C-50) obtained after immunising mice with a human colonic adenocarcinoma cell line. The antibody recognises two different structures: Lewis a ganglioside sialosyl (the common antigenic determinant with CA 19.9) and sialosyl-lacto-N-tetra-osylceramide (specific to CA 50). Lewis "a" and "b" negative phenotypes are therefore unable to synthesise CA 19.9 but do synthesise CA 50.

INDICATIONS FOR MEASUREMENT

CA 50 is a biological marker for gastro-intestinal cancers, particularly pancreatic and colorectal cancers. Measurement has no diagnostic use but is useful for prognostic assessment and monitoring treatment of patients suffering from these cancers, particularly for the early detection of recurrence or metastases. Its indications for use are therefore similar to those of CA 19.9. It is particularly useful for Lewis "a" and "b" negative phenotypes (3 to 7% of the population) who express CA 50 but not CA 19.9. CA 50 can also be measured in aspiration fluid (ascites). Combined with CEA measurement it is used to investigate peritoneal carcinomatosis of gastro-intestinal origin.

INFORMATION

SAMPLE

Preferably serum (dry tube). Do not use a tube with separator gel (some gels may increase the serum concentration of all carbohydrates or mucins).

Plasma taken into EDTA depending on the method. Refer to the manufacturer's recommendations. Discard jaundiced samples: hyperbillirubinaemia (> 100 µmol/l may result in an increase in serum CA 50 concentration.

Aspiration fluid (ascites).

A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Context of the measurement: Prognostic assessment, monitoring treatment or type of tumour.

Current treatment: Chemotherapy, radiotherapy, surgery, etc.: types and dates of treatment.

SAMPLE STORAGE AND TRANSPORT

Store the sample for 72 hours at + 4°C or 1 year at –20°C.

ASSAY METHODS

Immunometric "sandwich" method.

NORMAL EXPECTED VALUES

These vary depending on the technique.

As an indication: Usual serum values < 25 U/ml.

PATHOLOGICAL VARIATIONS

NB: Before interpreting results ensure that cholestasis or jaundice with hyperbilirubinaemia > 100 µmol/l is not present (falsely raises CA 50).

Increased serum CA 50 concentration in benign diseases:

– Transient increase in inflammatory diseases.

– Benign gastro-intestinal diseases: Acute pancreatitis, gallstones, hepatitis, cirrhosis, cholangitis and gastric ulceration.

Increased serum CA 50 concentration in malignant diseases

Gastro-intestinal cancers

– Pancreatic cancers and biliary tract cancers: The sensitivity of CA 50 measurement in pancreatic cancers is approximately 91%, i.e. slightly greater than that of CA 19.9 (74%). CA 19.9 however is more specific than CA 50 for biliary disease (92.4% vs 88.5%). The magnitude of the rise in the marker correlates with stage of disease, highest values (> 50000 U/ml) being seen in metastatic disease.

In general, the utility of CA 50 measurement is similar to that of CA 19.9, which is more widely performed. CA 50 appears to be particularly useful in the prognostic assessment and in monitoring treatment of Lewis "a" and "b" negative patients, who do not express CA 19.9.

– Colorectal cancers: Serum CA 50 concentrations are raised in 47% of patients with colorectal tumours and the rise is generally proportional to the stage of the disease. An increase in serum CA 50 concentrations during the months following surgical excision of a tumour suggests recurrence or metastases. This rise occurs in 66% of cases, 5 to 40 months before clinical or radiological signs, although remission after effective treatment only correlates with a fall in serum CA 50 values in approximately 1/4 of cases.

– Gastric cancers: CA 50 measurement has no utility in this situation (low sensitivity of approximately 50%).

Non-gastro-intestinal cancers

Serum CA 50 concentrations occasionally rise greatly in ENT, ovarian, lung, breast, prostate, renal and bladder cancers.

FOR FURTHER INFORMATION

- Troalen F., *CA 50*, Encycl Med Biol, Elsevier, Paris 2004.
- Funes A., *Quid Novi n° 1*, Marqueurs tumoraux, Laboratoire Cerba Ed, 1988.

CA 72.4

DEFINITION

CA 72.4 is a tumour marker belonging to the mucin family (polysaccharides) defined by its recognition by two monoclonal antibodies: CC 49 and B 723.

Synonym: TAG 72 *(Tumor Associated Glycoprotein).*

INDICATIONS FOR MEASUREMENT

– CA 72.4 serum is a prognostic indicator and marker used to monitor treatment in gastro-intestinal cancers, particularly stomach and mucinous ovarian tumours. It is of no diagnostic use because concentrations are often normal in early stages.

– CA 72.4 in an aspiration fluid (cyst, ascites, etc.) is a marker of malignancy. Regardless of site of the primary adenocarcinoma (ovary, breast, colon, pancreas, lung), it offers good specificity (approximately 95%) although poor sensitivity (50%). Measurement in ovarian cyst fluids may suggest mucinous histology.

INFORMATION

SAMPLE

Preferably serum (dry tube). Do not use a tube with separator gel (some gels may increase the serum concentration of all carbohydrates or mucins).

Plasma collected into EDTA or heparin depending on the method.

A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Current treatment: Chemotherapy, radiotherapy, surgery, etc. and also types and dates of treatment?

Treatment with interferon beta or gamma gives a large rise in CA 72.4.

SAMPLE STORAGE AND TRANSPORT

24 to 48 hours at + 4°C ; three months at -20° C.

ASSAY METHODS

Immunometric "sandwich" method.

NORMAL EXPECTED VALUES

Usual serum values are < 3.5 U/ml (95th percentile).

These vary depending on the method.

PATHOLOGICAL VARIATIONS

Rises in gastro-intestinal and ovarian cancers

Gastric adenocarcinomas

CA 72.4 is a prognostic indicator and is used to monitor treatment of gastric adenocarcinomas. It is more sensitive and specific than CEA (sensitivity 36%, specificity almost 100% vs 16% and 96% for CEA respectively).

– Prognosis: Serum CA 72.4 concentrations correlate with tumour size and are higher in advanced stages of the disease (stages III and IV).

– Monitoring treatment: CA 72.4 generally becomes negative 1 month after complete surgical excision of the tumour.

– Detection of recurrences and/or metastases: Serum CA 72.4 concentrations rise early in recurrence.

Colon cancers

CA 72.4 rises in colonic adenocarcinomas although it is less sensitive and less specific than CA 19.9 or CEA in this situation.

Mucinous ovarian tumours

– CA 72.4 is equivalent in sensitivity to CA 125 (approximately 65%) in detecting mucinous ovarian tumours although it appears to be more specific for the histological type (88% vs 66% for CA 125). It is therefore standard practice to measure both markers first line and then to monitor the change in the marker which is secreted preferentially.

– CA 72.4 is also of prognostic use, with serum concentrations correlating in line with the stage of disease.

– An early rise in serum concentrations is seen during follow up in almost 60% of recurrences and/or metastases.

Increases in other diseases

Increases are uncommon (< 10% of cases) and generally modest in:

– Benign gastro-intestinal disease: Peptic ulceration, inflammatory colitis (Crohn's Disease, ulcerative colitis) and cholestasis.

– Benign ovarian disease: Cystadenomas.

– Other metastatic cancers (excluding gastro-intestinal tract and ovary): CA 72.4 is increased in less than 30% of cases.

Occasionally very large rises (several tens of units) in treatment with interferon beta or gamma.

FOR FURTHER INFORMATION

Troalen F., *CA 72.4*, Encycl Med Biol. Elsevier, Paris.

CA 125

DEFINITION

CA 125 is a high molecular weight mucin antigen present in large amounts in coelomic embryonic epithelium. The marker is defined by recognition by monoclonal antibody OC125 which is used in the assay. It is absent in normal ovarian tissue but is expressed in more than 80% of non-mucinous ovarian epithelial cancers.

INDICATIONS FOR MEASUREMENT

CA 125 is the first line marker for non-mucinous ovarian epithelial tumours (or serous ovarian adenocarcinomas). Its major use is in monitoring treatment and following the progression of the tumours. It may also be useful in the etiological investigation of a pelvic mass of unknown origin and in monitoring treatment of benign gynaecological diseases such as endometriosis.

INFORMATION

SAMPLE

Preferably serum (dry tube). Do not use a tube with separator gel (some gels may increase the serum concentration of all carbohydrates or mucins).

Plasma collected into EDTA or heparin depending on the method. Refer to the manufacturer's recommendations.

Measurements may also be performed in an aspiration fluid (cyst, ascites, etc.).

A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Current treatment: chemotherapy, radiotherapy, surgery, etc: types and dates of treatment.

SAMPLE STORAGE AND TRANSPORT

Five days at + 4°C ; three months at -20° C.

ASSAY METHODS

Immunometric "sandwich" method.

NORMAL EXPECTED VALUES

These vary depending on the method.

As an indication: Usual serum values < 35 U/ml (95th percentile).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Increase in the 3rd trimester of pregnancy (< 50 U/ml).

CA 125 is hormone-dependent and concentrations may therefore rise slightly during follicle growth and in menstruation.

PATHOLOGICAL VARIATIONS

CA 125 is not a specific marker for the ovary and because of this cannot be used for screening or early detection of ovarian cancer. Values > 35 U/ml are found in approximately 1% of healthy people, 6% of patients with benign disease and in 28% of patients with non-gynaecological cancer.

Non-specific rises in serum CA 125 concentration

Serosal diseases:

– Pleural effusion, hepatitis, cirrhosis (particularly with ascites): Serum concentrations occasionally > 1000 U/ml. For this reason a raised CA 125 concentration in ascitic fluid does not distinguish non-malignant ascites from peritoneal ovarian carcinomatosis.

– Peritonitis, pericarditis and acute pancreatitis.

"Benign" gynaecological disease:

– Endometriosis: CA 125 is useful in monitoring medical or surgical treatment for this disease. Concentrations correlate well with response to treatment.

– Ovarian cysts: 20% of serous cysts are associated with raised CA 125 concentrations. CA 125 may be measured in cyst fluid and high concentrations (occasionally over 100000 U/ml) support serous histology without predicting malignancy (cystadenoma or cystadenocarcinoma).

– Uterine fibroids.

– Acute or chronic salpingitis with pelvic inflammation.

Non-ovarian malignant tumours:

– Breast, endometrial, gastro-intestinal tract or lung cancers.

– Hepatic metastases and hepatomas (serum CA 125 concentrations are occasionally extremely high).

Use of CA 125 in the investigation of pelvic masses

The likelihood of ovarian cancer rises with increasingly high CA 125 concentrations. At a cut off of 35 U/ml CA 125 has a sensitivity of 70%, compared to 94% at a cut off of 180 U/ml. Normal or low CA 125 values however do not exclude ovarian cancer.

Serum CA 125 concentrations in ovarian adenocarcinoma

Serum CA 125 concentrations are raised in approximately 82% of women with ovarian adenocarcinoma and correlate with clinical stage of progression of the disease in approximately 93% of cases.

Pre-operatively, the rise in CA 125 supports the diagnosis and provides information about extension of the disease.

Monitoring ovarian carcinomas

– CA 125 concentrations return to normal rapidly in 80 to 90% of patients after complete surgical resection. Concentrations that remain raised are usually only modestly so (< 60 U/ml), but represent a risk factor for recurrence.

– Serum concentrations used in monitoring treatment are an aid in the decision to perform secondary investigative surgery and raised values suggest significant residual peritoneal tumour or metastases. Low values, however, do not preclude the need for exploratory laparotomy to investigate for peritoneal micrometastases.

– The early kinetics of CA125 during induction chemotherapy for ovarian cancer is an important prognostic indicator. The half-life of CA 125 correlates closely with global survival, which is 48% at 5 years if the half-life is less than 13 days compared to only 13% if the half-life is between 13 and 20 days. This initial biological phase of CA 125 measured during the first three chemotherapy cycles is an early predictive indicator for the effectiveness of treatment and allows treatment to be tailored individually from the initial chemotherapy sessions.

– Measurement is particularly useful for the early detection of recurrence or metastases. Serum concentrations rise on average 4 to 8 months before clinical symptoms and the doubling time of the marker is a close reflection of the speed of growth of the tumour recurrence or metastases. The doubling time is approximately 4 months for chemosensitive disease compared to 1 month for the most resistant tumours.

FOR FURTHER INFORMATION

■ Bellet D., Bidart J.M., *Marqueurs biologiques des cancers. Vers une utilisation sélective pour la détection et la surveillance des tumeurs malignes*, Rev Prat 1989; 26 : 2350-4.

■ Beaudonnet A., Cohen R., *Cahier de formation Hématologie Immunologie Bioforma*, Paris 1995 : 48-54.

CADMIUM

DEFINITION

Cadmium (Cd) is classified as a heavy metal and is an element present in small amounts in the earth's crust. It is a light coloured malleable metal bound tightly to zinc and lead minerals. Like lead, cadmium is found in the diet (an adult ingests an average of 15 mg of Cd per day), in cigarette smoke (a cigarette contains an average of 1 mg of Cd) and in city air.

Cadmium is mostly obtained from the extraction of residues from the zinc industry. The other main sources of exposure are cadmium treatment of metals (iron, steel and copper), alloys with steel (manufacture of ball bearings), manufacture of nickel cadmium electrical batteries, the nuclear industry from neutron excess capture, manufacture of pigments for paints, and in the plastics industry as a stabiliser.

METABOLISM

Cadmium is absorbed particularly through the respiratory tract in an occupational environment and also through the gastro-intestinal tract (dirty hands at meal times or holding cigarettes). It is carried in the blood bound to haemoglobin and metallothionine, the synthesis of which is stimulated by cadmium exposure.

Cadmium is a highly cumulative toxin with a biological half-life in excess of 15 years. It accumulates above all in the lungs, kidneys, liver, pancreas, thyroid gland, testes and salivary glands. It is believed to enter the cell through the calcium channels. Total body cadmium in an adult who is not occupationally exposed is between 10 and 50 mg. Cadmium concentrations in the renal cortex are understood to have risen by a factor of approximately 50 since the beginning of the last century, following the various uses of Cd which have resulted in progressive environmental pollution in industrialised countries.

Cadmium excretion, although low, occurs in the urine and, in the absence of renal damage, urinary cadmium excretion is proportional to body load.

MECHANISM OF ACTION

Metallothionine, which is synthesised after cadmium enters cells, protects the cells against the toxicity of the metal. Once the synthesis mechanism is saturated, cadmium will cause cell damage. This explains for example, the toxic effect of cadmium on the renal tubule, resulting in impaired renal tubule function. Cadmium is also believed to interfere with the metabolism of some trace metals in the body (copper, cobalt and zinc). It also delays the intestinal absorption of calcium and prevents its deposition in the bone tissue.

SYMPTOMS OF ACUTE POISONING

Gastro-intestinal absorption results in acute poisoning which increases in severity with the amount ingested and may range from gastroenteritis with occasional bloody vomiting, diarrhoea and myalgia, or death with haemorrhagic gastroenteritis, anuria, parotitis, cardio-respiratory depression and death after a few days.

Acute poisoning from inhalation begins with prodromal symptoms similar to "smelters fever" (fever, rigors, flu like symptoms and headaches), followed by cough, dyspnoea, cyanosis and death after 1 to 3 days in 15 to 20% of cases.

SYMPTOMS OF CHRONIC POISONING

The predominant symptoms are:

Yellow cadmium teeth: Characteristic symptom of yellow pigmentation limited to the enamel.

Respiratory disorders: Rhinitis, bronchitis and emphysema. Very slow developing obstructive symptoms after exposure for approximately 20 years.

Renal disorders: Earlier than respiratory disorders, beginning with tubular proteinuria of Fanconi syndrome with glycosuria and aminoaciduria, defective acidification and reduction in renal concentration capacity. Phosphaturia, calciuria and defective uric acid reabsorption is also found. Increased calcium phosphate excretion may result in renal lithiasis (increased prevalence of calcium stones in workers exposed to cadmium).

Bone disorders: Osteomalacia, produced in the long-term by calcium loss and an effect on vitamin D due to impaired renal function.

Carcinogenic effect: Increased incidence of prostate and lung cancers in exposed workers.

INDICATIONS FOR MEASUREMENT

Measurement of calcium in the blood is an indication of recent exposure in subjects who have been exposed to small amounts and also of body load after exposure has stopped. Blood measurement should be used in preference during the first year of exposure or with variable exposure, and blood cadmium concentrations increase for 4 to 6 months and then stabilise. Smoking may interfere with the interpretation of results (blood cadmium concentrations rise in proportion to the amount of tobacco smoked). Concentrations increase with age.

Urinary cadmium concentrations above all reflect long term exposure and body load, provided that renal function is normal. Renal function is therefore monitored in parallel through measurements of albumin, β2 microglobulin and *Retinol Binding Protein* (RBP). There is a close correlation between urinary cadmium concentrations, extent of exposure and the risk of impaired renal function.

INFORMATION

SAMPLE

5 ml of heparinised whole blood and the sample can be taken at any time of the day.

20 ml of a non-acidified random urine sample preferably obtained at the start of a shift (Monday morning before arriving at work).

QUESTIONS FOR THE PATIENT

Do you smoke?

SAMPLE STORAGE AND TRANSPORT

Whole blood samples can be stored and transported to the laboratory at room temperature. Urine samples may be stored and transported to the laboratory at room temperature or between + 2 and + 8°C.

ASSAY METHODS

Electrothermal atomisation atomic absorption spectrophotometry (graphite furnace) with Zeeman correction.

Induction coupled plasma source – mass spectrometry.

REFERENCE VALUES

– Reference value in the general population:

Whole blood cadmium < 1 mg/l in non-smokers and < 2mg/l in smokers.

Urinary cadmium < 0.5 mg/g creatinine.

– Reference value in exposed subjects:

Urinary Cadmium < 5 mg/g of creatinine.

FOR FURTHER INFORMATION

- Lauwerys R., Toxicologie industrielle et intoxications professionnelles, *Cadmium*, 3rd edition, Masson: p136-149.
- Guide BIOTOX 2001–INRS.

CAFFEINE

DEFINITION

Like theophylline, caffeine is a compound belonging to the methylxanthine family. It is widely consumed around the world as coffee, tea, chocolate or cola. It is present in the composition of some analgesic or stimulant pharmaceutical products and is prescribed particularly as an IV injectable solution and oral solution for apnoea in premature new-born babies.

METABOLISM

Caffeine is completely and rapidly absorbed orally reaching maximum serum concentrations after 15 to 45 minutes. It is weakly bound to circulating proteins (15%) and spreads rapidly into the extra vascular space. It crosses the blood-brain barrier and concentrations in CSF being the same as those in serum. Large amounts also pass into breast milk with concentrations reaching 50% of those in serum. Caffeine is almost entirely metabolised in the liver in adults through oxidation, demethylation and acetylation with a half-life of elimination of 3 to 5 hours. Metabolic capacity is lower in new-born babies and caffeine is excreted unchanged in the urine. It has a far shorter half-life in new-born babies between 36 and 144 hours.

MECHANISM OF ACTION

Caffeine is an inhibitor of phosphodiesterase, the enzyme responsible for inactivating cyclic Adenosine MonoPhosphate (cAMP). Increase in cAMP produces the following pharmacological effects:

– Vasoconstriction of cerebral blood vessels.

– Relaxation of bronchial and gastro-intestinal smooth muscle fibres.

– Positive inotropic and chronotropic effects on cardiac muscle.

– Diuretic effects.

– Stimulation and strengthening of striated muscle.

SYMPTOMS OF POISONING

Signs suggestive of serious overdose (plasma concentration > 50 mg/l):

– CNS stimulation with seizures.

– Increased volume of urine passed with dehydration.

– Abdominal pain and gastro-intestinal haemorrhage.

– Acidosis, hypokalaemia, hyponatraemia and hyperglycaemia.

– Hypercatabolism, hyperthermia and very rarely rhabdomyolysis.

– Cardiac arrhythmias and ventricular tachycardia.

INDICATIONS FOR MEASUREMENT

Monitoring the amount of caffeine in the treatment of apnoea in premature new-born babies because of the large differences in individual kinetics. The desirable therapeutic range is between 8 and 15 mg/l. Respiratory response correlates with plasma concentrations.

INFORMATION

SAMPLE

Serum or heparinised plasma. The sample should preferably be taken immediately post dose. Do not use a tube with separating gel.

SAMPLE STORAGE AND TRANSPORT

The serum or plasma sample may be stored for a few days at 4°C and then frozen at – 20°C.

ASSAY METHODS

Immunoenzyme assay method.

High Performance Liquid Chromatography (HPLC).

REFERENCE VALUES

Desirable therapeutic range: 8 to 15 mg/l. Adverse neurological and cardiovascular effects develop at plasma concentrations of 20 mg/l or above.

FOR FURTHER INFORMATION

■ Allain P., *Les médicaments*, 3rd edition, CdM éditions, 2000.

CALCITONIN

DEFINITION

Calcitonin is a 32 amino acid peptide secreted mostly by the thyroid C cells (calcitonin cells or parafollicular cells) and additionally by the APUD cells (Amine Precursor Uptake and Decarboxylation), a component of the diffuse neuro-endocrine system cells. It is produced as pre-procalcitonin which is converted after successive cleavages into calcitonin. Its secretion is stimulated by gastrin, hypercalcaemia and glucagon.

It is present in different forms in the serum (precursors, polymerised and degraded forms) resulting in differences in immunoreactivity depending on disease and between subjects.

Synonym: Thyrocalcitonin, CT.

PATHOPHYSIOLOGY

Physiologically calcitonin is involved in the regulation of calcium-phosphate metabolism through a blood phosphate and calcium lowering effect, due to a reduction in bone calcium resorption and an increase in renal calcium clearance. Its effects antagonise those of parathyroid hormone, for which it is a physiological inhibitor.

In disease, large amounts of calcitonin are secreted into serum in medullary thyroid (C cell) cancers (MTC). It offers sufficient sensitivity and specificity for screening for MTC and post-operative follow up (assessment of residual tumour tissue).

MTC represents 5 to 10% of thyroid cancers. There are two forms; sporadic and familial. The sporadic form (approximately 75% of cases) peaks in incidence between the ages of 40 and 60 years old. The familial form (25% of cases) is part of the type-2 multiple endocrine neoplasias (MEN 2) due to constitutional mutations of the proto-oncogene RET. There are three clinical forms; MEN2A (60% of cases) is an association of MTC, pheochromocytoma and hyperparathyroidism; MEN2B (5%) is an association of MTC, pheochromocytoma, ganglioneuromatosis and Marfan dysmorphia and finally isolated familial MTC (35% of MEN2) which only involves MTC. Genetic screening is possible in familial cases enabling treatment before the malignant stage.

MTC is generally discovered from a nodule, thyroid adenopathy, and diarrhoea or flushing. The laboratory diagnosis is based on measurement of calcitonin possibly combined with measurement of CEA and the pentagastrin test.

INDICATIONS FOR MEASUREMENT

Screening for medullary thyroid cancers (MTC) in the presence of suggestive clinical symptoms (thyroid nodule, flushing and diarrhoea) or in a familial context of MEN2; isolated serum measurements or after pentagastrin stimulation (a gastrin analogue).

Therapeutic monitoring for MTC.

INFORMATION

SAMPLE

Dry tube (serum).

SAMPLE STORAGE AND TRANSPORT

Serum can be stored for 24 hours at +4°C. If a measurement is performed after more than 24 hours the sample should be frozen at –20°C (ideally within 4 hours of sampling). Samples can be stored for several months at –20°C.

Transport: Freeze at –20°C if the measurement is to be performed more than 24 hours later.

ASSAY METHODS

Recommended method: Immunometric method using monoclonal antibodies only recognising the mature CT molecule (32 aa), not interfering with the other forms of CT (notably procalcitonin).

NORMAL EXPECTED VALUES

These have been determined by the GTE (*Groupe des tumeurs endocrines*; http://www.sf-endocrino.net), from a file of all patients with MTC:

– Baseline values < 10 pg/ml

– Normal response to the pentagastrin stimulation test

< 30 pg/ml.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Serum calcitonin concentrations are higher in infants, during pregnancy and during breast-feeding.

PATHOLOGICAL VARIATIONS

Increased serum calcitonin concentrations in MTC

Calcitonin is a sensitive specific marker for MTC. A rise in serum concentration (> 10 pg/ml) suggests C cell disease (although it may occur in other circumstances, cf. below). Baseline values rise with tumour mass (and may be normal with a microcarcinoma).

In the investigation of a thyroid nodule: The sensitivity and specificity of calcitonin measurement in the diagnosis of MTC in this context are 70 and 98 % respectively. A baseline value of > 35 pg/ml is almost pathognomonic for MTC. A pentagastrin test increases the sensitivity of the screening result if the baseline value is moderately raised (< 35 pg/ml).

Pentagastrin test

The aim of this test is to improve the efficiency of MTC detection by stimulating calcitonin secretion with IV injection of pentagastrin, a synthetic gastrin analogue.

The test involves injecting 0.5 micrograms/kg of Peptavlon® over 3 minutes into a fasting subject, lying down, under close medical monitoring (high risk of side effects such as cramps, nausea, vomiting, large fall in blood pressure and tachybradycardia). Blood samples are taken at T-5min, T0, T3 (calcitonin peak), T5 and T10 minutes.

Interpretation of the pentagastrin test:

– CT peak (T3min after pentagastrin injection) < 10 pg/ml: Normal subject (no stimulation).

– CT peak < 30 pg/ml: 96% of healthy adults.

– 30 < CT peak < 50 pg/ml: Suspected MTC (these values are only seen in 4% of healthy adults).

– CT peak > 100 pg/ml with moderately raised basal serum calcitonin (< 35 pg/ml): C cell disease, precancerous hyperplasia or micro-cancer.

In the context of family screening: The key investigation to screen for subclinical forms in related subjects is the pentagastrin test.

In the absence of a known family mutation or in cascade family screening (rare situation): Serum calcitonin < 30 pg/ml after pentagastrin stimulation is considered to be normal and values of > 100 pg/ml generally indicate MTC or precancerous C cell hyperplasia.

In the presence of a known family mutation or in cascade family screening, a basal or pentagastrin-stimulated serum calcitonin > 10 pg/ml indicates C cell disease. Values of < 10 pg/ml after pentagastrin test do not exclude the diagnosis although disease if present in this situation is limited.

Therapeutic monitoring of MTC

Serum calcitonin falls rapidly after surgical excision, becoming undetectable in a few hours if excision has been complete. Baseline and pentagastrin stimulated values must remain low and a further rise may precede recurrence of the cancer by several months or a year.

- **Increased serum calcitonin concentrations other than in MTC**

In benign disease (values generally < 40 pg/ml)

– Hyperthyroidism and thyroiditis.

– Hyperparathyroidism.

– Chronic renal insufficiency.

– Cirrhosis and acute pancreatitis.

In other malignant diseases

– Neuro-endocrine tumours and pheochromocytoma (baseline values occasionally raised but little or not increased after pentagastrin stimulation).

– Bronchial carcinomas, liver, pancreatic, colonic and breast cancers.

FOR FURTHER INFORMATION

- Izembart M., *Les marqueurs*. In : Bounaud M.P., Duron F., Ingrand J., Izembart M., Piketty M.L., Talbot J.N., L'exploration de la thyroïde, Bioforma Ed, Paris 1999 :103-4.
- Izembart M., *Thyrocalcitonine*, Encycl Med Biol, Elsevier, Paris 2003.
- Charrié A., Bertholon-Grégoire M., *Thyroïde et cancer thyroïdien*, Workshop during the XVIIIth journées nationales de Biologie, Lyon, June 2003.
- *Cancer médullaire de la thyroïde et Néoplasies Endocrininennes Multiples de type 2 (MEN2), livret de recommandations pour la prise en charge diagnostique et thérapeutique*. Conseil scientifique du GTE, document co-ordonated by Dr Patricia Niccoli-Sire, Ed January 2006.

CAMPYLOBACTER

DEFINITION

The *Campylobacter* are gastro-intestinal bacteria which mostly cause intestinal infections. They are Gram negative bacilli characterised by their curved morphology (comma-shaped), very great mobility and micro-aerophilic respiratory metabolism. The *Campylobacter* genus belongs to the *Campylobacteriacae* family and currently includes 17 species, those most involved in human disease being: *Campylobacter jejuni*, *Campylobacter fetus* and *Campylobacter coli*.

INTRODUCTION

EPIDEMIOLOGY

Species belonging to the *Campylobacter* genus are found in the gastro-intestinal tract of humans and animals, particularly poultry, pigs, sheep and goats. They are transmitted through contaminated food or water. Human-to-human infection from animals or the environment is extremely rare. *Campylobacter* infection usually develops as sporadic cases although more rarely, epidemics are seen after drinking raw unpasteurised milk or contaminated water.

SYMPTOMS

- ***Campylobacter jejuni:*** Is responsible for bacterial intestinal infections.

  It is an invasive bacterium, the molecular pathogenic mechanism of which is not yet known. It is only known that it produces 2 toxins, including an enterotoxin similar to cholera toxin.

  Following an incubation period in the order of 1 to 3 days, patients develop:

  – Gastro-intestinal symptoms, such as diarrhoea which may or may not be accompanied by blood in stools, abdominal pain and vomiting.

  – More systemic symptoms are fever, asthenia and anorexia.

  Generally the infection resolves spontaneously in approximately a week although immediate complications such as appendicitis, peritonitis, cholecystitis and post-infectious complications such as reactive arthritis and more rarely the Guillain-Barré syndrome (reversible polyradicular neuritis occurring 3 weeks after the enteritits) may occur.

- ***Campylobacter coli:*** Has identical pathogenic potential to *C. jejuni*.
- ***Campylobacter fetus:*** Is responsible for septicaemia originating from the gastro-intestinal tract occurring in people with a weakened immune system (pregnant women and people who are immunosuppressed because of a treatment or disease).

SEARCH INDICATIONS

Diagnosis of community or non-community acquired enteritits in adults or young children.

Diagnosis of septicaemia in immunosuppressed subjects.

Confirmatory diagnosis of Guillain Barré syndrome.

Differential diagnosis with other bacterial intestinal infections such as salmonella, shigella, Yersinia, etc.

INFORMATION

SAMPLE

Stools: Collect the stool sample as soon as it is produced, into a clean container and place the equivalent of a large walnut sized specimen in a sterile container.

Venous blood for blood culture: Withdrawn by venepuncture after thorough asepsis at the sampling site, using a disposable device, preferably to be withdrawn before the antibiotic phase. Record sample time.

Serum: Blood collected into a dry tube.

Various samples: Food.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

Context and source of possible immunosuppression?

Current treatments?

SAMPLE STORAGE AND TRANSPORT

Stool samples: Should be sent at room temperature as soon as possible or stored at + 4°C for a maximum of 12 hours.

Blood cultures: Should be sent at room temperature as soon as possible. The bottles are placed in an incubator at 37°C as soon as possible.

Serum: Stored at + 4°C.

TEST METHODS

BACTERIOLOGICAL DIAGNOSIS

- **Direct fresh examination** can reveal the characteristic "midge in flight" mobility of *Campylobacter*.
- **Culture:** Is performed on stool samples or blood cultures.

  This requires a selective enriched culture medium. It is incubated in a micro-aerophilic atmosphere for 1 to 2 days at 37°C and the colonies obtained are grey and translucent.
- **Identification of bacteria belonging to the *Campylobacter* genus** is performed from morphology, mobility, respiratory type and biochemical features (catalase and oxidase-positive). The genus diagnosis is confirmed from identification of the species based on the result of hippurate hydrolysis and the antibiotic sensitivity profile.
- **Molecular methods (PCR):** Are used for direct testing for bacteria in food and are reserved for specialist laboratories.

SEROLOGICAL DIAGNOSIS

This is useful for post infectious complications particularly in the Guillain Barré syndrome and is performed using the complement fixation reaction (CFR) or by an ELISA method.

TREATMENT

In intestinal infections: Antibiotic treatment is debatable as Campylobacter enteritis resolves spontaneously. It is only justified to accelerate recovery and shorten the carrier period (macrolide, tetracyclin or fluoroquinolone).

In systemic infections: Parenteral treatment with gentamicin combined with another antibiotic (fluoroquinolone or β-lactamine).

FOR FURTHER INFORMATION

■ Cabie A., Bouchaud O., Coulaud J.P., *Infections à Campylobacter*, Encycl Med Chir maladies infectieuses 8027A10; 1996 : 6 p.

■ Dumoulard B., Wallet F., Glowacki F., Courcol R.J., *Bactériémie à Campylobacter fetus subsp fetus chez une patiente infectée par le virus de l'hépatite C*, Ann Biol Clin, 2004; 62: 587-89.

CANDIDIASIS

DEFINITION

The candidiases are very common opportunistic infections caused by saprophytic yeasts belonging to the *Candida* genus. Around fifteen species of *Candida* are pathogenic to human beings and most infections are caused by *Candida albicans* but *Candida glabrata*, *Candida parapsilosis*, *Candida tropicalis*, *Candida krusei* may also be responsible. They are uncapsulated, unpigmented and unicellular yeasts which reproduce by budding.

INTRODUCTION

EPIDEMIOLOGY

Candida does not exist in nature and live as saprophytes on human and animal mucous membranes. They are opportunistic yeasts which only become pathogenic in the presence of triggering factors which alter the local or general equilibrium of the person, such as age (prematurity or old age), physiological states (pregnancy or menopause), pre-existing diseases (diabetes, malignant blood diseases, AIDS, etc.), drugs (antibiotics, corticosteroids, oestrogen-progestogen combinations and psychotropic agents), local factors (moisture, skin maceration and skin trauma) or iatrogenic factors (catheters, probes, etc.).

C. albicans and ***C. glabrata*** are pathogenic, gastro-intestinal and genital tract yeasts.

C. parapsilosis is a commensal skin yeast.

C. tropicalis is found on the skin and mucous membranes.

SYMPTOMS

There are two types of clinical candidiases (superficial and deep).

Superficial candidiasis:

This occurs as the result of fungal colonisation of the gastro-intestinal or genital skin or mucous membranes. The main cause is *Candida albicans*.

Cutaneous candidiasis: Weeping, itchy intertrigo affecting all of the main skin folds (inguinal, axillary, infra-mammary, abdominal and buttocks) and small skin folds (hand and foot interdigital, commisure of the lips.

Gastro-intestinal candidiasis: This affects the oropharyngeal (oral thrush, anguilar cheilitis, stomatitis and glossitis), oesophageal (due to immunodeficiency) or intestinal (post-antibiotic diarrhoea) mucous membranes.

Urogenital candidiasis: Vulvitis, vaginitis and cervicitis which are common in women (during pregnancy, when taking oral contraceptives or after the menopause); urethritis and balanitis in men (common in diabetics).

Ungual candidiasis: Perionyxis and onyxis.

Deep candidiasis:

This often follows superficial candidiasis starting from the skin, gastro-intestinal or genito-urinary tract and spreads contiguously or via the blood stream. Exogenous infections are also seen, in which the species responsible are those other than *C. albicans*.

Non-septicaemic visceral candidiasis: Respiratory candidiasis, urinary candidiasis and gastro-intestinal candidiasis, secondary to spread from skin or mucosal candidiasis, and occurring in the presence of underlying diseases such as ulcers or neoplasia.

Septicaemic visceral candidiasis: In immunosuppressed patients, the yeasts colonise the blood and cause distant infections in most organs such as the kidney, liver, spleen, lungs, eye, endocardium, meninges and more rarely, the bone and joints.

Allergic candidiasis:

This involves skin reactions such as eczema, urticaria and erythema or respiratory disorders (such as asthma).

INDICATIONS FOR MEASUREMENT

Diagnosis of superficial candidiasis in immunocompetent or immunosuppressed patients.

Diagnosis of deep candidiasis in immunosuppressed patients.

Differential diagnosis with other mycoses of similar appearance.

INFORMATION

SAMPLE

Moist skin lesions, mouth, anus and vagina: Taken onto a sterile wet slide. Recover white coatings if possible, if not, scrape an erythematous area vigorously.

Dry skin samples: Taken with a curette or vaccination needle.

Ungueal lesions: Cutting or scraping the nail or pressing on the periungual nail bed to recover the pus.

Sputum: Collect sputum after a mouthwash. Ideally, induced sputum should be recovered (BAL or bronchial aspirates).

Urine: Collected after careful cleansing.

Serum: To test for antibodies and antigens.

Blood cultures.

Tissue biopsies.

Others: Stool, CSF, aqueous or vitreous humour and abscess aspirate.

QUESTIONS FOR THE PATIENT:

Age?

Clinical symptoms?

Are you pregnant?

Underlying diseases?

Are you taking medicines?

Other causes of weakened immune system?

SAMPLE STORAGE AND TRANSPORT

Samples must be transported as quickly as possible to the laboratory.

DIAGNOSTIC METHODS

MYCOLOGICAL DIAGNOSIS

- **Direct examination:** Involves testing for budding yeasts and filaments on fresh samples or stained slides from various samples such as stool, urine, nails, etc.

  This allows confirmation and quantification of the yeast present.

- **Culture:** On Sabouraud agar spiked with antibiotics (chloramphenicol or gentamycin) without cycloheximide.

  – The yeast is identified from morphological criteria which enable the genus of Candida to be diagnosed (filamentous and pseudofilamentous forms obtained after 24 hours following reinsemination into PCB medium) and physiological criteria to be identified (assimilation and fermentation of sugars, enzyme-based methods and tetrazolium reduction) which allows to diagnose the species.

  – Yeasts are counted in the urine and also in other biological fluids.

- **Fungal sensitivity profile:** The MIC (minimal inhibitor concentrations) are measured in liquid (bioMérieux®) or solid (E test®) medium.

- **Molecular biology:** DNA detected from biological samples using real-time PCR method (epidemiology and molecular studies).

- **Histological diagnosis:** On tissue samples.

IMMUNOLOGICAL DIAGNOSIS

- **Testing for circulating antibodies:** (Two types of methods are used):

  – *Screening:* Indirect haemagglutination, IFI, ELISA and electrosyneresis,

  – *Confirmation:* Immunoelectrophoresis.

  Any positive screening reactions must be confirmed.

- **Testing for circulating antigens (mannan or heat labile antigen):** Useful in diagnosing candidiasis in immunosuppressed patients and in monitoring the effectiveness of treatment. Agglutination and ELISA methods are available. Testing for circulating antigen is relatively insensitive and requires rapidly frozen serum.

INTERPRETATION

The diagnosis of candidiasis is based above all on clinical and biological evidence. The diagnosis of superficial candidiasis is easier than systemic candidiasis as the clinical symptoms are more suggestive and the samples are easier to obtain.

Biological results are interpreted taking account of the species isolated, sampling site, number of fungal bodies and the patient's immune system:

–*In immunocompetent patients:* Isolation of *Candida* in a sample is not always pathologically significant even if it contains *C. albicans*. The yeasts must be seen on direct examination and large numbers must be isolated in culture. Isolation of *Candida albicans* from skin, nails, urine and CSF is pathogenic. Conversely, isolation even in large amounts in faeces or sputum is not strictly pathogenic. Finally, the presence of *Candida* in the mucous membranes should be considered significant if clinical symptoms are suggestive and if large numbers of yeasts are isolated. Serological results are difficult to interpret because of the saprophytic nature of *Candida* (colonisation cannot be distinguished from infection). However, seroconversion or a significant rise in the antibody titre in a second sample, represents evidence in favour of active candidiasis.

– *In the immunosuppressed:* Observation of budding yeasts and filaments on direct examination or isolation of a yeast from a closed site is of pathological significance as is isolation of yeasts (*C. albicans* or others) from colonisation sites because of the potential risk of spread. A single positive blood culture is indicative of septicaemia. Testing for circulating antibodies is often fruitless or provides limited information although testing for circulating antigens may be useful, although current techniques lack sensitivity and specificity.

TREATMENT

The choice of treatment is based on the site of the candidiasis, the patient's immune status and studying the yeast's antifungal sensitivities.

Superficial candidiasis: Nystatin, amphotericin B, imidazoles: miconazole, ketoconazole, fluconazole, econazole and isoconazole.

Deep candidiasis: IV amphotericin B, flucytosine or a combination of both of these, miconazole, ketoconazole, or an association of ketoconazole and flucytosine.

Prophylactic measures are used in "at risk" subjects: Systematic administration of antifungal agents and regular laboratory monitoring.

FOR FURTHER INFORMATION

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- Crickx B., *Infections cutanées et muqueuses à Candida albicans*, Rev Prat, 49; 1999: 2149-2152.

CANNABIS

DEFINITION

Cannabis or hemp is a plant belonging to the cannabinaceae family, Urtical order. It was previously used therapeutically as an analgesic and to combat insomnia but its use is now illegal in France.

In practice, it is used above all "recreationally" for its psychoactive properties. Several preparations are offered from two varieties: *cannabis sativa sativa* and *cannabis sativa indica* (Indian hemp).

– The plant (marijuana, grass, kif, ganja, etc.) is used in the form of a powder prepared from dried leaves and flower tips. Depending on the preparation it can be smoked pure in a hookah pipe or mixed with tobacco (joint or splif).

– Hashish (hash or shit) is a yellowish or brownish powder prepared by threshing and sieving leaves and flower tips then compressing them into "bars" or "soaps". The resin is generally used mixed with tobacco and smoked and more rarely incorporated into cakes.

– The oil is obtained from extracting the resin with 90°C alcohol and then exposing it to sunlight to evaporate the alcohol. It takes the form of a viscous liquid which is generally mixed with cakes and often with chocolate.

The two main active substances in these preparations are 9-delta tetrahydrocannabinol (9THC, the most psychoactive) and 11hydroxy 9-delta tetrahydrocannabinol.

Synonyms: Indian hemp, marijuana, hash, shit, grass, joint or splif.

METABOLISM

Absorption after inhalation	Approximately 18% of THC passes into the blood
Plasma peak	7 to 8 minutes
Cmax	50 to 200 ng/ml
Metabolism of 9-THC	Hepatic, by oxidation and glucuronide conjugation
Clearance	Urinary, mostly THC-COOH (11-nor-9delta-tetrahydrocannabinol- carboxylic acid), and partially in bile
Clearance ½ life	Approximately 8 days

INFORMATION

CIRCUMSTANCES OF POISONING

Cannabis is mostly smoked or eaten, usually in isolation and sometimes repeatedly but without true dependency (rave parties, between friends, etc.). True cannabis addicts whose entire personality is organised around taking cannabis are rare (5 to 10% of users). Despite its widespread use, (depending on the source, the number of users ranges from 1 to 5 million in France), few serious accidents occur as the acute toxicity of cannabis is limited.

It is however a genuine problem in the context of road safety as it is reported to be found in the blood of 8 to 15% of drivers involved in road traffic accidents (20% in those under 30 years old), whether or not combined with alcohol.

PHARMACOLOGY

The target cells for cannabis have recently been identified and are the CB1 and CB2 receptors.

The CB1, membrane receptor is mostly located in the cerebellum, hippocampus and frontal cortex. It is also found in small amounts in the uterus, gonads, spleen and heart. The CB2 receptor which is also a membrane receptor is present above all in the blood cells, explaining the effect of cannabis on cellular immunity. Binding of cannabinoids to their receptors results in an effect on the calcium and potassium ion channels causing a reduction in synaptic neurotransmitter release and inhibition of neuronal excitability.

The effects sought by users vary according to dose and extent to which doses are repeated. Generally, the "cannabis high" caused by 9THC at doses > 25 mg involves a feeling of joy, happiness and physical wellbeing, excitation and dissociation of ideas, disorientation in time and space, increased sexual desire and experience of sensations, and an overall shift towards more pleasurable sensory perceptions.

TOXICITY

Cannabis has cardiovascular effects involving tachycardia and orthostatic hypotension, gastro-intestinal effects (vomiting, diarrhoea and abdominal pain at high dose), adverse effects on reproduction function and on the immune system. However, the predominant effects that may have dramatic consequences, particularly in car drivers or in numerous everyday circumstances are the psychological effects. Cannabis has significant neurobehavioural toxicity, altering perception of time and distance, disturbing vision, causing mood disorders with euphoria, depersonalisation and loss of inhibition and disturbance of short-term memory. Tolerance develops in regular smokers. Although physical dependency is weak, psychological dependency develops more markedly producing compulsion for repeated use.

The lethal dose in human beings is not known.

INDICATIONS FOR MEASUREMENT

– Cannabis screening is proposed for drivers involved in an accident with physical injury and is mandatory in France for those involved in a fatal accident.

– Medico-legal circumstances (dysfunctional behaviour resulting in prosecution).

– Company screening for drug use particularly for at risk jobs (train drivers, repair or maintenance work requiring absolute control of behaviour otherwise placing other people's lives in danger).

– Doping tests, because of its sedative effects (used by athletes to help them rest before competitions) and for its anxiolytic properties which are very popular in those performing precision sports (riding, shooting, etc.).

– Monitoring heroin addicts being treated with buprenorphine or methadone substitution. Cannabinoids are tested for regularly in the urine particularly in the follow-up of patients

on methadone, because of the possible pharmacological interactions and as part of the overall management of these patients.

INFORMATION

SAMPLE

Urine: 40 ml of urine collected at the laboratory, preferably into 3 plastic bottles (2 bottles for subsequent tests to be frozen at –20°C). Outside of a medico-legal context a single sample taken under the same conditions into an empty bottle for urine cytology is acceptable. Some of the cannabinoids however may be adsorbed onto the plastic tube wall and because of this, silylated glass containers are recommended. Ensure the sample is not fraudulent (substituted, addition of water or adulterating agents) by measuring pH, urine density and confirming sample temperature as soon as possible after it has been produced (temperature > 30°C). Close the bottles firmly (seal them if the analysis is being requested in a legal context), identify them accurately and record the date, time and place of the collection.

Blood: Reserved mostly for tests performed in a medico-legal context (road traffic accidents, etc.).

The sample is taken into a tube containing fluoride, with or without anticoagulant.

QUESTIONS FOR THE PATIENT

If possible, reason for test, other medicinal products or psychotropic agents taken concomitantly.

SAMPLE STORAGE AND TRANSPORT

Urine may be stored at room temperature at + 4°C for one week.

Serum or plasma must be decanted promptly and frozen at - 20°C if the analysis is to be postponed.

ASSAY METHODS

Urine screening: Immunochemical methods (FPIA and EMIT are the most commonly used). The sensitivity of these methods ranges from 20 to 100 ng/ml of THC-COOH. The positivity threshold recommended by the European Union is 50 ng/ml (NB: All positive screening methods must be confirmed with a confirmatory method on blood). The risk of cross-reaction with related or unrelated substances that may cause falsely-positive results exists.

Specific confirmatory method: Gas-phase chromatography linked to mass spectrometry (reference method).

NORMAL EXPECTED VALUES

Negative screening test if cannabis has not been taken (sensitivity threshold of EMIT screening method: 50 ng/ml).

INTERPRETATION

The substance tested in the urine is THC-COOH (11-nor 9-delta tetrahydrocannabinolcarboxylic acid). The threshold of 50 ng/ml recommended by the European Union for cannabis screening methods in the urine can exclude passive smokers (maximum urine concentrations invariably < 10 ng/ml). Interpretation can occasionally be difficult when methods with a different positivity threshold are used. On average, THC-COOH can be detected in the urine for 7 days in occasional smokers (once to twice per week), although it may be detected for 15 to 30 days in regular smokers (5 - 6 "cigarettes"/day).

Attention should be paid to the high risk of drug addicts falsifying urine samples. This can be done *In Vivo* or *In Vitro* and is intended to reduce the actual cannabis concentration. The main methods used *In Vivo* are drinking very large amounts of liquid or taking diuretics before passing urine ("flushing" effect) or taking ibuprofen, aspirin, metronidazole or vitamin B2. *In Vitro*, various substances can be added to urine to interfere with the analysis, including GC-MS. These are alkalis (caustic soda, potassium hydroxide, etc.), weak acids (lemon juice, vitamin C or vinegar), oxidising agents (bleach, hydrogen peroxide, etc.), detergents, liquid soaps, table salt, benzlkonium chloride, glutaraldehyde, etc.

A few pitfalls can be avoided by examining the urine before analysis (appearance, colour, temperature, pH, density, osmolality, turbidity and smell).

A concentration of > 1 ng/ml in the blood is considered to be significant, i.e. it may explain psychological disturbances.

FOR FURTHER INFORMATION

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- Mura P., *La recherche et le dosage des cannabinoïdes : pourquoi et comment ?* Revue française des laboratoires 2000 ; 322 : 35640.
- Mura P., Piriou A., *Cannabis*. In : Toxicologie et pharmacologie médicolégales, coordinateur P. Kintz, Collection Option/Bio, Elsevier Ed, Paris 1998 : 543654.
- Mura P., Piriou A., *Le Cannabis*. In : Alcool, médicaments, stupéfiants et conduite automobile, coordinateur Patrick Mura, Collection Option/Bio, Elsevier Ed, Paris 1999 : 59674.

CARBAMAZEPINE

DEFINITION

Carbamazepine is a molecule with mood regulating and anticonvulsant properties marketed in France under the name Tégrétol®. It is indicated for use in the treatment of epilepsy, to prevent recurrences of depression in manic depressive psychosis, in the treatment of mania or hypomania and for facial neuralgia.

Synonyms: Tégrétol®, Tégrétol®LP.

INDICATIONS FOR MEASUREMENT

The carbamazepine dose/steady state concentration ratio varies with the dose administered and between individuals. Measurement in blood can be used to confirm that the dosage is effective and below the toxicity threshold. This allows for better dosage adjustment.

Measurement is indicated when:

1) Treatment is ineffective.

2) Signs of overdose are present, such as drowsiness, dizziness, ataxia, visual disorders, dry mouth, etc. or in acute poisoning, symptoms of which develop 1 to 3 hours after ingestion and are neuromuscular, cardiovascular, respiratory and gastric with consciousness disorders which may extend to profound coma or seizures (young children).

3) When associated with other medicinal products or in some clinical situations liable to alter the metabolism of carbamazepine:

– Serum carbamazepine concentrations are reduced (with a risk of epileptic seizures), as a result of increased hepatic metabolism when it is associated with:

- Other anti-epileptics: Phenobarbital, phenytoin and primidone.

NB: When associated with phenobarbital and primidone there is a gradual reduction in plasma concentrations of carbamazepine and its active metabolite without apparent change in efficacy on epilepsy; when associated with phenytoin there is a reciprocal fall in plasma concentrations and increased metabolism, without any apparent change in efficacy on epilepsy.

- Rifampicin (by enzyme induction).

– Increased plasma carbamazepine concentrations as a result of inhibition of its hepatic metabolism when associated with the following medicinal products: Valproic acid, valpromide, acetazolamide, cimetidine ≥ 800 mg/d, (initial days of treatment), clarithromycin, clonazepam, danazol, dextropropoxyphene, digoxin, diltiazem, erythromycin, felbamate, fluoxetine, fluvoxamine, josamycin, isoniazid, verapamil and viloxazin.

– Reduced binding of carbamazepine to plasma albumin increasing the free active fraction with risk of overdose (carbamazepine is normally 80% bound to plasma proteins, mostly albumin). These situations include:

- Any fall in blood albumin concentration (malnutrition, renal loss, etc.).

- Jaundice (competition with bilirubin).

- Association with other medicinal products which are extensively bound to plasma proteins (by competition): Salicylates, non-steroid anti-inflammatory drugs and phenytoin.

- Pregnant women (increased circulating free fraction).

INFORMATION

■ SAMPLE

Serum or EDTA or heparinised plasma. The sample must be taken ***before the dose of the drug*** (trough levels) and always at the same time. For therapeutic monitoring, the sample should not be taken until the pharmacokinetic steady state has been reached after 5 half- lives, in other words after 5 to 6 days for carbamazepine. In suspected overdose the sample is taken when clinical symptoms are present.

■ ESSENTIAL INFORMATION

Any order for a drug assay must include the reasons for the request (investigation for efficacy or toxicity), the sample time, the date when the treatment was started and/or dosage was changed, dosing details (amount administered, frequency, route of administration) and the subject's age, height and weight when possible.

■ QUESTIONS FOR THE PATIENT

Are you taking other medicines *(cf. above, list of compounds liable to alter plasma carbamazepine concentrations)*?

Are you pregnant?

■ SAMPLE STORAGE AND TRANSPORT

24 hours at room temperature; 7 days at + 4°C; or –20°C beyond this time. Transport at + 4°C.

ASSAY METHODS

Chromatographic methods (gas-phase chromatography coupled to mass spectrometry and HPLC) although considered "comparison methods" (in the absence of reference methods) are not widely used.

The methods which are currently used are immunological competitive, homogeneous or heterogeneous phase methods. These do not allow to distinguish carbamazepine from its metabolites, particularly its main active metabolite, the 10, 11-epoxide.

REFERENCE VALUES

The therapeutic range for steady state trough concentrations is between 4 and 12 µg/ml (17 to 51 µmol/l). Concentrations above 15 µg/ml (63 µmol/l) are considered to be toxic.

Conversion factor: 1 µg/ml = 4.2337 µmol/l

1 µmol/l = 0.2362 µg/ml.

FOR FURTHER INFORMATION

■ Mialon A., *Dosage des médicaments*, tome II, Cahier BIOFORMA N° 18, Paris, January 2000, carbamazepine chapter: 4353.

■ Dictionnaire Vidal®.

CARBOHYDRATE-DEFICIENT TRANSFERRIN

DEFINITION

Transferrin is a glycoprotein present in plasma and produced by the liver. HPLC chromatographic separation has revealed 8 different isoforms depending on the number of terminal sialic acids (from 0 to 7), bound to the transferrin carbohydrate groups. In healthy people, tetrasialylated transferrin predominates (approximately 80%), whereas the mono and disialylated forms represent only approximately 2% of total transferrin. The half-life of transferrin in serum is 7 days, desialylated transferrin unlike other desialylated glycoproteins in the body is not removed by hepatic asialoglycoprotein receptors and it therefore has a longer half-life in the circulation of between 14 and 17 days.

Patients suffering from chronic alcohol abuse have an altered distribution of the sialylated transferrin isoforms, with a relative rise in the hypo or desialylated forms at the expense of the tetra or pentasialylated forms, whereas total transferrin concentration remains normal.

Synonyms: *Carbohydrate Deficient Transferrin* (CDT), CDTect, decarboxytransferrin, hyposialylated transferrin, desialylated transferrin and sialic acid deficient transferrin.

INTRODUCTION

Excessive consumption of 50 to 80 g of alcohol per day (equivalent to 0.75 l of wine, 1.5 l of beer or 20 cl of 40% spirit) causes partial desialylation of the carbohydrate chains resulting in an increase in the percentage of de- or hypo-sialylated transferrin (CDT) in serum. Measurement of these desialylated fractions has therefore been proposed as a marker of chronic alcohol abuse.

Unlike other markers of alcoholism, CDT is not influenced by a number of other diseases including pancreatitis, pancreatic cancer, myocardial infarction, brain tumour, diabetes, etc. or by enzyme-inducing drugs *(cf table below)*.

INDICATIONS FOR MEASUREMENT

Early diagnosis (after 1 month) of chronic alcohol abuse.

Monitoring alcohol abstention and testing for abstinence.

INFORMATION

SAMPLE

Serum (dry tube): the patient should preferably be fasting before the sample is taken.

Discard haemolysed samples (artefactual increase in CDT) or clouded samples (if the measurement is performed using turbidimetry). Lipid removal is recommended as lipids cause a reduction in serum CDT.

QUESTIONS FOR THE PATIENT

Context of request (detection or monitoring alcohol disease or investigation for metabolic disease).

SAMPLE STORAGE AND TRANSPORT

The sample should be centrifuged promptly after the sample is taken.

Serum can be stored for 30 hours at room temperature for 1 week at +4°C and for several months at –20°C.

Transport at +4°C.

ASSAY METHODS

Isoelectric focussing, anion exchange chromatography: HPLC or microcolumns (commercial kits are available) and capillary electrophoresis.

NORMAL EXPECTED VALUES

Results can be expressed as U/l or as a percentage. As an indication: CDT < 2.6% (ion exchange chromatography).

CDT < 1.3% (capillary electrophoresis).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Values are higher in abstinent women than in men (if CDT values are expressed as a percentage they are independent of sex).

Several genetic transferrin variants exist, the Tf-C1 variant being the predominant variant in the Caucasian population. CDT may be falsely negative in Tf-B or Tf-D variants in an excessive drinker or falsely positive when monitoring abstinence in an abstinent patient.

PATHOLOGICAL VARIATIONS

- ***Chronic alcoholism:*** CDT is a more specific and less sensitive marker of alcoholism than GGT in excessive drinkers. When diagnostic difficulties arise, and particularly when confounding variables interfere with interpretation of the GGT, CDT can be used to confirm alcohol exposure (cf. table). CDT, however, is not an acute marker of intoxication; it rises after consumption of at least 50 to 80 g of pure alcohol per day for a month and changes in the CDT reflect the cumulative situation over previous months. Serum concentrations are not associated with the daily amount of alcohol consumed.

  It is also a good marker to monitor patients during alcohol withdrawal. Following withdrawal, serum concentrations fall in the initial days and remain low for 3 to 5 weeks. Return of concentrations to normal values depends on other factors, particularly the patient's liver status (cf. table).

  During detoxification, CDT can be used to identify approximately 76% of relapses compared to 33% for GGT. 95% of relapses can be identified using a combination of these two markers.

- ***Congenital disorders of glycosylation (CDG1):*** CDG1 or type 1 glycoprotein glycosylation abnormality is a genetic disease with an incidence of approximately 1/25000. It is occasionally tested for in children but also and increasingly often nowadays in adults by neurologists. Affected people have hyposialylation of N-glycoproteins including transferrin and have psychomotor retardation and/or multi-organ disease. In this situation, CDT values may be greatly increased. Clinical details are required to interpret results.

- ***Other causes of rises in serum CDT in disease:*** apart from alcohol consumption, serum CDT is increased in many pathological situations *(see table)*.

FOR FURTHER INFORMATION

- Seta N., *Transferrin desialylated*, Encycl Med Biol, Elsevier, Paris, 2003.

Comparative features of biological markers for alcohol consumption

	Sensitivity	Specificity	False positives	Drugs interfering with the test
MCV	50 - 60%	90%	• Smoking: • Pregnancy • Regenerative reticulocytosis • Folate deficiency • Age	• Macrocytosis and folate deficiency - Antifolates (methotrexate, Bactrim®), antiHIV (AZT, DDI), - Cytotoxics (mercaptopurine, 5FU…)
GGT	70 - 80%	50 to 90% (in the absence of liver disease)	• Non-alcoholic hepatobiliary disease • Diabetes • Obesity • Dyslipidaemia • Hyperthyroidism • Pancreatic disease	• Enzyme inducers; barbiturates, AVK, oestrogen-progestogens, haloperidol, imipramine, most anticonvulsants…
ASAT	20 - 30%	Low		
CDT	30 à 70%	89 to 100%	• Hepatocellular insufficiency secondary to primary biliary cirrhosis, auto-immune or viral cirrhosis, chronic active hepatitis, drug-induced liver disease, hepatocellular carcinoma, etc. (variable results). • Smoking, obesity, hypertension • Congenital metabolic abnormalities of protein glycosylation • People with a genetic transferrin variant Tf B or D (< 1%) • False + cases reported in people with hyperferritinaemia and hypotransferrinaemia	

CARBOXYHAEMOGLOBIN

DEFINITION AND SYNONYMS

Carboxyhaemoglobin (HbCO) is a complex formed between haemoglobin and carbon monoxide (or CO). Carbon monoxide is a colourless, odourless, insipid gas with a density similar to that of air which is poorly soluble in water and fairly soluble in some organic solvents such as ethyl acetate or ethanol. It is produced from the incomplete combustion of carbon-containing substances (combustion engines or incorrectly regulated gas heaters or water heaters, fire and tobacco smoke) and can be released by many industrial procedures (metallurgy and chemicals industry). It is a common pollutant of the air in large towns where concentrations commonly exceed 20 ppm. Carbon monoxide is an extremely inflammable gas which can form explosive mixtures with air.

In human beings, carbon monoxide is absorbed through the lungs and binds 80 – 90% to haemoglobin with an affinity approximately 200 times greater than that of oxygen forming a stable but reversible complex, which is known as carboxyhaemoglobin. Twenty per cent of carbon monoxide binds to myoglobin and 1% to cytochromes and catalases. Carbon monoxide crosses the placenta and the blood-brain barrier. Carboxyhaemoglobin concentrations rise rapidly from the start of exposure up to three hours after poisoning begins, reaching a plateau at eight hours. It is removed mostly through the lungs and after exposure has stopped, carboxyhaemoglobin concentrations decline with a half-life of 3 to 5 hours.

INFORMATION

Carbon monoxide poisoning is the leading cause of death from poisoning in France and in most Western countries. There are estimated to be around 8,000 cases of poisoning annually in France.

The symptoms of hyperacute or massive poisoning, involves limb paralysis, comas and seizures which progress rapidly to death without treatment. Conversely, acute or subacute poisoning is characterised by mild and variable functional symptoms from nausea and vomiting which may mimic food poisoning to severe headaches with temporal throbbing. Asthenia, dizziness plus mood and behavioural disorders may occur. Electrocardiographically, repolarisation disorders are seen. Neurological complications (Meynière syndrome, reduced intellectual capacity, personality and behavioural disorders) which may or may not be reversible and cardiac complications may be seen. The severity of these complications correlates with the severity and duration of poisoning. The presenting signs of chronic poisoning are similar to those of early subacute poisoning (headaches, dizziness, asthenia and occasionally gastro-intestinal disorders).

Carbon monoxide is highly foetotoxic and may cause foetal death or serious neurological complications if the mother suffers severe poisoning.

INDICATIONS FOR MEASUREMENT

Measurement of carboxyhaemoglobin in exposed workers can be used to assess the extent of exposure (on the same day of exposure) when exposure is relatively constant. A fairly close correlation exists between atmospheric carbon monoxide concentrations and blood carboxyhaemoglobin concentrations and the risk for health. Blood carbon monoxide and carboxyhaemoglobin concentrations are related by the equation below:

HbCO (%) = Blood CO concentration (ml/100 ml) x 100/1.39 x Haemoglobin concentration (g/100 ml)

Carbon monoxide can also be measured non-invasively in expired air from exposed subjects using direct reading instruments. This parameter also correlates with same day exposure to carbon monoxide. Carboxyhaemoglobin is related to carbon monoxide in expired air by the equation shown below:

HbCO (%) = CO in expired air (ppm) x 0.00115

Carboxyhaemoglobin measurement is used in accidental or intentional poisoning to assess the extent of poisoning and to decide on further management. Conversely, measurement is of limited use in monitoring patients once the diagnosis has been made.

It should be noted that carboxyhaemoglobin measurement may also be useful in monitoring dichloromethane (or methylene chloride) poisoning as its metabolisation produces carbon dioxide and carbon monoxide leading to prolonged poisoning. In this case the half-life of carboxyhaemoglobin is extremely long.

INFORMATION

SAMPLE

Samples must be taken into EDTA or lithium heparin anticoagulant (whole blood). Sodium fluoride can be used to minimise *In Vitro* production of CO by possible bacterial contamination.

In exposed workers, samples must be taken at least three hours before exposure begins and within 15 minutes after exposure ends.

In accidental or intentional poisoning the sample should be taken when the patient is seen by the emergency services.

QUESTIONS FOR THE PATIENT

Type of occupational exposure?

When was the sample taken with regard to the occupational exposure period?

Is the patent exposed to methylene chloride?

Does the patient smoke?

Does the patient live in an area with high air pollution?

SAMPLE STORAGE AND TRANSPORT

Samples can only be stored for a few hours at + 4°C.

ASSAY METHODS

The most widely used method is spectrophotometry. Gas-phase chromatography, electrochemical and infra-red spectrophotometric methods can also be used.

NORMAL EXPECTED VALUES

The reference value for carboxyhaemoglobin in the general population is < 1% in non-smokers, rising to 5 to 10% in people who smoke one to three packets daily. The guideline end of shift value in France is < 3.5% which is the same as in North America, the United States and Quebec, but different to the guideline value in Germany (< 5%).

Reference values for carbon monoxide in the general population are < 0.4 ml/100 ml in non-smokers and < 2 ml/100 ml in those who smoke one packet daily). The guideline value in France is 0.7 ml/100 ml immediately at the end of a shift (no guideline values are set in other countries).

PATHOPHYSIOLOGICAL VARIATIONS

The relationship between extent of poisoning and symptoms is summarised in table I.

HbCO en %	Symptoms
0,4 to 0,7	None (levels generally found in non-smokers living by the sea)
1 to 2	None (levels found generally in non-smokers living in towns)
5 to 7	None (levels found generally in smokers, returning to normal after breathing in open air for approximately 12 hours)
10	Headaches and respiratory disorders
35	Serious poisoning
50	Severe poisoning – possibly fatal, particularly in aggravating circumstances (child or respiratory disease)
66	Coma, death

Table I: Relationship between extent of poisoning (carboxyhaemoglobin levels) and symptoms in poisoned patients *(from Fiche de toxicologie n° 140 – Oxyde de carbone; Société française de toxicologie analytique; www.sfta.org).*

Results must be interpreted taking account of smoking habit, extent of exposure to air pollution, whether or not the patient has had oxygen therapy and possible exposure to methylene chloride or haemolysis. Exposure to 50 ppm of methylene chloride produces an HbCO level of 1.5 to 2.5% at end of shift in a non-smoker.

FOR FURTHER INFORMATION

■ Mathieu-Nolf M., Lhermitte M., Mathieu D., *Monoxyde de carbone*, Traité d'Encyclopédie Médico-Biologique.
www.bioconsulte.com
■ Fiche Biotox, *Oxyde de carbone sanguin*. www.inrs.fr
■ Fiche Biotox, *Carboxyhémoglobine*. www.inrs.fr
■ Fiche toxicologique, *Oxyde de carbone* n° 47. www.inrs.fr

CARNITINE

DEFINITION

Carnitine is a small molecular weight organic molecule involved in mitochondrial β-oxidation of long chain fatty acids. It is present in almost all tissues in the body in free and bound or acylated form. Carnitine is filtered by the kidney and more than 90% is reabsorbed. Measurement is useful in the diagnosis and monitoring of certain congenital or acquired metabolic diseases.

Synonym: Free and total carnitine, beta-hydroxy-gamma-trimethylaminobutyric acid.

INTRODUCTION

Carnitine enables long chain fatty acids (C > 14) activated in the cytoplasm to be transferred into mitochondria. Acylcarnitine is involved in intracellular homeostasis of free coenzyme A which is involved in various oxidation reactions, particularly oxidation of fatty acids and branched amino acids.

INDICATIONS FOR MEASUREMENT

Investigation of congenital carnitine deficiency as part of the investigation of cardiomyopathy.

Investigation for secondary deficiency, due to inadequate intake or high losses (excessive excretion of acylcarnitines, renal dialysis, tubule disease).

Therapeutic monitoring of patients suffering from organic acidurias receiving L-carnitine supplementation.

INFORMATION

■ SAMPLE

Blood: Heparinised plasma, EDTA must not be used, do not use tubes with a phase separating gel.

Urine: 24 hour urine collection or first early morning urine sample (all of the urine passed over night).

■ QUESTIONS FOR THE PATIENT

Are you fasting?

Are you being treated with valproic acid, as this medicine increases urinary carnitine excretion and reduces its plasma concentration?

In women: Are you taking oral contraceptives?

This produces approximately a 15% fall in plasma carnitine concentrations.

■ SAMPLE STORAGE AND TRANSPORT

Blood: Centrifuge and decant the sample promptly and freeze at -20°C within an hour after sampling. Transport frozen.

Urine: Freeze at –20°C within an hour after collection.

ASSAY METHODS

"Routine" radio-isotopic or chemical method with spectrophotometric reading.

Reference method is isotope dilution tandem mass spectrometry and this method can distinguish between the free and acyl fractions of carnitine (total carnitine measured radio-isotopically = free + acyl).

NORMAL EXPECTED VALUES

These may vary slightly between assay methods and laboratories.

For reference, by tandem mass spectrometry:

– In blood:

Free carnitine concentration:

M/F at birth: 13 to 27 µmol/l.

M/F from 2 to 7 days old: 12 to 18 µmol/l.

M/F from 8 to 28 days old: 18 to 38 µmol/l.

M/F from 1 to 12 months old: 29 to 43 µmol/l.

M/F 1 year to adult: 30 to 50 µmol/l.

Total carnitine concentration:

M/F at birth: 25 to 47 µmol/l.

M/F from 2 to 7 days old: 21 to 29 µmol/l.

M/F from 8 to 28 days old: 26 to 48 µmol/l.

M/F from 1 to 12 months old: 40 to 56 µmol/l.

M/F 1 year to adult: 43 to 65 µmol/l.

– In urine:

M/F: Free carnitine: 13 to 16 mmol/mol creatinine.

M/F: Total carnitine: 34 to 47 mmol/mol creatinine.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

■ **In blood:** Variations in total plasma carnitinaemia:

By age: *cf* above

By sex: "Normal" values fall slightly at the end of the menstrual cycle and throughout the cycle in a woman taking oral contraceptives. In pregnant women, plasma carnitine falls gradually to approximately 50% of the baseline value in pregnant women at the end of pregnancy.

By length of fasting: Plasma total carnitine concentrations increase gradually up to 15 to 18 hours of fasting and then return to normal.

■ **In urine:** Total carnitine concentrations increase with age. Normal values in newborn babies under a week old are approximately one tenth of those in adults.

■ PATHOLOGICAL VARIATIONS

Reduced plasma total carnitine concentration is seen in different situations:

■ **Reduced carnitine biosynthesis:**

Inherited carnitine deficiency: Deficiency of an enzyme involved

in the biosynthesis pathway, tissue transporter deficiency, resulting in reduced total, free and acylcarnitines.

NB: Primary inherited carnitine deficiency is extremely rare and is due to defective tubular carnitine reabsorption, causing a very large fall in blood concentrations and massive urinary carnitine excretion.

Secondary deficiencies are far more common and are seen in organic acidurias (massive urinary loss of esterified carnitine), in Reye's syndrome or after poisoning with compounds which require Coenzyme A for their mitochondrial catabolism. They may also occur as a result of inadequate intake, increased requirements or massive urinary excretion *(cf. below).*

Deficiency of synthesis precursors: Malnutrition, malabsorption (total free and acylcarnitine are normal or reduced), dialysis, cirrhosis, long-term unsupplemented total parenteral nutrition (reduced total and free carnitine and normal acylcarnitine).

Renal insufficiency: Reduced renal enzyme activity (reduced total and free carnitine, normal or increased acylcarnitine);

- **Increased urinary excretion:**

  Renal insufficiency, dialysis, tubule disease, patients treated with valproic acid resulting in a reduced total and free carnitine, normal or increased acylcarnitine.

- **Increased requirements**

  – Pregnant or breast-feeding women: Reduced total and free carnitine, normal acylcarnitine.

  – Diabetes: reduced total and free carnitine, increased acylcarnitine.

FOR FURTHER INFORMATION

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CAROTENES

DEFINITION

Carotenes are part of a family of compounds containing 8 isoprenoid units. Six major carotenoids have been isolated from blood. They are beta-carotene, alpha-carotene, zeaxanthine, luteine, lycopene and beta-cryptoxanthine. Beta-carotene is quantitatively the most important. Carotenes are obtained from the diet, mostly in green vegetables (salad leaves, spinach etc.) and carrots but also from some fruits (blueberries, apricot, orange, etc.), plus from butter and dairy products. Hydrolysis of β carotene in the intestinal mucosa results in the formation of retinol (vitamin A).

INTRODUCTION

Apart from their role as vitamin A precursors (beta-carotene, alpha-carotene and beta-cryptoxanthine), the carotenoids have specific properties. β carotene traps free radicals reducing perioxidation reactions. Some carotenoids have photo-protective effects by stimulating melanin synthesis. They are all anti-oxidants.

INDICATIONS FOR MEASUREMENT

Measurement of β carotene as a vitamin A precursor can be used as an indirect assessment of a patient's vitamin A status. Plasma β carotene concentrations are proportional to dietary intake.

Because of its anti-oxidant properties, measurement can be used to assess a patient's anti-oxidant defence status. In this situation a carotenoid profile can also be obtained including measurements of lutheine, zeaxanthine, canthaxanthine, beta cryptoxanthine, lycopene, and alpha carotene.

INFORMATION

SAMPLE

Serum (dry tube). As carotenes are labile, the sample must be protected from light, centrifuged and the serum frozen at – 20°C within an hour after sampling.

QUESTIONS FOR THE PATIENT

Suspected disease?

Current treatment?

SAMPLE STORAGE AND TRANSPORT

When not exposed to light, samples can be stored frozen at – 20°C for several weeks.

ASSAY METHODS

The carotenoid profile is determined by high performance liquid chromatography (HPLC) after extraction on a C18 pre-column, and read spectrophotometrically in the UV range at 325 nm. Beta carotene is assayed by HPLC.

REFERENCE VALUES

NORMAL EXPECTED VALUES

For reference: beta carotene (by HPLC).

Men: 0.04 to 1.02 µmol/l, i.e. 26.0 to 551.0 µg/l

Women: 0.14 to 1.92 µmol/l, i.e. 77.0 to 1036.0 µg/l

Conversion factor: µmol/l → mg/l = 537.63.

PATHOLOGICAL VALUES

Hypocarotenaemia

– Inadequate intake.

– Fat malabsorption (coeliac disease, cystic fibrosis, billiary tract atresia, chronic pancreatitis, cirrhosis, etc.).

Hypercarotenaemia

– Hyperlipidaemias.

– Hypothyroidism and diabetes.

– Excessive intake (dietary and medicines).

FOR FURTHER INFORMATION

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CATECHOLAMINES AND METHOXYL DERIVATIVES

DEFINITION AND SYNONYMS

Catecholamines are neuromediators synthesised in nerve cells and in the adrenal medulla. They include ***dopamine*** (DA), ***noradrenaline*** (NA) and ***adrenaline*** (A). They are biogenic amines derived from the catechol core.

They are synthesised from an amino acid, phenylalanine and are stored in specific vesicles in neurones and adrenal medullary chromaffin cells. Adrenaline accounts for almost 80% of the catecholamines, stored in the chromaffin cells. Peripheral and central noradrenergic neurone synaptic vesicles store primarily NA.

Dopaminergic neurones are found mostly in the central nervous system.

Catecholamines have a very short biological half-life, in the order of a few minutes. They are catabolised by 2 main enzymes:

– MonoAmineOxydase (MAO);

– Catechol-O-Methyl Transferase (COMT).

Depending on the tissues these 2 enzymes may act either alternatively or in succession. The metabolism of NA and A results in the production of methoxyamines or metanephrines (***normetanephrine*** or ***normetadrenaline***) (NMN), ***metanephrine*** (or ***metadrenaline***) (MN)) and ***vanillmandelic acid*** (VMA). The metabolism of DA results in the formation of 3-methoxytyramine (MT) and its derivative ***homovanillic acid*** (HVA).

70-80% of catecholamines and methoxyamines are found in conjugated form in the plasma and in the urine in human beings.

In usual practice, catecholamine metabolism is investigated by measurements of:

– Catecholamines: DA, NA and A.

– Metanephrines: NMN, MN and MT.

– Acid catabolites: HVA and VMA.

Generally, the catabolites, metanephrines and acid catabolites are considered to better reflect catecholamine secretion than the catecholamines themselves and their 24 hour urinary excretion fluctuates less.

INFORMATION

PHYSIOLOGICAL EFFECTS ON ADRENALINE AND NORADRENALINE

On the cardiovascular system

– Increased rate, speed of conduction and force of contraction of cardiac muscle (effects mediated by beta-adrenergic receptors).

– Sympathetic stimulation producing a rise in blood pressure due to direct stimulation of the heart and vasoconstriction (effects mediated by alpha-adrenergic receptors).

On metabolism

Adrenaline and the sympathetic system have important metabolic effects causing hyperglycaemia, hyperlactataemia, hyperlipidaemia and increased oxygen consumption.

On hormone secretion

Increased secretion of renin and glucagon and reduced secretion of insulin and thyroid hormones.

PHYSIOLOGICAL EFFECTS OF DOPAMINE

On certain central nervous system nerve endings.

On the corpus striatum: Regulation of DA/acetylcholine (particular role in Parkinson's disease).

In the hypothalamus by modulating the action of hypothalamo-pituitary axis hormones.

PATHOLOGICAL ROLE

The two main indications for examining catecholamine metabolism are screening and monitoring of neuro-endocrine tumours, pheochromocytoma and neuroblastoma.

A third indication in psychiatry has also been proposed.

INDICATIONS FOR MEASUREMENT

PHEOCHROMOCYTOMA (PC)

These are usually benign, adult tumours.

Clinically, these are characterised by the classical triad of pallor, sweating and headaches, associated with arterial hypertension which is refractory to all treatments. Tumours develop in the adrenal medulla but may also develop in a site with a persisting islet of chromaffin cells.

They occur on average around the age of 40 and constitute approximately 1% of the causes of hypertension. Clinical features of the disease are due to excess catecholamine secretion. PC is frequently hereditary and may be associated with other neuro-endocrine tumours such as medullary thyroid cancer and multiple endocrine neoplasia (MEN).

Biochemically, the typical forms of PC excrete mostly NA, with smaller amounts of A. Their laboratory diagnosis is therefore based on measuring NA and A, and particularly their metabolites (as catecholamines are rapidly metabolised).

Generally an increase in catecholamines, metanephrine and VMA excretion in the urine is found and whilst the VMA level may remain within normal limits, the sensitivity and specificity of metanephrine measurement are almost 100% for PC diagnosis.

Measurement of free blood or urinary catecholamines and particularly urinary metanephrines are therefore essential for diagnosing the disease.

NB: An increase in urinary DA excretion also appears to be seen in rare cases of malignant PC.

The PC is treated by surgical excision of the tumour after localising it by ultrasound, tomodensitometry or MIBG (meta-iodobenzylguanidine) scintigraphy.

NEUROBLASTOMAS (NB)

These are malignant tumours of young children which usually develop between 3 months and 5 years of age and carry a poor prognosis if not diagnosed early. NB can be

found at any site in the body, predominantly retroperitoneally (75 %). Clinical symptoms are therefore relatively non-specific and depend on the site of the tumour and of its metastases (abdominal pain, vomiting, diarrhoea, respiratory features, neurological disorders etc.).

Biochemically the predominant catecholamines in the tumour tissue are DA and NA. Logically therefore, raised urinary concentrations of DA, HVA and VMA are found (DA is converted to NA which is catabolised in the tumour into VMA).

The three essential laboratory measurements for the diagnosis of NB are therefore DA, HVA and VMA. These parameters provide a diagnosis in almost 95% of cases of NB.

NB: Predominant excretion of HVA appears to be a poor prognostic marker.

Treatment is with chemotherapy and surgical excision of the tumour.

INFORMATION

■ SAMPLE

Blood: Venous blood withdrawn from the antecubital fossa in the morning with the subject fasting and after lying down for at least 20 minutes. It is recommended that venous access be established before the sample to avoid the rise in catecholamines from stress associated with the sampling procedure. The sample (approximately 5 ml) is drawn into lithium heparin or EDTA and must be centrifuged and frozen within an hour.

Urine: 24 hour urine acidified with 12N hydrochloric acid to obtain a urinary pH of between 2 and 3, then stored at +4°C.

■ QUESTIONS FOR THE PATIENT

Ensure that the patient has rested completely for the 20 minutes before the sample is taken (fasting, no physical exercise or smoking).

Correct diet to be followed for 72 hours before the sample is taken, avoiding foods which may interfere with the measurement, such as bananas, chocolate, tomatoes, citrus fruits, vanilla and advise only a modest intake of coffee and tea.

L. DOPA treatment (anti-parkinsonian) interferes with measurement of endogenous dopamine.

■ SAMPLE STORAGE AND TRANSPORT

Plasma catecholamines and metanephrines are only stable for a few hours after the sample is taken, at + 4°C. If measurement is to be postponed it is essential the samples are frozen at –20°C.

ASSAY METHODS

High performance liquid chromatography (HPLC) with electrochemical detection

This is now ***THE*** reference method which provides differential measurement of each of the catecholamines and their different catabolites. It is sensitive and specific.

The sample purification phase differs depending on the compounds to be measured and influences the quality of the result.

Other methods (not commonly used)

– Fluorimetric: Lacks sensitivity and specificity.

– Radioenzymatic: Specific and relatively sensitive although it takes a long time to perform and requires the handling of radio-isotopes.

NORMAL EXPECTED VALUES

(In adults)

Catecholamines: *Usually measured as free form*

	URINE (nmol/24 hours)	PLASMA (ng/l)
Noradrenaline	90 - 500	< 185
Adrenaline	< 120	< 675
Dopamine	300 to 3000	< 190

Metanephrines

	URINE (nmol/24h)
Normetanephrine	750 to 2000
Metanephrine	250 to 1200
3-Methoxytyramine	600 to 1300

Acid metabolites

	URINE (µmol/24h)
Vanillmandelic acid (Adult)	< 33
Homovanillic acid (Adult)	< 41

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Basal catecholamine levels vary greatly and their secretion may be influenced by many factors, including circadian rhythm, standing position, physical activity, stress, emotional state, obesity, hot/cold, etc. Results may be slightly above normal in children because of stress, particularly when the sample is taken.

Catecholamine concentrations may also be influenced by many foods, such as coffee, tea, tobacco, alcohol, bananas, chocolate and citrus fruits.

It is important to be aware and take account of these factors when validating a catecholamine metabolism profile.

■ PATHOLOGICAL VARIATIONS

– Plasma catecholamine and catecholamine metabolites are frequently raised in patients with kidney and heart failure although urine levels are reduced.

– Plasma and urinary catecholamines are raised in arterial hypertension, myocardial infarction and situations of extreme stress. In this case measurements need to be repeated later in order to exclude pheochromocytoma.

FOR FURTHER INFORMATION

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CEA

DEFINITION

Carcinoembryonic antigen (CEA) is a high molecular weight glycoprotein belonging to a cell surface glycoprotein family produced in excess by different types of cancer. It is an oncofoetal antigen normally present in the intestine, liver and pancreas of the foetus during the first 2 months of gestation (hence its name carcinoembryonic antigen). CEA is mostly cleared by the liver and its elimination kinetics follow a two phase model with an initial half life of 3.2 days followed by a second half life of 11 days.

Synonym: Carcinoembryonic antigen.

INTRODUCTION

CEA is involved in cell adhesion.

INDICATIONS FOR MEASUREMENT

CEA is a non-specific antigen expressed by various healthy body tissues and by many cancer tissues. Because of its lack of sensitivity and specificity it cannot be used for screening or diagnostic purposes. Its indications are:

– Follow up of adenocarcinomas: staging assessment, monitoring of treatment and early identification of recurrence or development of metastases, but more specifically, for colorectal, breast and lung adenocarcinomas. It can also be useful for monitoring adenocarcinomas of the liver, pancreas, ovaries and medullary thyroid cancer, occasionally combined with more specific markers.

– Prognostic assessment.

CEA in broncho-alveolar fluid may be helpful in the diagnosis of lung cancer, particularly when this is not visible endoscopically (such as when masked by pneumonia).

INFORMATION

SAMPLE

Serum or plasma.

Various aspiration fluids (broncho-alveolar, ascites, etc).

No circadian rhythm is described.

A fasting sample is not required. Haemolysis or hyperlipidemia however can interfere with the assay.

QUESTIONS FOR THE PATIENT

Current treatment? Chemotherapy, radiotherapy, surgery, types and date of treatment?

SAMPLE STORAGE AND TRANSPORT

24 hours at +4°C or -20°C beyond this.

ASSAY METHODS

"Sandwich" immunometric method.

NORMAL EXPECTED VALUES

Serum: CEA < 5 µg/l. These may vary depending on the method used.

PATHOPHYSIOLOGICAL VARIATIONS

NON-SPECIFIC VARIATIONS

CEA > 5 µg/l in 4.4% of healthy people.

The serum concentration of CEA generally remains < 10 µg/l in the following situations:

– Smoking (average in smokers: 7.5 µg/l)

– Benign gastro-intestinal (viral hepatitis, cirrhosis, Crohn's disease, ulcerative colitis, polyps, pancreatitis) and lung diseases

– Chronic renal failure.

INCREASES IN MALIGNANT DISEASE

Colorectal cancer

In the initial assessment: A rise in CEA may correlate with disease stage. However, approximately 1/5 of patients with advanced stage cancer (Dukes D) have a normal serum CEA concentration. Pre-operatively, CEA is a prognostic indicator which is independent of age, sex, site, histological type and Dukes classification. A direct relationship has been described between pre-operative serum CEA and relapse after curative surgery. In addition, survival rates are significantly better in patients with normal pre-operative serum CEA compared to those with a raised value.

Monitoring after initial treatment: A raised serum CEA after 6 weeks supports the persistence of tumour tissue. Persistently raised CEA values beyond 6 weeks may however be due to the hepatotoxicity of adjuvant chemotherapy (5FU-levamisole) (21% of patients) without active recurrence of the disease.

Detection of recurrence: Raised CEA over several measurements.

Monitoring palliative treatment of recurrence: CEA concentrations correlate with response to treatment.

Breast cancer

CEA is raised in 12% of cases of non-metastatic breast cancer and in 35 to 40% of cases of metastatic cancer, correlating with tumour progression.

Lung cancer

CEA is raised at the time of diagnosis in 33 to 62% of cases regardless of histological type (the highest values are seen in adenocarcinomas). CEA has a recognised prognostic value in patients suffering from small cell lung cancer and measurement in combination with other markers such as NSE or TPA is useful to monitor specific treatment.

A CEA over a threshold of 1 µg CEA/mg albumin is suggestive of lung cancer in broncho-alveolar fluid.

Other cancers

Serum CEA concentrations rise in various types of cancer: stomach, pancreas, liver, thyroid, lung, ovaries, colon, rectum, neuro-endocrine tumours, lymphomas and melanomas. Measurement is often used jointly with other more "specific" markers for the cancer concerned.

FOR FURTHER INFORMATION

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- ANAES/Service des références Médicales. *Marqueurs sériques dans les cancers du sein et les cancers colo-rectaux*. September 1997. www. anaes.fr.

CELLULAR TESTS FOR ALLERGIES

DEFINITION

These tests are of limited interest in routine practice and only have a place in the context of a well-documented allergological investigation for certain allergens.

They never have predictive value for the reintroduction of an allergen.

INTRODUCTION

Contact with an allergen results in the synthesis of specific IgE which, through their receptors, produce a hypersensitivity reaction with the release of mediators, including histamine and leukotrienes.

Current means of studying this hypersensitivity reaction *in-vitro* are directed at the basophil (flow cytometry and study of the receptors) and the mediators (tests for the release of histamine and leukotrienes).

INDICATIONS FOR TESTING

– No indications in routine practice.

– As a complementary investigation, only in a well-documented context, for example when a search for specific IgE in the blood cannot be performed in-vitro (this is the case of allergens which do not exist in combination with a carrier).

– Cellular exploration after an interval following an anaphylactic reaction, but it the test has no predictive value.

– Investigation of cross-reactions.

INFORMATION

SAMPLE

Whole blood collected on different anticoagulants depending on the tests employed.

Collection after an interval following an anaphylactic reaction (4 to 6 weeks).

Cessation of antihistamine and corticoid drug treatment several days or weeks before the test is performed, depending on the drugs.

The sample delivery time must be less than 24 hours (do not freeze). Send a sample of the allergen to be investigated with the blood sample.

ESSENTIAL INFORMATION

Symptoms?

Allergens suspected?

Other investigations carried out?

SAMPLE STORAGE AND TRANSPORT

Whole blood, viable for 24 hours on average, but cell functionality varies according to the patient.

METHODS

The principle of the tests is always the same: study of cellular reactivity in-vitro in the presence of an allergen at different concentrations.

- **Basophil degranulation test (BDT):** historically the first cellular test developed - should no longer be used, even after enrichment of preparations, because of inadequate sensitivity and specificity.
- **Histamine release test (HRT):** measurement of histamine released, research protocol – no standardised method – the best-known test, however, with good sensitivity and specificity for allergy to venom and curare.
- **Leukotriene release test (CAST):** measurement of leukotriene C4 or C4+D4+E4 – research protocol – no standardised method – no standardised interpretation – may allow investigation of intolerance to aspirin and to non-steroid anti-inflammatories.
- **Flow cytometry (FCM):** measurement of expression of activated basophil receptors (CD63, CD203) - research protocol – no standardised method – no standardised interpretation.
- **Lymphoblastic transformation test (LTT):** research protocol – no standardised method – no standardised interpretation – previously suggested in studying delayed hypersensitivity – still used by certain groups in allergies to drugs and cows' milk proteins. Should be replaced by new cellular methods, such as ELISPOT with cytokine measurement.

UNITS AND REFERENCE VALUES

Units: specific to each group and each test.

Normal expected values: specific to each group and each test.

FOR FURTHER INFORMATION

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CERULOPLASMIN

DEFINITION

Ceruloplasmin is a glycoprotein of 135 kDa which carries copper around the body. 95% of copper in the plasma is transported by ceruloplasmin.

INTRODUCTION

Ceruloplasmin is synthesised by the liver, initially in the form of apoceruloplasmin, and then integrates 6 atoms of copper per molecule, which gives it a blue colour and an enzymatic activity (holoceruloplasmin). The activity is oxidasic with respect to iron and certain neuromediators (adrenalin, noradrenalin and serotonin).

Although its principal role is to carry copper, ceruloplasmin is also involved in iron metabolism (cellular excretion of iron) and as an antioxidant in collagen synthesis.

INDICATIONS FOR MEASUREMENT

A ceruloplasmin measurement is indicated when screening for Wilson's disease, Menkes disease and congenital aceruloplasminaemia.

INFORMATION

SAMPLE

Serum stored at +4° C, preferably taken from a fasting subject.

Reject hyperlipaemic samples.

SAMPLE STORAGE AND TRANSPORT

Plasma or serum can be stored for 1 week at +4° C and several months at -20° C.

Transport at + 4 °C or frozen, if the sample is already in the frozen state.

ASSAY METHODS

Immunoturbidimetry and immunonephelometry are the methods most frequently used, measuring overall apoceruloplasmin and holoceruloplasmin.

Radial immunodiffusion (seldom used).

An **enzymatic method** is available which measures holoceruloplasmin only, in its functional form with the copper atoms.

NORMAL EXPECTED VALUES

In immunoturbidimetry and immunonephelometry:

Adults: 0.20 to 0.60 g/l.

Newborn babies and infants: 0.10 to 0.40 g/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL AND IATROGENIC VARIATIONS

Age-dependent: concentrations are lower in the newborn, due to hepatic immaturity.

Oestrogens: ceruloplasmin rises during pregnancy and when oestrogens are taken, including oral contraceptives and hormone replacement therapy during the menopause.

PATHOLOGICAL VARIATIONS

Reduced levels

Wilson's disease

Wilson's disease, or hepatolenticular degeneration, is an inherited disease with autosomal recessive transmission. In this disease, the ceruloplasmin level is usually reduced (< 0.20 g/l), or even nil (exceptionally normal in 5% of cases): cupraemia is also low, while cupruria is greatly increased (frequently 10 times the normal level) within 24 hours. The illness is characterised by defective biliary elimination of copper and its accumulation in the liver, then in the brain, cornea, kidneys and other tissues. Clinical signs are hepatic (cytolysis, hepatitis, hepatic insufficiency and cirrhosis), with neurological manifestations (problems with tonus and motricity, and psychiatric difficulties), as well as ophthalmological, haematological and renal impairment.

Menkes disease

Menkes disease is a serious neurodegenerative condition, with sex-linked (X chromosome) recessive transmission, which in its classical form, results in death before the age of 5. It causes defective intestinal absorption of copper and therefore a deficiency in free copper, resulting in low or zero cupraemia and ceruloplasmin levels. Most of the symptoms are explained by a deficiency in the activity of enzymes which have copper as a cofactor.

Aceruloplasminaemia

This is an autosomal recessive disorder in which ceruloplasmin is inhibited by various mutations of its gene situated on the long arm of chromosome 3. The disease is characterised by the appearance of diabetes with cerebral ataxia, extrapyramidal disorder and signs of dementia. As in Wilson's and Menkes diseases, levels of ceruloplasmin and cupraemia are low, while urinary copper is normal. A disappearance of the ferroxidasic activity of ceruloplasmin is observed, resulting in changes in iron metabolism: reduction in sideraemia, increased ferritin and accumulation of iron in tissues such as the liver, the pancreas and the central grey nuclei.

Ceruloplasmin is also lowered in nephritic syndromes, exsudative enteropathies, chronic hepatitis and severe malnutrition.

Increase

Ceruloplasmin levels rise in inflammatory syndromes, in acute or chronic infections, during oestrogen treatments and in poisoning by copper salts by inducing its synthesis.

FOR FURTHER INFORMATION

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CHLAMYDIA PNEUMONIAE AND PSITTACI

DEFINITION

Chlamydia pneumoniae and *psittaci* are bacteria in the *Chlamydiaceae* family of the Chlamydiales order, with obligate intracellular parasitism. The family includes two bacterial genera: *Chlamydia*, group *trachomatis* and *Chlamydia*, group *psittaci*.

The Chlamydia genus, group *trachomatis*, encompasses the species *C. trachomatis* (humans), *C. muridarum* (mice and hamsters) and *C. suis* (pigs).

The Chlamydia genus, group *psittaci*, is made up of the species *C. psittaci* (birds), *C. abortus*, *C. felis* (cats), *C. caviae* (guinea pigs), *C. pecorum* and *C. pneumoniae*.

Two forms appear during their development: elementary bodies (resistance) and reticulate bodies (replication). Their intracellular cycle is slow and results in specific cytopathogenic effects (vacuoles or intracellular inclusions).

C. pneumoniae is an extremely widespread pathogen, being found in virtually the whole of the human population, which is its only natural reservoir. *C. psittaci* causes zoonotic disease which can be transmitted to humans. These two agents are responsible for human atypical interstitial pneumonia.

INTRODUCTION

■ *CHLAMYDIA PSITTACI*

This organism is responsible for zoonotic disease in birds, which constitute the reservoir. Human contamination is accidental. "Psittacosis" refers to contamination from parrots, budgerigars and canaries, while the term "ornithosis" is used in connection with wild birds and poultry.

After 10 to 15 days of incubation, the typical signs are an influenza-like syndrome with fever, sweating, photophobia and bronchopulmonary impairment. In contrast to *C. pneumoniae*, extra-pulmonary signs are present, including myalgia in the pectoral and pelvic girdles, mediastinal ganglia, hepatic cytolosis and splenomegaly. Delayed diagnosis is associated with mortality due to hepatic, cardiac, renal and neurological complications.

■ *CHLAMYDIA PNEUMONIAE*

C. pneumoniae causes upper or lower respiratory impairment by strictly human-to-human transmission (droplets in suspension). TWAR is the only strain responsible for human infections.

Clinical manifestations may range from pharyngitis and sinusitis to community pneumonia (6 to 22 % of pneumonias). The majority of infections are benign, with the exception of certain forms which occur in debilitated subjects. They mainly occur in adolescents, young adults and the elderly. Polynucleosis is generally moderate. Without treatment, this may become prolonged and persistent. Certain authors have associated the presence of specific *C. pneumoniae* antibodies with chronic illnesses (atherosclerosis, asthma and chronic bronchial pneumonia).

SEROLOGY INDICATIONS

Serological diagnosis of *C. pneumoniae* and *C. psittaci* is indicated when atypical interstitial pneumonia is suspected.

INFORMATION

■ SAMPLE

Samples for serodiagnosis are collected in dry tubes. They must not be haemolysed or lipaemic. Pathological interpretation of the serology necessitates two samples separated by 3 weeks (slow rise in antibodies).

■ QUESTIONS FOR THE PATIENT

Exposure to animal contamination (farm or pet birds)?

Previous non-documented interstitial pneumonia and similar cases among contacts?

■ SAMPLE STORAGE AND TRANSPORT

Serum can be stored for 24 hours at ambient temperature, at +2° C to +8° C for 7 days, or for several months at -20° C.

ASSAY METHODS

The three methods available are complement fixation (increasingly abandoned because not species-specific), microimmunofluorescence (MIF) and the immunoenzymatic technique (ELISA). The technique using complement fixation on the lipopolysaccharide (LPS) common antigen, allows monitoring of deep systemic *C. psittaci* infections and 25 % of *C. pneumoniae* infections. In most cases, the MIF method uses, as antibodies, purified elementary bodies of specific strains.

NORMAL EXPECTED VALUES

The seroprevalence of *C. pneumoniae* is high in the general population, except in pre-school children, in whom it is practically nil. Seroprevalence is 10% in children of 5 to 10 years, 30% to 40% in adolescents and 80% in the elderly. Almost half of the adult patient population is seropositive.

The seroprevalence of *C. psittaci* is very low.

PATHOLOGICAL VARIATIONS

In the case of C. psittaci, an IgG titre ≥128 in IIF is significant for concluding that a sample is seropositive. For ELISA techniques, the threshold is a ratio, which must be above 1:1. To reach a conclusion of a recent or current infection, a second sample, taken after an interval of 3 weeks, must show either seroconversion or a multiplication of the IgG titre by 4 in IF. There are false positives with the antigens of *Legionella*. A titre ≥ 32 is considered suspicious if the signs and the context suggest psittacosis or ornithosis. Early antibiotic treatment may delay the appearance of antibodies.

For *C. pneumoniae*, the TWAR antigen is the most specific. The three categories of immunoglobin can be monitored i.e. IgG, IgA and IgM. IgM immunoglobulins appear within three weeks of the acute phase (and disappear after 2 to 6 months). IgG and IgA immunoglobulins appear after 6 to 8 weeks. IgG's decrease slowly, while IgA's persist for several years after infection (> 3 years in 60% of cases). Antibodies are not

protective and reinfections occur throughout life. In cases of reinfection, there is a rise in IgG's and IgA's after 1 or 2 weeks, whereas IgM's are more variable. Significant titres are considered to be 512 for IgG's and 16 for IgM's. IgA's offer little added value in diagnosing acute infections and are mediocre markers of chronic infection. Seroconversion does not necessarily occur in the acute phase in children (70% seronegative, in spite of a positive culture), due to the late appearance of antibodies.

Because of the absence of kits using a species-specific recombinant antigen, cross-reactions between *C. psittaci* and *C. pneumoniae* antibodies are virtually inevitable.

TREATMENT

At the present time, macrolides, fluoroquinolones and cyclins are prescribed for a period of 10 to 15 days.

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CHLAMYDIA TRACHOMATIS

DEFINITION

Chlamydia trachomatis (strains with serotypes D to K) is responsible for widespread, sexually-transmitted urogenital tract infections, as well as neonatal infections. The infection may pass unnoticed in women and a diagnosis may only be established when complications occur. These complications include salpingitis, with a risk of tubal sterility or ectopic pregnancies. Durand-Nicolas-Favre disease, or *lympho-granuloma venereum* (LGV), is caused by the serotypes L1, L2 and L3. This disease is widespread in tropical regions and sporadic in developed countries.

The bacterium (genus *Chlamydia*, family *Chlamydiaceae)* responsible for these infections has an obligate intracellular multiplication cycle. Two bacterial forms (the infectious "elementary bodies" and non-infectious "reticulate bodies") play a part in the sequence of events of the intracellular cycle.

Although the isolation of *Chlamydia trachomatis* in cell cultures remains the current reference method (carried out in reference laboratories), direct diagnosis of infection by *Chlamydia trachomatis* uses the detection of antigens or the genome of *Chlamydia trachomatis* directly on urogenital samples. The serological techniques constitute an indirect diagnostic approach, whose interpretation in urogenital pathology is still problematic, and are only of interest in the presence of certain clinical indications (upper urogenital tract infections in men or women).

SYMPTOMS

The clinical forms are frequently asymptomatic and delayed diagnosis may result in irreversible damage, particularly in women (sterility) and chronic damage (manifestations in the joints).

Urogenital tract infections

Lower urogenital tract infections in men: subacute urethritis producing a non-purulent discharge, sometimes with a burning sensation when urinating. Asymptomatic forms represent approximately 50 to 90 % of cases.

Lower urogenital tract infections in women: cervicitis with whitish, greenish or yellow leukorrhea, although it may be totally asymptomatic in 50 to 90 % of cases.

- **In adults,** infection by *Chlamydia trachomatis* can also affect epithelial cells of the rectum or conjunctiva (anorectitis and conjunctivitis). Trachoma, the most common form in developing countries, is a chronic keratoconjunctivitis of immune origin which eventually leads to blindness. Transmission is through dirty hands, dust and flies.
- **In the newborn baby,** infection by *Chlamydia trachomatis* can be responsible for conjunctivitis and pharyngitis and may affect the epithelial cells of the lower respiratory tract.

SEARCH INDICATIONS

Screening or clinical suspicion of upper or lower urogenital tract infections in men or women.

Suspicion of an ocular infection or pneumopathy in a newborn baby.

INFORMATION

SAMPLE

Urogenital tract samples: Male urethral samples are taken by deep, insistent swabbing of the endourethral mucosa, at a distance of 3 to 4 cm from the meatus. Endocervical samples are collected using a speculum, after careful removal of secretions present at the cervical orifice. A plastic swab or a brush inserted into the endocervical canal allows endocervical epithelial cells to be scraped away. In women, systematically combining this with urethral sampling, even in the absence of urethral symptoms, increases the probability of diagnosis.

Collecting the first jet of urine: This type of sampling only permits a search for chlamydial antigens or nucleic acids and is not suitable for identifying *Chlamydia trachomatis* by culture. In women, the first jet of urine is not a substitute for endocervical swabbing.

Other samples: Other types of sample may be dictated by the clinical circumstances (endometrium or Fallopian tube biopsy, conjunctival, pharyngeal or rectal swabs, sperm collection, epididymial puncture, inguinal lymph node puncture, or peritoneal liquid).

Serum: Serum or plasma (EDTA or citrate), freshly decanted.

CONDITIONS OF STORAGE AND TRANSPORT

Urogenital tract samples collected for cell culture purposes necessitate strict conditions of storage and transport, in order to preserve the infectious capability of the chlamydial elementary bodies. Placed in a transport medium (2-SP) and transported at 4° C, the samples must be inoculated for cell culture within 24 hours.

When searching for antigens or nucleic acids in urethral or endocervical samples, swabs discharged into a 2-SP medium are stored at 4° C and transported within 48 hours. For longer periods of storage, it is preferable to freeze the sample at -20° C or (better) -80° C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

Direct diagnosis of *Chlamydia trachomatis* infections of the urogenital tract is based on:

– Either a search for bacterial bodies (cellular inclusions) after cell culture

– or the detection of bacterial antigens by direct immunofluorescence or by enzymoimmunology

– or detection of the bacterial genome by using the methods of molecular biology.

- **Cell culture:** The isolation of *Chlamydia trachomatis* in cell cultures (using HeLa 229 or McCoy cell lines) continues to be the reference diagnostic method. Although the specificity of isolation in cell cultures is high (100%), sensitivity varies from 80% to 100%.

- **Antigen detection:** Finding chlamidial antigens in smears, using polyclonal or monoclonal antibodies specific to the *Chlamydia trachomatis* species or to the *Chlamydia* genus, using immunofluorescence techniques with detection sensitivity of 80% to 90% and specificity of 98-99% relative to the culture. Certain rapid EIA tests present lower sensitivity relative to the culture.
- **Molecular methods:** The identification of nucleic acid sequences specific to *Chlamydia trachomatis* (ribosomal RNA or plasmidic DNA) in samples uses simple hybridisation methods, without a previous amplification stage, or genetically-engineered amplification (PCR [polymerase chain reaction], LCR [ligase chain reaction], TMA [transcription mediated amplification], SDA [strand displacement amplification], or TaqMan™ real-time PCR). The sensitivity of molecular methods is higher than that of cell culture (in excess of 95%), while specificity approaches 100%. There is however no commercially-available kit that is capable of assessing the number of bacteria present in the analysis sample. For endocervical and urethral samples and urine, molecular methods based on genetically-engineered amplification *in-vitro* have high specificity and better sensitivity than enzymoimmunological methods and cell culture.

  Molecular methods, because of their known performance in terms of sensitivity, specificity and the possibility of using them on samples which are non-invasively collected (urine) or unsuitable for cell culture (sperm, urine and vulval or vaginal samples), are the techniques of choice for diagnosis and screening of *Chlamydia trachomatis* infections of the lower urogenital tract.

INDIRECT DIAGNOSIS

Indirect diagnosis is based on serological methods which reveal the presence of circulating antibodies.

In routine practice, the methods used are microimmunofluorescence (MIF) and enzyme-linked immunosorbent assay (ELISA). Microimmunofluorescence, the reference technique, detects and titres IgG, IgA and IgM antibodies directed against antigens of the *Chlamydia trachomatis* species and serotype. This method uses as an antigen, a suspension of the elementary bodies of the various serotypes of *Chlamydia trachomatis*. Certain ELISA kits use the Major Outer Membrane Protein (MOMP) (or a peptide specific to it) and are reputed to be specific to *Chlamydia trachomatis*.

The value of serodiagnosis is limited by the variability of responses from one individual to another, the weak antibody response in lower genital infections, and the existence of cross-reactions between different species in the case of MIF techniques. Seroconversion, which is the appearance of antibodies between an early sample and a later one, is rarely observed. However, a rise of an antibody titre of at least 4 dilutions between two samples may be observed, which would strongly suggest a deep active infection. The most frequently observed case is an elevated titre isolated in IgG. Although the observation indicates that the subject has been in contact with the organism, it does not in any way predict an active infection: certain individuals can retain a high IgG titre for years after the infection. Specific IgM's are only rarely observed, due to their fleeting existence, but their presence indicates a recent active infection. A search for IgM's is vital if pneumopathy due to Chlamydia is suspected in a new-born baby. Certain authors recommend searching for specific IgA's, which could be a better marker than IgM's for diagnosing active infections. Although it is generally accepted that IgA levels generally rise early in a deep Chlamydia trachomatis infection, the dynamics of falling IgA's seems to be too variable between individuals to attribute value to them in diagnosis or in recovery after treatment.

The interpretation difficulties in serodiagnosis of *Chlamydia trachomatis* infections are linked to the existence of cross-reactions, particularly with the Twar strain of *Chlamydophila pneumoniae*. In numerous epidemiological studies, the presence of antibodies against the Twar strain has given rise to over-assessment of serological results in urogenital pathology. This means that a serological diagnosis of *Chlamydia trachomatis* infection remains of limited interest in the present state of technology.

TREATMENT

Initial treatment of a urogenital tract infection is based on a single dose of azithromycin or on the administration of cyclins (doxycycline or minocycline) for one week. The disappearance of clinical signs after 7 days is a sign of recovery. The success or failure of treatment after a period of 5 weeks can be assessed by molecular methods (time necessary for the elimination of chlamydial nucleic acids).

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CHOLESTEROL
(HDL, LDL, VDL)

DEFINITION AND INTRODUCTION

Blood lipids comprise free cholesterol, esterified cholesterol, free fatty acids, phospholipids and triglycerides. 30% of cholesterol is of dietary origin, the remainder being endogenous through liver synthesis.

Cholesterol is carried in the bloodstream in the form of various lipoproteins: chylomicrons, VLDL, LDL and HDL.

Chylomicrons are the form in which dietary lipids (triglycerides and cholesterol) are transported. They contain 85-95% of triglycerides. Synthesised by the intestine after food is eaten, they are normally absent from the serum of a fasting subject.

Very Low Density Lipoprotein (VLDL) is the form in which triglycerides of hepatic origin are transported and corresponds to an intermediate form which will be catabolised by serous lipoprotein lipase (LPL) into Intermediate Density Lipoprotein (IDL), then by hepatic LPL into LDL.

Low Density Lipoprotein (LDL) is the form in which cholesterol is carried from the liver to the cells of the body. LDL is derived from VLDL and is rich in cholesterol. It possesses APOB and APOE proteins, of which the receptors are found in all cells of the body (APOB receptors) and in hepatic cells (APOE receptors). Excess LDL in the arteries oxidises and may be deposited in the form of atheromatous plaques. A high concentration of LDL cholesterol is a factor in cardiovascular risk.

High Density Lipoprotein (HDL), the form in which excess cholesterol is returned to the liver, is capable of capturing cholesterol at the surface of cells. HDL is rich in cholesterol and in apolipoprotein A-I. A high concentration of HDL-cholesterol is a factor for protection against cardiovascular risk.

Official recommendations in France are as follows: an initial lipids analysis should include a determination of concentrations of total cholesterol and triglycerides.

Cholesterol	Triglycerides	Consequences
< 2 g/l (5.1 mmol/l)	< 2 g/l (2.3 mmol/l)	Normal result No measurement of fractions No monitoring before age 45 in men and 55 in women.
> 2 g/l (5.1 mmol/l)	and/or > 2 g/l (2.3 mmol/l)	Measurement of fractions Monitoring after strict fasting.

If the patient is in a cardiovascular risk group, investigation of a lipids abnormality is recommended. A normal result should be followed up after three years, or after one year in diabetic patients. A pathological result must be confirmed in a second sample collected after 12 hours of strict fasting.

INDICATIONS FOR MEASUREMENT

Investigation for primary or secondary dyslipoproteinaemia.

Monitoring of dyslipidaemia.

Monitoring of cholesterol-reducing treatments (or of hypercholesterolaemic side-effects of certain drugs).

INFORMATION

SAMPLE

Total cholesterol measurement can be performed on serum or plasma (heparinised or EDTA). Measurements of the LDL, HDL and VLDL fractions are essentially carried out on serum.

Meals have little effect on total cholesterolaemia. In a context of lipids analysis, the measurements are generally carried out on a patient after a fast of 12 hours, because of the measurement of triglycerides (assessment of VLDL). Fasting is necessary for measuring the LDL, HDL and VLDL fractions.

The sample must be collected from a patient in a seated position. A standing position tends to raise cholesterol concentration through haemoconcentration. A recumbent position reduces the concentration by approximately 10%.

The cuff should not remain in place for too long: beyond 2 minutes, it causes a 5% rise in cholesterol concentration.

QUESTIONS FOR THE PATIENT

Pregnancy: term?

Habits: dietary, tobacco and alcohol?

Sporting activities?

Medication: corticotherapy, oestrogen, progestins and antiepileptics?

SAMPLE STORAGE AND TRANSPORT

Whole blood can be stored for one week at ambient temperature.

Serum or plasma can be stored at +4° C for one week and at – 20°C for 3 months.

ASSAY METHODS

– Total cholesterol: colorimetry, gas/liquid phase chromatography (reference).

– Measurement of LDL, VLDL and HDL cholesterol: gel electrophoresis. Official French recommendations are for HDL cholesterol to be measured by a direct method. If HDL-cholesterol is < 0.77 mmol/l (0.30 g/l), the Pathologist can check the result, by carrying out a measurement of apolipoprotein A-I. LDL-cholesterol is usually calculated by using the Friedewald equation:

$$LDL = CT - \left[HDL + \frac{TG\ (g/l)}{5} \text{ ou } \frac{TG\ (mmol/l)}{2,2} \right]$$

Where CT= total cholesterol and TG = triglycerides.

This formula is not applicable if TG > 3.4 g/l (3.9 mmol/l). The Pathologist may then, measure either apolipoprotein B or LDL-cholesterol by an automated direct enzymatic method.

Measurement of VLDL can only be done by gel electrophoresis.

NORMAL EXPECTED VALUES

Normal values depend on age, gender, circadian rhythms (lower at night) and diet.

Usual values of total cholesterol, regardless of age and gender, can be suggested: 4.10 to 6.20 mmol/l (1.60 to 2.40 g/l).

Indicative values according to age:

Age	Triglycerides	Total cholesterol	LDL-cholesterol	HDL-cholesterol
< 20 years	0.65 to 1.5 mmol/l	3.1 to 4.8 mmol/l 1.20 to 1.85 g/l	3.5 to 4.5 mmol/l	1 to 1.6 mmol/l
20 to 30 years		< 5.2 mmol/l < 2 g/l		
30 to 40 years		< 5.7 mmol/l < 2.2 g/l		
> 40 years	0.7 to 1.8 mmol/l	< 6.2 mmol/l < 2.6 g/l		1.1 to 1.8 mmol/l

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL AND IATROGENIC VARIATIONS

In view of the variability between individuals, the result obtained should be conformed in two or three samples.

Total cholesterol

– Increase of 0.50 mmol/l on average every 10 years from 30 to 60 years.

– Before the menopause, cholesterol concentrations are lower in women than in men. During the first weeks of pregnancy, cholesterolaemia can rise by more than 40%.

– Obesity, alcohol and certain medicines can influence cholesterolaemia, where levels are lower in the case of statins and fibrates, and increased in the case of oral contraceptives and the antiepileptics phenobarbital and phenytoin.

HDL-cholesterol

– Concentrations of HDL-cholesterol are higher in women before the menopause.

– Lowered by smoking, obesity and progestins.

– Increase: physical exercise (?), oestrogens and corticotherapy.

PATHOLOGICAL VARIATIONS

Hypocholesterolaemia

– Secondary hypocholesterolaemias are common: hepatic insufficiency, infectious diseases, digestive impairment (malabsorption), hyperthyroidism and malnutrition.

– Primary hypocholesterolaemias are more rare and usually caused by a mutation of one of the proteins involved in lipoprotein metabolism: Tangier disease (absence of HDL) and abetalipoproteinemia (absence of VLDL).

Hypercholesterolemia

Hypercholesterolaemia can be secondary to thyroid deficiency (myxoedema), nephrotic syndrome (with hypertriglyceridaemia), biliary cirrhosis, chronic or acute pancreatitis, an attack of gout, diabetes or medication (diuretics, progestins, corticoids and cyclosporin).

Hypercholesterolaemia may also be primary, although this is much more rare (familial or polygenic disorder).

Pure hypercholesterolaemia (Type IIa in the Fredrickson classification) is a dominant autosomal disorder with possible manifestations of tendinous xanthoma. Mixed hypercholesterolaemia (Fredrickson Type IIb) is a condition of genetic and environmental origin. LDL-cholesterol is elevated; HDL-cholesterol is raised if triglycerides are low.

Hypercholesterolaemias due to hyperHDLaemia are rare familial dominant autosomal disorders, associated with a protective effect for cardiovascular disease.

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CHOLINESTERASE

DEFINITION

Cholinesterase is an enzyme which catalyses the hydrolysis of acetylcholine into choline and an acetate group.

Two types of cholinesterase are found in humans:

– Cellular cholinesterase, with a strong specific enzymatic activity on acetylcholine and acetylbetamethylcholine. The latter is mainly found in erythrocytes and nerve cells.

– Plasma cholinesterase, with weak enzymatic activity (also known as pseudocholinesterase).

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

The physiological role of cholinesterase is a reduction of the activity associated with acetylcholine and its derivatives. Measurement is indicated whenever there is a suspicion of poisoning by organophosphates with a cholinesterase-inhibiting activity. Organophosphates are pesticides used in agriculture (diazinon, chlorpyrifos, parathion, malathion, etc.). They inhibit cholinesterase in cholinergic synapses and prevent the breakdown of acetylcholine. The excess of acetylcholine produces muscarinic and nicotinic effects (bradycardia, hypersalivation, diarrhoea and muscle paralysis). It also allows preoperative assessment of patient sensitivity to anaesthesia using curariforms (succinylcholine). In patients with reduced or non-existent cholinesterase activity (hereditary deficit), the curare-like effect persists and requires the use of controlled ventilation. Succinylcholine doses can then be reduced in these patients: at a level of 0.04 to 0.06 mg/kg, 90% of neuromuscular blocking can be obtained.

INFORMATION

SAMPLE

Serous cholinesterase: heparinised serum or plasma (do not use oxalates or fluorides).

Globular cholinesterase (erythrocytes): whole blood EDTA.

QUESTIONS FOR THE PATIENT

Existence of known hepatic or neuromuscular impairment?

Existence of prolonged curare-like effects during anaesthesia or documented congenital deficit?

Possibility of poisoning by organophosphate insecticides?

Medication: cholinesterase inhibitors:

– Carbamates.

– Rivastigmine, used in the treatment of Alzheimer's disease.

SAMPLE STORAGE AND TRANSPORT

When serous concentration of cholinesterase is to be measured, centrifuge and decant the serum rapidly after collection.

Transport at +4° C.

ASSAY METHODS

Enzymatic measurement of cholinesterase activity.

If a deficit of genetic origin is suspected, use molecular biology.

NORMAL EXPECTED VALUES

Indicative levels:

– Serous cholinesterase: 1900 to 3800 UI/l.

– Globular cholinesterase (erythrocytes): 24 to 36 UI/g of haemoglobin.

PATHOLOGICAL VARIATIONS

A reduction in cholinesterase activity may be linked to:

– Impaired liver function (viral hepatitis, cirrhosis, hepatocellular carcinoma or severe liver failure).

– Anaemia, malnutrition or neuromuscular impairment (neuromuscular syndrome and dermatomyositis).

– A congenital deficit (often revealed by prolonged curare-like effects during general anaesthesia).

– Organophosphate poisoning: an observed reduction in serous activity is followed by a reduction in globular activity.

Increased activity is observed in cases of nephritic syndrome.

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CHROMIUM

DEFINITION

Chromium is a silvery-white metal with high resistance to wear. Its name comes from the Greek "*chroma*", meaning "colour", of which is a reference to the fact chromium compounds have various colours.

Trivalent chromium (Cr^{3+}) is an essential trace element for sugar metabolism. Chromium deficiency in humans results in glucose intolerance. Chromium is found particularly in spices (thyme and black pepper), brewer's yeast, liver and egg yolk.

In its hexavalent state (Cr^{6+}), chromium is a powerful oxidant, toxic for the body. Chromium is mainly used in making corrosion-resistant metal alloys (stainless steel), chromium plating, lithography, the textile industry, printing, photography, paints, tanning, and synthesis processes (polymerisation, oxidation of hydrocarbons, aldehyde and ketone production). Traces of chromium are present in many products (cement, bleach and magnetic tapes) and can cause skin allergies. It is also found in tobacco smoke.

METABOLISM

Trivalent chromium is an essential trace element and is present in body tissues. The absorption of chromium depends on solubility and valency: trivalent derivatives are absorbed to a lesser degree than hexavalent compounds, which can penetrate the body by any route. Occupational absorption is mainly respiratory and cutaneous, but can also be digestive (poor hygiene).

Chromium transported in the bloodstream is fixed to transferrin, and may bind non-specifically to other proteins.

Chromium VI distributes to the liver, kidneys, spleen and lungs. It can pass through biological membranes and then be reduced to the highly reactive chromium V, and finally to chromium III in all tissues. In particular, it penetrates red blood cells, which then retain it throughout their lifetime in the form of chromium III.

Excretion is essentially urinary in the form of chromium III, with low levels of faecal excretion. Elimination occurs in 2 stages, depending on where the chromium is stored. The half-life for rapid elimination is 7 hours, or from 2 weeks to several months for slow elimination. Chromium accumulates throughout the week, or throughout the year in the most exposed subjects.

MECHANISM OF ACTION

Its action in carbohydrate metabolism is due to its potentiation effect on insulin (a role which has been demonstrated in animals). Chromium is also believed to play a part in the metabolism of lipids, through the same mechanism.

CLINICAL SIGNS OF INTOXICATION

■ ACUTE INTOXICATION

The ingestion of chromium salts results in massive inflammation of the digestive tract, with necrosis, from the mouth to the jejunum. This can rapidly lead to death through collapse (the lethal oral dose is estimated to be from 1 to 3 g). If the outcome is not fatal, the situation evolves towards hepatic and renal necrosis.

■ CHRONIC INTOXICATION

The principal signs of long-term exposure are the following:

– ***Cutaneous symptoms*** showing eczematous dermatitis, of which is mainly found on the forearm ("chromium bracelet"). This is common in subjects exposed to cement. Ulcers may present, of which are referred to as "chromium holes".

– ***Irritation of the mucosae,*** resulting in atrophy of the nasal mucosae, bronchitis, oesophagitis and gastritis.

– ***Bronchial cancers:*** this risk is thought to be mainly caused by soluble chromates such as those of calcium, strontium and zinc.

INDICATION FOR MEASUREMENT

Chromium measurements are used in occupational medicine for the purposes of monitoring exposed workers.

The level of intraerythrocytic chromium reflects the intensity of exposure to chromium VI (the most toxic form) during the lifetime of the erythrocytes. Its use is restricted to the monitoring of exposed subjects.

Chromuria, in contrast, in a sample collected at the end of a working shift, is the best indicator of recent exposure to chromium VI, and also probably to chromium III. In cases of heavy exposure, chromuria also reflects chronic exposure and sampling at the beginning and end of a shift allows a good assessment of exposure during the day.

After heavy exposure, chromuria may remain more elevated than in the general population, even for several months after exposure has ceased.

INFORMATION

■ SAMPLE

5 ml of heparinated whole blood; 20 ml of non-acidified urine.

Whole blood: sample collected at the end of a shift and the end of a week.

Urine: sample collected at the beginning or end of a shift and at the end of a week.

■ QUESTIONS FOR THE PATIENT

Enquire about the consumption of beer before sample collection, and the use of tobacco.

■ SAMPLE STORAGE AND TRANSPORT

Samples of whole blood can be stored and sent to the laboratory at ambient temperature. Samples of urine can be stored and sent to the laboratory at ambient temperature or between +2° C and +8° C.

ASSAY METHODS

Electrothermal atomic absorption spectrophotometry (graphite oven) and Zeeman correction.

REFERENCE VALUES

Reference values in the general population

Serous chromium: < 0.50 µg/l

Total blood chromium: < 1.00 µg/l

Urinary chromium: < 1.00 µg/g of creatinine.

For exposure to chromium VI, fumes or water-soluble aerosol

Total permissible urinary chromium = 30 µg/g of creatinine at the end of a shift and the end of a week (10 µg/g rise in creatinine during a shift).

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CHROMOGRANIN A

DEFINITION - INTRODUCTION

The chromogranins (chromogranins A, B and C) are soluble acid proteins contained in the secretory granules of neuroendocrine cells and secreted by tumours derived from these cells. Chromogranin A (CgA) is ubiquitous throughout neuroendocrine tissues and is co-expressed by polypeptide neurotransmitters and hormones, particularly the catecholamines. The chromogranins are involved in various intra and exracellular biological processes, although their chemical role is still poorly understood. Within the cell they are involved in the regulation of the storage and secretion of hormones and peptides. Outside of the cell they are understood (after cleavage) to be precursors of different hormone components (pancreastatin, chromostatin or prochromacin with anti-bacterial activity).

Synonym: *CgA, Protein Secretory I.*

INDICATIONS FOR MEASUREMENT

Serum CgA is a laboratory marker used for the diagnosis and monitoring of treatment of neuroendocrine tumours, particularly those located in the gastro-intestinal or bronchial tract, pheochromocytomas and thyroid medullary cell cancers. It is also useful in the prognosis and monitoring of prostatic tumours with a neuroendocrine component (hormone-resistant tumours).

INFORMATION

SAMPLE

Serum (dry tube); haemolysed samples should be rejected.

A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Type of tumour and current treatment: chemotherapy, radiotherapy and surgery (types and date of treatment).

Are you being treated with a proton pump inhibitor (omeprazole, etc.), an H2 anti-histaminergic agent (ranitidine) or a corticosteroid? These medicines increase serum CgA concentrations.

SAMPLE STORAGE AND TRANSPORT

Store <3 hours between + 2 and + 8°C; – 20°C beyond this time.

Send frozen at –20°C.

ASSAY METHODS

Radioimmunometric method.

NORMAL EXPECTED VALUES

Usual serum values are between 20 and 100 ng/ml. These may vary depending on the method used.

PATHOLOGICAL VARIATIONS

Non-specific rises

– Renal insufficiency: serum chromogranin A concentrations are generally proportional to the extent of renal insufficiency. It cannot be used as a marker in patients with renal insufficiency, although when the serum CgA concentration is normal the patient can be reassured that they do not have a neuroendocrine tumour.

– Achlorydria: chronic atrophic gastritis or treatment with proton pump inhibitors (omeprazole, lansoprazole, pantoprazole and rabeprazole) or H2 anti-histaminergic agents (ranitidine, cimetidine, famotidine and nizatidine) significantly increases serum chromogranin A concentrations.

– Corticosteroid treatment: moderate increase in serum chromogranin A concentrations (generally < 2 times the upper limit of normal).

Increase in neuroendocrine tumours or tumours with a neuroendocrine component

– Gastro-entero-pancreatic neuroendocrine tumours (neuroendocrine tumours of the stomach, proximal duodenum, biliary tract, distal intestine, pancreas, gastronoma, insulinoma, glucagonoma, somatostatinoma, VIPoma or PPoma).

–Neuroendocrine tumours of the bronchial tract.

–Tumours derived from neural crest cells: pheochromocytoma, paraganglioma, neuroblastoma and thyroid medullary cell carcinoma.

–Tumours with neuroendocrine differentiation: advanced prostatic tumours.

<u>Neuroendocrine tumours of the gastro-entero-pancreatic (GEP) tract</u>

Numerous authors have reported raised chromogranin A concentrations in patients suffering from these tumours characterised by secretion (with increased urinary concentration) of 5hydroxyindolacetic acid (5HIAA; cf. corresponding chapter). Although chromogranin A is not specific for carcinoid tumours, it can be a sensitive marker for early diagnosis of these diseases. In patients with carcinoid syndrome (flushing, diarrhoea and cardiovascular disorders) who have "borderline" urinary 5HIAA concentrations, a normal serum chromogranin A concentration offers evidence in favour of a non-endocrine cause.

Some authors have also reported a relationship between serum chromogranin A concentrations and tumour mass: some false negative results may therefore be obtained in small tumours.

Highest serum chromogranin A concentrations are seen in patients suffering from pancreatic carcinoid tumours. In diagnostic terms, CgA is the most sensitive and specific laboratory marker for these tumours and its concentration correlates with extension of the disease. It is also used for postoperative follow-up and to assess the response to medical treatment.

<u>Pheochromocytoma</u>

CgA is a sensitive (sensitivity = 90%) and specific (specificity = 92%) marker for the diagnosis of pheochromocytoma and according to some authors is a better marker than urinary catecholamines or their catabolites.

Serum CgA concentration is useful to confirm (at high values), but particularly exclude (normal CgA) pheochromocytoma. In practice, it is an excellent marker to exclude chromaffin cell disease in patients suffering from hypertension.

Small cell lung cancer

CgA is reported to be a better marker than NSE in patients suffering from this type of cancer (diagnostic sensitivity of 61% vs 57% for NSE), particularly in non-advanced disease. It is also only raised in 14% of cases of non small cell lung cancer compared to 22% for NSE (improved specificity). In practice, its measurement may be combined with the measurement of NSE in this situation.

Medullary thyroid cell carcinomas (MTC)

Serum CgA concentrations are raised in patients suffering from these tumours, with the highest values being seen in metastatic disease. It is not, however, a good diagnostic or follow-up marker for MTC and it is not widely used in this situation (calcitonin is the laboratory marker of choice).

Non-neuroendocrine tumours

Some cancers which are not derived from neuroendocrine cells may contain neuroendocrine components, of which applies particularly to some breast and prostate cancers. Whilst studies conducted in breast cancer have concluded that CgA is of no use, the situation is different in prostate cancers.

Prostate cancers containing neuroendocrine differentiation are more aggressive and are usually associated with a poor prognosis. A relationship between raised serum CgA concentrations and progression of prostate cancer has been found in many studies. Some authors have also shown that patients with high serum CgA concentrations have a shorter survival rate than those with normal CgA. The progression of prostate cancer can be described schematically as two successive phases: hormone-dependent followed by hormone-resistant. In cancers which respond to hormone treatment, a rise in serum CgA concentrations is seen in 7 to 15% of patients, compared to 48 to 71% (versus 28 to 30% for NSE) in advanced hormone-resistant cancers. Generally, CgA is found to rise in more patients than NSE prostate cancer monitoring (64% vs 24.4%). In practice, a rise in serum CgA concentrations in patients suffering from advanced or relapsed prostate cancer is a poor prognostic indicator and suggests hormone resistance. Whilst no specific treatments are currently available for cancers with neuroendocrine differentiation, clinical trials are underway with the expectation of new treatments, and patients with raised serum CgA may benefit from preventative radio-isotopic treatment against bone metastases.

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CIRCULATING ANTICOAGULANT - LUPUS ANTICOAGULANT

DEFINITION

The circulating anticoagulants (CAC) are acquired coagulation inhibitors. They can be divided into two groups, which have different clinical expressions:

– Antibodies directed specifically against a coagulation factor (anti-factor antibodies) predisposing to bleeding (see corresponding section).

– Non-specific CAC directed against a coagulation phase. The commonest of these are the lupus anticoagulants (named as such as they were initially discovered in patients with systemic lupus erythematosus); these predispose to a risk of thrombosis. These immunoglobulin antibodies belong to the large family of anti-phospholipid antibodies (APL). The pathogenic antibodies persist and are found in the plasma of patients for more than three months. Transient non-specific CAC are also occasionally found in plasma of patients of all ages with inter-current infection. These CAC are not associated with any risk of thrombosis or bleeding.

Synonyms: circulating lupus (or lupus type) anticoagulants (CAC), lupus anticoagulant (LA), anti-prothrombinase antibody (former name).

INTRODUCTION

The APL family includes a set of heterogeneous auto-antibodies (Ab) grouping together with the LA, detected from prolongation of phospholipid-dependent coagulation tests and other Ab, mostly anti-cardiolipin or ACL and the anti-β2 glycoprotein I Ab (anti- β2GPI), detected immunologically (by ELISA). They were initially called "anti-phospholipid" (APL) antibodies as their targets were found to be different negatively charged phospholipid antigens: cardiolipin and phosphatidylserine etc. In reality the antibodies are directed against proteins, which have affinity for anionic phospholipids, i.e. β2GPI, prothrombin and annexin V. Considerable clinical evidence and animal models indicate that the APL are pathogenic.

The APLS is an autoimmune disease characterised by APL in the plasma of patients who suffer recurrent vascular thromboses and/or obstetric complications. The diagnostic criteria for patients with APLS were produced by international consensus at Sapporo in 1999 (Wilson WA, 1999), and then revised in Sydney in 2005 (Miyakis S, 2006). APLS is defined by the presence of **at least one clinical and at least one laboratory criteria** out of the following:

Clinical criteria	**1.** Vascular thrombosis: At least one clinical episode of arterial venous or small vessel thrombosis in any territory. The thrombosis must be confirmed by imaging, Doppler ultrasound or histology except for superficial venous thromboses. When histological confirmation is performed the thrombosis must be present in the absence of significant signs of inflammation of the vessel wall.
	2. Obstetric disease: ***(a)*** At least one unexplained fetal death of a morphologically normal fetus during or after the 10th week of pregnancy (normal fetal morphology must be confirmed on ultrasound or by direct fetal examination) or ***(b)*** At least one premature birth of a morphologically normal newborn baby at or before 34 weeks of pregnancy because of severe pre-eclampsia, eclampsia or severe placental failure or ***(c)*** At least three spontaneous miscarriages before the 10th week of pregnancy with no anatomical or maternal hormonal abnormality and no maternal or paternal chromosomal abnormality.
Laboratory criteria	**1.** The presence of anti-cardiolipin or anti-β2 glycoprotein I IgG and/or IgM isotype antibody at moderate or high titres in at least one of two samples taken at least 12 weeks apart measured using a standardised ELISA method demonstrating anti-cardiolipin β2-GPI dependent antibodies
	2. The presence of lupus circulating anticoagulant found in at least one of two samples taken at least six months apart under conditions defined by the Société Internationale d'Hémostase et Thrombose *(cf. below)*

International consensus for the classification of APLS from Wilson et al, (Arthritis Rheum 1999; 42: 1309-1311) revised by Miyakis et al, (J. Thromb. Haemost. 2006; 295-306).

Identification of APL is an essential stage of the diagnosis of APLS and is based on three different tests:

– A phospholipid-dependent coagulation test to identify lupus anticoagulants or LA,

– An ELISA test identifying the ACL,

– An ELISA test identifying the anti-β2GPI Ab

These tests are different but not independent and do not all have the same clinical significance. ACL mostly recognises β2GPI and the LA group together the anti-β2GPI Ab and the anti-prothrombin Ab. Clinically, there is considerable evidence suggesting that the anti-β2GPI are pathogenic and several studies have clearly shown that the coagulation test which identifies LA is the test of choice to identify clinically significant APL, particularly DRVVT. LA+ and anti-β2GPI+ patients are more at risk of recurrent thrombosis than LA+ or anti-β2GPI+ patients.

Outside of the APLS, lupus anticoagulants can be seen in various clinical circumstances including lymphoproliferative disease, malignancy, infectious diseases (syphilis, HIV and acute viral infections), some drugs and even in people without any disease (particularly the elderly and young children).

They may be transient, particularly in infections (especially in children) in which case they are not thrombogenic or permanent when they are associated with a significantly increased risk of thrombosis and/or obstetric complications.

SEARCH INDICATIONS

Measurement of LA must be relevant, i.e. restricted to patients with significant likelihood of having APLS:

– Low: venous (VT) or arterial (AT) thrombosis in the elderly,

– Moderate: fortuitous discovery of prolonged APPT in an asymptomatic person, provoked venous thrombosis in the young, early spontaneous miscarriage < 10 weeks of pregnancy,

– High: unexplained VT or AT in the young (< 50 years old). Thrombosis at an unusual site, late fetal loss (> 10 weeks of pregnancy), any thrombosis or obstetric complication occurring in a context of autoimmune disease.

If the APL is positive the patient is at high risk of recurrent thrombosis or obstetric complication and therefore requires long term anti-vitamin K (AVK treatment) and hence exposure to a risk of bleeding. Caution is required therefore to avoid excessive diagnoses.

INFORMATION

These have been precisely defined and the results of the test depend on their being strictly observed.

■ SAMPLE

Samples should if possible be taken when the patient is not on anticoagulant therapy. Plasma is obtained from blood collected into a citrate tube (0.109 M or 0.129 M). Blood can also be collected into a CTAD (citrate, theophylline, adenine, dipyridamole) tube, which provides for better sample storage (to be preferred when the tube transport time is more than 2 hours and less than 3-4 hours). No other anticoagulant may be used.

Tests on frozen-thawed plasma samples are permitted if the sample is centrifuged twice in order to obtain a platelet poor plasma (PPP < 10 G/l platelets), i.e. after centrifuging the primary tube for 15 minutes at 2000 g at room temperature and then centrifuging the plasma obtained from the first sample a second time for 10 minutes at over 2500 g, at room temperature and then freezing at ≤ -70°C, thawing in a water bath at 37 °C, for no more than 5 minutes.

Plasma filtration (0.22 µm filters) is not recommended as this can damage other coagulation proteins (FVIII) and artificially prolong the APPT. Ultra-centrifugation is also not recommended as this may fragment platelets and expose PL which neutralise the APL.

A fasting sample is not required and a light low fat snack is permitted.

■ QUESTIONS FOR THE PATIENT

Clinical context: past history of venous and/or arterial thrombosis or repeated foetal loss?

Are you taking any of the following drugs?

– Unfractionated heparin, low molecular weight heparin, hirudin and derivatives, dabigatran, rivaroxaban (cf "Interpretation" section).

– Phenothiazines, certain antibiotics, and interferon alpha: lupus anticoagulant has been described in people taking these treatments.

■ SAMPLE STORAGE AND TRANSPORT

Maximum time before testing: 2 to 4 hours at laboratory temperature (do not store at + 4°C, because of the risk of activating factor VII). If the test is to be performed later separate the platelet poor plasma and store at -20°C for up to 3 weeks or at – 70°C beyond this time.

Transport: PPP, frozen at – 20°C within 2 hours of sampling.

TESTING FOR IDENTIFYING LUPUS ANTICOAGULANT

SSC/ISTH CRITERIA: 2009 RECOMMENDATIONS *(*Pengo V, *Thromb Haemost)*

1- Screening tests

It is recommended that two tests be used (no more, no less), based on different principles.

- Russell viper venom test (dRVVT) 1st line because of its specificity for β2GP1-dependent LA. By directly activating FX, this short - circuits FVII, the contact phase factors, FVIII and FIX.

- Sensitive APTT using silica as the activator and low phospholipid concentration.

The following tests are not (or are no longer) recommended: APTT with kaolin or ellagic acid activator (relatively insensitive), the dilute thromboplastin time (variability between thromboplastins), tests using snake venom such as ecarin or textrarin (lack of well standardised commercial test), and the KCCT (poor reproducibility).

2- Mixture test

The aim is to demonstrate lack of correction of the coagulation time if the prolongation is due to an inhibitor, although caution is required in low LA (dilutional false negatives)

The mixing test uses a pool of normal plasma, the preparation of which has been specifically validated for LA testing either prepared in the laboratory (after double centrifugation) or commercial, frozen or lyophilised. In the latter situation, the supplier's specifications (normal titres of factors, absence of platelets) should be checked. An equal volume mixture is prepared without pre-incubation (false positives have been described due to a rise in pH at 37°C).

3- Confirmatory test

This is performed by increasing the concentration of PL in the abnormal screening test or using hexagonal phase PL.

The role of "integrated" APPT or dRVVT tests

These involve performing a screening test (low PL concentration) in parallel with a confirmatory test (high PL concentration) and does not in principle require the mixing test. These are increasingly offered by Companies, although their use remains contentious.

Is the mixing test still useful?

This question has been raised because of the possibility of integrated tests and the risk of failure to detect low LA. The mixing test remains useful when the confirmatory test does not correct the abnormal screening test, particularly in patients on AVK or with factor deficiency or an anti-factor Ab or in the specific case of very potent LA, which are not neutralised by confirmatory test phospholipids.

INTERPRETATION

Calculation of cut-offs

These must be defined from results obtained in a population of healthy people (at least 40 people under 50 years old), locally for a reagent-analyser couple (do not use predetermined cut-offs).

The cut-off is the 99th percentile (i.e. the level at which 1% of the "normal" population has a result above the cut-off), rather than using mean ± 2 standard deviations (in which case 2.5% of the normal population will have results > cut-off) for each of the tests used.

The cut-offs should be determined for the screening test from the coagulation time of the patient (P) or the ratio P+C/C; for the mixing test from the coagulation time P+C or the P+C/C ratio, or a correction index can be calculated (which is different from the Rosner index): [((C+P)- C)/P] x 100.

The mean percentage individual correction should be calculated in the confirmatory and integrated tests:

A- [(screening time– confirmatory time)/screening time] x 100

B- Screening time/confirmatory time ratio

C- Standardised ratio (reduces between batch variability = (screening time P/screening time normal plasma (NP))/(confirmatory time P / confirmatory time NP).

Any positive results should be confirmed within 12 weeks of the first test and interpreted against the patient's full APL profile (patients are at high risk of APLS if an LA is associated with another APL). Beware of current anti-coagulant treatments and tests performed in the acute phase of a thrombotic episode (interference of CRP, risk of false positives).

Sensitivity to anti-coagulant therapy

- Testing for LA on unfractionated heparin: some commercial reagents for the dRVVT or APTT contain a heparin inhibitor (polybrene or heparinase) at a titre of up to 0.6 to 0.8 U/ml (check that the thrombin time is normal).

- Patients on low molecular weight heparin (LMWH): it is recommended that LA be tested at least 12 h after the last dose (the interference depends on the anti-IIa/anti-Xa ratio of the LMWH).

- Patients on AVK, the test should ideally be performed 1 to 2 weeks after stopping the AVK. It is only recommended if the INR is < 1.5. If the INR is between 1.5 and 3, the LA test is acceptable providing that a mixing test is performed.

- Patients on anti-IIa (dabigatran), there is currently no answer. A recent article on rivaroxaban (Merriman 2011) showed that LA testing in treated patients produced many false positives. LA testing is not therefore recommended in patients on rivaroxaban.

Interferences

Anti-factor antibodies (anti-FVIII, anti-FV) can produce a false positive LA (although the clinical context is different). LA can also interfere with chronometric FVIII measurements (patient and control lines are not parallel) and therefore with the Bethesda unit titration. In this case a chromogenic FVIII assay is required.

Reporting of results

A report showing the quantitative results and an interpretation should be produced. Results should not be reported as "equivocal" or "borderline": a repeat test is preferred.

The risk of venous thrombosis associated with lupus anticoagulant (persisting for at least 2 months) is increased by a factor of 6 to 12 compared to the general population depending on the clinical situation. The diagnosis of APLS can only be made from persistence of lupus anticoagulant retested 12 weeks later. The current consensus is that patients with APLS should be treated with an anti-vitamin K agent with a target INR of 2.5 (acceptable range 2 to 3).

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CIRCULATING IMMUNE COMPLEXES

DEFINITION

Circulating immune complexes (CIC) are antigen-antibody aggregates of varying size, bound non-covalently to the corresponding antigens. They are formed as a result of classical immune system protective reaction to the introduction of a foreign antigen. CIC are normally removed rapidly by the macrophage system. In some pathological situations the CIC are incompletely removed and are deposited on the vascular endothelium causing damage, in particular by activating the complement system. High CIC concentrations are found in different pathological, autoimmune, infectious and neoplastic situations.

INTRODUCTION

The nature, size and concentration of antigen and antibody in the immune complex, influence its capacity for removal and therefore its pathogenic potential. The antigens involved may be exogenous (whole micro-organisms, toxins, viruses and allergens) or endogenous (rheumatoid factor, circulating proteins or enzymes). The complement system and its erythrocyte CR1 receptors are involved in removal of CIC and activation of classical pathway proteins enables the complexes to be dissolved before being carried by erythrocyte CR1 receptors to the phagocyte cells. Persisting CIC may be due to complement C2 or C4 protein deficiency often seen in systemic lupus erythematosus or to a reduced density of erythrocyte CR1 receptors which can be seen in different situations (systemic lupus erythematosus, rheumatoid arthritis, blood dyscrasias, etc.). CIC deposition on tissues can activate the complement and trigger a series of destructive events.

INDICATIONS FOR MEASUREMENT

Investigation of a CIC-related disease:

– Systemic lupus erythematosus.

– Rheumatoid arthritis.

– Glomerulonephritis.

– Neoplasia (Hodgkin's lymphoma, leukaemias).

– Bacterial, parasitic and virus infections.

Their presence is relatively non-specific and does not suggest any particular diagnosis. They are therefore of very limited use.

INFORMATION

SAMPLE

1 ml of serum. Discard lipaemic, haemolysed or icteric samples.

SAMPLE STORAGE AND TRANSPORT

The samples can be stored for 7 days at between + 2 and + 8°C. Beyond this time store at – 20°C.

ASSAY METHODS

Different methods are available based on the physico-chemical or biological properties of the CIC. Methods based on binding of CIC to complement fraction C3 (Raji cell methods or conglutinin test) revealed by a radiolabelled antiglobulin are no longer used.

Current tests use the property of CIC to bind to C1q via the Fc fragment of the immunoglobulins. The wells of an ELISA micro-titre plate are coated with C1q, to which the CIC present in the sample bind. Binding of the complex can be revealed by adding an enzyme labelled anti-globulin or by adding cells labelled with protein A or labelled monoclonal rheumatoid factors.

Results are expressed in µg/ml of aggregated immunoglobulin equivalents against a standard curve prepared with heat-aggregated immunoglobulins. Normal values vary depending on the kit used.

INTERPRETATION

Raised CIC concentrations can be seen in many pathological, autoimmune and infectious situations and are therefore very non-specific for a particular disease. CIC testing can guide the clinical diagnosis in precise situations.

FOR FURTHER INFORMATION

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CITRIC ACID

DEFINITION

Citric acid (or 3-hydroxypentanedioic acid-3-carboxylic acid) as its citrate ion is an essential compound in cellular energy metabolism. Most of it is generated from glucose although other metabolic pathways can generate citrate and more comes from ingested food. It is an organic acid which, in semen, is a marker for prostate secretory activity. It is also found in the urine. It is used by the kidneys to regulate pH. Most citrate filtered by the glomeruli is reabsorbed in the proximal tubule. Its main role is to chelate cations.

INTRODUCTION

- **Seminal fluid**

  A full semen biochemical work-up includes assays of the following markers: Fructose (produced by the seminal vesicles), carnitine, α1-4 glucosidase (produced by the epididymis), citrate, acid phosphatase and zinc (produced by the prostate). Such a work-up has two purposes: to check the condition of the genital system and, if there is a problem, to identify its focus. Citrate is the main anion in seminal plasma.

- **Urine**

  Fluctuations in the citrate concentration in the urine reflect cellular citrate needs and reveal acid-base balance problems. Abnormally low levels are seen in acidosis and abnormally high levels in alkalosis.

INDICATIONS FOR MEASUREMENT

- **Seminal citrate:**

  An investigation of male fertility used as a second-line test in a patient with azoospermia, oligospermia or asthenospermia at semen analysis.

- **Urinary citrate:**

  Distal renal tubule acidosis complicated by renal lithiasis, usually with calculi composed of calcium phosphate. Many factors are involved in the pathogenic mechanism underlying calculus formation, including high levels of calcium and phosphate in the urine, high urinary pH, and low citrate excretion.

- **Serum citrate:**

  Rare with no current application.

INFORMATION

SAMPLE

Sperm is collected in the laboratory by masturbation, into a sterile recipient which is then kept at 37°C for 20-30 minutes to liquify the fluid. The sample is then centrifuged to remove the spermatozoa from the seminal plasma which is then frozen and kept at -20°C until the testing.

24-hour urine collection without acidification but with a preservative (to prevent citrate breakdown by bacteria): Sample can be stored for 1 week at +4°C.

Serum (Dry tube): Sample can be stored for 1 week at +4°C.

ESSENTIAL INFORMATION

The subject should have abstained from sexual relations for 3-5 days before providing the sperm sample in order to avoid variations in certain parameters which depend on this interval (notably the sperm concentration).

SAMPLE STORAGE AND TRANSPORT

Sperm should be tested immediately after liquefaction or within one hour of collection.

Urine and serum samples can be kept at +4°C for up to one week.

ASSAY METHODS

Enzymatic method based on citrate lyase: Citrate is the first substrate in a cascade of enzymatic reactions leading to the formation of NADPH, the concentration of which is measured as an end point (by UV spectrophotometry at 340 nm).

NB: protein in the seminal plasma has to be removed using perchloric acid and then the sample has to be neutralised before testing.

NORMAL EXPECTED VALUES

Concentrations can be expressed in mg/l or µmol/l (µg x 5.21 = µmol).

Serum: 25-135 mg/l

Urine: 175-650 mg/24h

Sperm: 2-8 mg/l

PATHOLOGICAL VALUES

Sperm

Prostatitis: Citrate decreased.

Cancer of the prostate: Citrate increased.

Prostate palpation: Citrate increased

Urine

The most markedly reduced citrate concentrations are seen in congenital and acquired forms of distal renal tubular acidosis. Moderately reduced concentrations are often seen in patients with kidney stones.

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CLOSTRIDIUM BOTULINUM
(Toxin testing)

DEFINITION

Testing biological fluids and food for the toxin responsible for causing the disease known as "botulism". The toxin is also called "botulinum neurotoxin" (BoNT). It is tested for in a context of community food poisoning or in the prevention of biological terrorism. Botulinum toxin is currently also now used for therapeutic purposes.

INTRODUCTION

BACTERIUM AND NEUROTOXIN

Botulism is a disease caused by *Clostridium botulinum*, an obligate anaerobic Gram positive bacillus. The bacteria are ubiquitous, found throughout the earth and in water and have highly resistant spores. A very potent neurotrophic toxin is secreted during bacterial growth (lethal dose 1 ng/kg), which is very rapidly inactivated in 5 minutes at 85°C. Different serotypes of *Clostridium botulinum* produce different neurotoxins: A, B, C-alpha, D, E, F and G. Human botulism is generally caused by the A, B, D and E toxins.

The botulism neurotoxins are peptides consisting of two chains with a molecular weight of 150 kD. They are zinc metalloproteinases which block the release of neurotransmitters into the cholinergic synapse. The toxin circulates systemically in the blood before being internalised by neurones and irreversibly blocking cholinergic transmission.

EPIDEMIOLOGY AND CONTAMINATION

The disease is rare but ubiquitous.

- ***Indirect exogenous contamination*** (food botulism): The food eaten contains the preformed toxin. The foods include preserves from small-scale specialist producers, particularly foods of low acidity, with high water content and low preparation temperatures (< 120°C). The foods usually responsible are preserves of vegetables (United States, type A), potatoes, fish, beef stews etc. In Europe the foods responsible are usually meats such as ham (France, type B). Type E has been found in seafood and fish.
- **Direct exogenous contamination** (inoculation botulism): Occurs when the bacteria are inoculated through a wound which is associated with subcutaneous abscess containing *Clostridium botulinum*, which produces the neurotoxin. In this case the disease is associated with fever. The main at risk group is drug addicts.

CLINICAL SYMPTOMS

The incubation period is from 5 hours to 5 days. Initial signs are gastro-intestinal (90%), visual disorders (ptosis and diplopia) and ENT disorders (dysphonia and dysphagia). The predominant sign is development of symmetrical descending flaccid paralysis from the head to the trunk and then extremities.

THERAPEUTIC USE OF BOTULINUM TOXIN

Type A botulinum toxin is used for its atropinergic properties in plastic surgery procedures. It produces a lifting effect on the face which last for 3 to 4 months.

INDICATIONS FOR MEASUREMENT

Clinical suspicion of botulinum toxin poisoning, combined with culturing.

INFORMATION

SAMPLE

The samples may be serum, gastric fluid, stool, vomit, the suspect food, anatomical specimens and wound swabs or environmental samples for bioterrorism.

Blood samples are collected into a dry tube (15-20 ml for adults, 2 ml for children). Food must be sent in its original container or in a sterile container.

QUESTIONS FOR THE PATIENT

Ensure that toxin A has not been administered therapeutically.

Ensure that the antitoxin has not been administered before the samples are collected.

SAMPLE STORAGE AND TRANSPORT

The samples must be transported promptly to the laboratory:

– At ambient temperature for smears and anatomical specimens.

– At +4°C for other samples.

Serum and stool must be frozen if the transport period is superior to 3 days too long. Sample freezing has no impact on detecting the toxin.

ASSAY METHODS

The reference method is an *in-vivo* neutralisation test. The patient's serum is administered by intraperitoneal injection into a mouse and a second mouse is administered with the same serum and a botulinum antitoxin. The test lasts 4 days. Toxin typing (A, B, D, and E) may then be performed using specific seroneutralisation.

Other *in vitro* methods used are ELISA enzymatic tests which are as sensitive and specific as the reference test.

Molecular biology methods may replace culture techniques (niche PCR or real-time PCR) by detecting the gene coding for the toxin although these appear to overestimate the amount of toxin produced. They would appear to be more useful for inoculation botulism and testing in food.

NORMAL EXPECTED VALUES

No neurotoxin is present in normal biological specimens (serum, stool, gastric fluid, skin samples).

PATHOLOGICAL VARIATIONS

The presence of the neurotoxin in biological samples (serum, gastro-intestinal fluid) is proof of the disease, confirmed by a positive anaerobic culture. The presence of the toxin in food is not sufficient to confirm the disease.

Toxaemia is positive in 75% of cases and persists for 15 to 30 days. The toxin may be excreted in faeces, in which *Clostridium botulinum* may be found for up to 1 month after the onset of symptoms.

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CLOSTRIDIUM DIFFICILE

DEFINITION AND SYNONYMS

Clostridium difficile is an obligate spore-forming anaerobic Gram positive bacillus responsible for 15 to 25% of post-antibiotic diarrhoea and more than 95% of cases of pseudomembranous colitis (PMC). It is the leading cause of nosocomial adult infectious diarrhoea. The toxin-producing strains (pathogenic) should be distinguished from the non toxin-producing strains (non-pathogenic).

INTRODUCTION

Clinical forms

Twenty-five to 80% of children under 2 years old and 2 to 5% of adults (but 10 to 20% in hospital) are healthy carriers. *C. difficile* infections (CDI) can present with varying degrees of severity from simple diarrhoea (without mucosal involvement), moderate diarrhoea without systemic signs to pseudomembranous colitis (7 to 9% of C. difficile diarrhoeas), characterised endoscopically by the development of pseudomembranes lining the colonic wall which may be complicated by toxic megacolon with a risk of perforation and septic shock. The mortality rate in uncomplicated disease is 0.6 to 3% rising to 35 to 50% in pseudomembranous colitis (PMC). The infections recur in approximately 20% of cases.

In view of the severity of these infections the Directorate General for Health in France provided a ***definition of severe C. difficile infection*** in 2006:

A patient suffering from CDI meeting one or more of the following criteria:

– For community origin, admission to a health care organisation for the treatment of CDI.

– Admission to intensive care for the treatment of CDI or its complications (complications requiring support of vital functions).

– Leukocytosis (≥ 20 000/mm^3).

– Surgery (colectomy) for megacolon, perforation or refractory colitis.

– Death attributable to *C. difficile* within 30 days after the onset of symptoms.

Pathophysiology of CDI

Several stages are required for CDI to develop:

1: Change in the bowel flora with breakdown of the barrier effect.

2: Establishment of a strain of C. difficile with the assistance of adhesion factors.

3: Production of the major virulence factors, A and B toxins.

4: Inadequate immunity against *C. difficile*.

Three main groups of strains can be found:

– strains Tox A-, Tox B-: non pathogenic

– strains Tox A+, Tox B+: pathogenic

– strains Tox A- (truncated protein), Tox B+: pathogenic (2% of strains)

A third toxin has also been described, the binary toxin which consists of two sub-units of cdtA and cdtB, which have not been shown to date to play a role in virulence.

Transmission of *C. difficile*

This is either endogenous from bowel carriage or, usually, exogenous from the spread of spores in the environment of patients and healthy carriers, from surfaces, furniture, medical material and the hands of medical and paramedical care staff. Spores may persist for several months on inert surfaces and may spread very quickly within a department.

New features of the CDI

The beginning of the millennium saw:

1: An increase in the incidence and severity of the CDI. Incidence increased by a factor of 3 in Canada and in the United States between 1991 and 2003 and increased incidence of complications (from 7% in 1991-1996 to 18.2% in 2003).

2: Failure of metronidazole (MTZ) treatment. Failure rate x 2.5 between 1991 and 2003.

3: Cases of community-acquired CDI. The incidence of these cases increasing from 1 case/100,000 people (1994) to 22 cases/100,000 people (2004) in the United Kingdom.

Community-acquired CDI is defined by the following four criteria:

– Symptomatic patient (gastro-intestinal problems),

– Diarrhoea on admission or developing 48 hours after admission,

– Positive toxin(s) in faeces,

– No hospitalisation within the previous 3 to 12 months.

4: Emergence of CDI in low risk populations

5: Animal role. The hypothesis of transfer of animal strains to human beings.

6: Dietary role. In Canada, 10 to 20% of batches of meat intended for human consumption (beef, veal, pork, turkey) are contaminated.

EMERGENCE OF AN EPIDEMIC 027 STRAIN

Molecular characterisation of this hypervirulent epidemic strain showed deletions in the *tcdC* regulator gene which could result in high production of A (16 times more) and B (23 times more) toxins. Other hypotheses explaining the hypervirulence of the 027 clone include in particular improved capacity of the B toxin to bind to its receptor (modification of the *TcdB* C-terminal domain) and an increase in spore-forming capacity.

Epidemic 027 strains are resistant to fluoroquinolones (MIC for ciprofloxacin, moxifloxacin and gatifloxacin > 32mg/l) due to mutations of the *gyrA* and *gyrB* genes. They are also resistant to erythromycin (MIC > 256 mg/l) and some are resistant to clindamycin, although are still sensitive to metronidazole and vancomycin.

The strain spread from Canada and the USA and affected France in January 2006 (Nord-Pas de Calais).

In practice: In the absence of phenotypic characteristics the 027 strain cannot be identified in the laboratory although it should be suspected if a severe form of the disease or clustered cases of *C. difficile* infections are found. In this case stool must be cultured to isolate the strain and send it for expert analysis to a reference laboratory.

SEARCH INDICATIONS

Specialist stool culture with testing for *C. difficile* and the A and/or B toxins must be performed for diarrhoea during or following antibiotic therapy, PMC, a suggestive epidemiological context and for diarrhoea in any patient hospitalised for at least 3 days. Children under 2 years old are often healthy carriers of the organism, including toxin-producing strains. If such a strain is isolated it may be responsible for the diarrhoea or may just be a colonising organism (this should be discussed with the paediatrician).

NB: A single dose of antibiotic is occasionally sufficient to produce CDI, which may occur up to 2 months after stopping the antibiotic in question.

INFORMATION

SAMPLE

C. difficile testing is performed on diarrhoea stool. Colonic biopsies may also be used for culture although testing for the A and B toxins on a homogenised colonic biopsy is not recommended as it lacks sensitivity. A rectal swab must not be used.

QUESTIONS FOR THE PATIENT

The patient should be asked about possible risk factors for acquiring CDI, mostly antibiotic therapy (oral carries greater risk than parenteral), often the beta lactams, clindamycin and more recently the broad spectrum fluoroquinolones which have become a major risk factor and occasionally the tetracyclines, sulphonamides which have become a major risk factor plus occasionally the tetracyclines, sulphonamides and macrolides and rarely the aminoglycosides, metronidazole, vancomycin, and chloramphenicol. Other treatments have also been incriminated, including cancer chemotherapy, proton pump inhibitors, laxatives and enemas. Other causative factors are hospitalisation (particularly long duration and/or in a department of intensive care and long stay departments) age > 65 years old, gastro-intestinal procedures (endoscopy, naso-gastric canula, gastro-intestinal surgery).

SAMPLE STORAGE AND TRANSPORT

The sample must be cultured promptly. If EIA testing for toxins is to be performed at a later stage the stool can be stored for 72 hours at + 4°C (although the test should preferably be performed as soon as possible as if the result is positive, patient isolation and management measures will be taken). The stool samples can be frozen at –20°C if toxins are tested by EIA but not if cytopathic activity is to be tested (the cytotoxic activity of the B toxin is destroyed on freezing).

DIAGNOSTIC METHODS

Antigen detection: *Identification of glutamate dehydrogenase (GDH)*

This is performed directly on stool samples using an immunoenzymatic method. Its positive predictive value (PPV) is low (50-60%), although the negative predictive value (NPV) is high (99,6%), making it a good screening method.

Detection of A and B toxins (to be preferred)

This may be performed by direct detection on stool samples or detection on culture if the stool test is negative (toxigenic *culture).*

- *Detection of the B toxin:* Cytotoxicity test (reference method)

This involves testing the cytopathogenic activity of a 1/10 dilution of the ultrafiltered stool sample on culture cells, where in the presence of the B toxin the cells become rounded in 24 hours. The specificity of the effect must be confirmed by neutralisation with a *C. difficile* B toxin antiserum or *C. sordellii* antitoxin antiserum. This test cannot be performed routinely by most laboratories.

- *Detection of A and B toxins: Immunoenzymatic tests*

These are immunochromatography or sandwich methods using polyclonal or monoclonal antibodies. They are performed in 96 well plates or as single tests to detect either the A toxin or the A and B toxins.

Isolation of *C. difficile*

This is performed on selective media: CCFA (Georges medium) = cycloserine (500 mg/l), cefoxitin (16 mg/l), fructose, neutral red, egg yolk or media derived from CCFA= cycloserine (250 mg/l), cefoxitin (8 mg/l), 5% sheep blood, etc (bioMérieux, AES, Biorad, Oxoid…etc.).

This can be combined with a medium promoting spore germination: Sodium taurocholate (Sigma refT-4009) 0.1% or lysozyme 5 mg/l, useful to recover strains from samples after long transport or storage periods. These media are not commercially available and must be prepared in the laboratory.

0.1-0.2 ml of diarrhoea stool or a 1/10 stool dilution should be inseminated. For media promoting spore germination it is useful to inseminate the stool sample after alcoholic or thermal shock to remove vegetative forms and only leave the spores present. It is then easier to read the media.

The medium is incubated for 48 h at 37°C anaerobically.

Identification of *C. difficile*

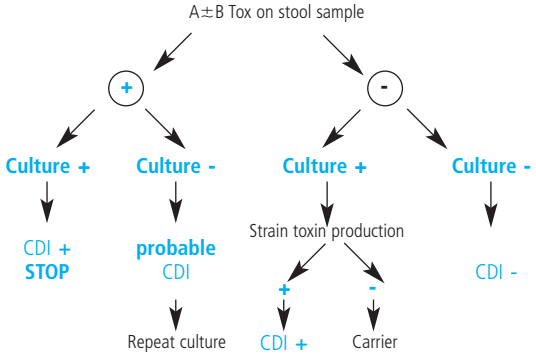
– *Macroscopic appearance:* On CCFA agar + blood, the colonies are flat, greyish in colour and star shaped with no haemolysis and chartreuse yellow fluorescence on UV (360 nm: Wood lamp) and a sintered glass appearance under a binocular magnifying glass.

– *Microscopic appearance:* Gram positive bacillus with deforming subterminal spores. Presumptive identification is based on the appearance of the colonies, the smell (horse manure) and UV fluorescence and confirmation from biochemical characteristics (lecithinase and lipase negative, no indole production, esculin +, gelatine +, glucose, fructose, levulose and mannitol fermentation) and enzymes present on a bioMérieux Rapid ID32A® (two positive tests: proline arylamidase and leucine arylamidase).

– *Other methods developed by Oxoid* (*C. difficile* test® kit): following agglutination using latex particles sensitised by anti *C. difficile Ab*. The NPV of the test is good (96%), although false positives occur (cross-reactions with *C. sordellii*, *C. glycolicum*, *C. bifermentans*).

There are currently no recommendations for the laboratory diagnosis of CDI. A decision algorithm is suggested below:

Decision Algorithm: diagnosis of CDI



RESULTS

The isolation rate ranges from 5 to 70% in healthy adults depending on the study and is less than 3% in healthy newborn babies. Isolation rates are 10 to 25% in asymptomatic patients receiving antibiotics or in hospitalised patients, 5 to 10% being toxin-producing strains. Toxin-producing strains are found in 10 to 25% of cases of post-antibiotic diarrhoea and the endoscopic isolation rate of toxin-producing *C. difficile* in pseudomembranous colitis is 95 to 100%.

INTERPRETATION

Not all strains are pathogenic. Some toxin-producing strains cause diarrhoea and then colitis as a complication. Culture is not therefore sufficient to conclude that the *C. difficile* is responsible for the features of the disease.

Conversely, finding toxins directly in stool samples is an excellent marker that the *C. difficile* is responsible. Cell culture tests are useful to record the cytopathic activity of the B toxin. Immunoenzymatic tests are useful to test for the presence of the A toxin. Testing for the common antigen (glutamate dehydrogenase) is inadequate to provide conclusion as to the cause of the diarrhoea.

TREATMENT

Firstly, stop the antibiotic responsible and avoid antiperistaltic treatments.

- **First episode, uncomplicated form**

  Metronidazole: 500 mg x 3/day *per os* 10 days or vancomycin: 125 mg x 4/day *per os* 10 days.

- **Recurrence**

  Further course of metronidazole or vancomycin. A probiotic such as *Saccharomyces boulardii* can be added.

- **More than 1 recurrence**

  Decreasing doses of vancomycin (*per os*): 125 mg/6 h for 7 days then 125 mg/12 h for 7 days, 125 mg/24 h for 7 days, 125 mg on alternate days for 7 days and 125 mg every 3 days for 14 days.

- **For severe disease**

  Vancomycin: 125 mg *per os* x 4/day for 14 days (IV not recommended). No microbiological control to assess efficacy (which is judged from clinical improvement).

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CLOZAPINE

DEFINITION

Clozapine is a neuroleptic belonging to the diazepine class. It is marketed in 25 and 100 mg scored tablet form and is indicated for the treatment of chronic severe schizophrenia (present for at least two years) in patients who are resistant or severely intolerant of the conventional neuroleptics, and in the treatment of psychotic disorders occurring during Parkinson's disease if the usual treatment strategy fails. The dosage must be adjusted individually, using the lowest effective dose in each patient.

Clozapine acts by moderate blockade of the D1, D2, D3 and D5 dopaminergic receptors and potent blockade of the D4 receptor. It also inhibits the alpha-1 and 2 adrenergic, H1 histaminergic, cholinergic, muscarinic and serotoninergic receptors. It has potent anti-psychotic activity on both productive and deficient syndromes. Amongst the neuroleptics its characteristic features are its rapid intense sedative action, scarcity of extra-pyramidal side effects and lack of major rise in serum prolactin.

Clozapine poisoning can occur secondarily to chronic overdose or acute intentional suicidal poisoning.

PHARMACOKINETICS

Mean absolute bioavailability	Approximately 55% (after first pass hepatic metabolism).
Plasma peak (Tmax)	1 to 6 hours.
Protein binding	95%.
Metabolism	Hepatic by N-oxidation and N-demethylation: Only the N-methylated derivative is pharmacologically active (weaker activity and shorter duration).
Plasma half-life of elimination	Variable: Average 12 hours after single dose.
Elimination	50% in urine, 40% in bile.

INDICATIONS FOR MEASUREMENT

Clozapine measurement in blood is indicated if treatment is ineffective or in suspected chronic poisoning when side effects or signs of toxicity develop. Large inter-individual variability in the pharmacokinetics of the compound is seen, where for the same dose, some patients may have very low serum concentrations, and in others the toxic range may be reached. Changes in the metabolism of the compound may also occur secondary to environmental effects (smoking reduces serum clozapine concentrations by approximately 20%) or therapeutic interactions. Association with rifampicin, phenytoin, carbamazepine or phenobarbital carries a risk of the antipsychotic treatment failing as a result of increased hepatic metabolism of clozapine (with reduced plasma clozapine concentrations); association with fluvoxamine, fluoxetine, cimetidine, fluoroquinolones or erythromycin carries a risk of overdose (with a rise in plasma clozapine concentrations).

The main secondary effects in chronic poisoning (which usually reverses when the treatment is stopped) are drowsiness, lethargy, neurological disorders (extra-pyramidal or pseudo-Parkinsonian syndrome) and orthostatic hypotension. Clozapine can also cause severe neutropaenia with a risk of agranulocytosis (which is unrelated to serum concentrations, estimated incidence: 0.46%). There is also a risk of long-term secondary effects (after treatment for several months or years) of tardive dyskinesia or "*rabbit syndrome*" (involuntary rhythmical movements of the face, tongue and lips).

The clinical symptoms of acute poisoning are neurological (drowsiness, confusion, hallucinations, delusions, extra pyramidal syndrome, hyperreflexia, seizures and hypersalivation), cardiovascular (hypotension, collapse, tachycardia and arrhythmia) and respiratory disorders (respiratory depression or even arrest and dyspnoea). Finally, as applies to all of the neuroleptics it carries a risk of the malignant neuroleptic syndrome, a rare complication but one with a high mortality rate (15 to 20%). The syndrome involves a combination of fever ≥38°C, generalised extra pyramidal hypertonia or simple neck muscle rigidity and neuro-autonomic and consciousness disorders.

INFORMATION

SAMPLE

Serum or plasma taken into EDTA or heparin. Avoid tubes with separator gel.

The sample **should be taken immediately before the next dose** (trough concentration) for suspected inefficacy or chronic poisoning or when signs of overdose dare present in acute poisoning.

QUESTIONS FOR THE PATIENT

What context is the measurement being requested in (suspected chronic overdose, acute intentional or suicidal poisoning)? If possible, how much was taken, and the date and time of the dose(s)?

Are you taking other medical treatments? Rifampicin, phenytoin, carbamazepine or phenobarbital all carry a risk of the antipsychotic treatment being ineffective; fluvoxamine, fluoxetine, cimetidine, fluoroquinolones and erythromycin carry a risk of clozapine overdose.

SAMPLE STORAGE AND TRANSPORT

Centrifuge and separate the plasma or serum promptly. If the analysis is to be performed later; freeze within 4 hours after the sample is taken.

Transport at +4°C.

ASSAY METHODS

High performance liquid chromatography with UV, UV diode bar or mass spectrometry detection: Gas phase chromatography coupled with mass spectrometry.

NORMAL EXPECTED VALUES

Target steady state trough concentration: 50 to 700 ng/ml although large inter-individual variability of concentrations is seen for the same dose. For therapeutic monitoring, the literature proposes that trough concentrations be maintained above a threshold value of 350 ng/ml although some patients respond clinically to lower values and others to far higher values (> 1 000 ng/ml).

Signs of toxicity generally occur above 1000 ng/ml. Serum clozapine concentrations of between 1,200 and 13,000 ng/l have been reported in the literature in fatal poisoning.

There is no antidote in acute poisoning. Treatment involves decontaminating the bowel with activated charcoal and symptomatic management (ventilatory assistance, vascular filling, anticonvulsants, etc.).

FOR FURTHER INFORMATION

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COBALT

DEFINITION

Cobalt (Co) is a relatively rare element in the earth's crust. It is a light, bluish metal with magnetic properties and cobalt blue was used in the early ages as a dye in pottery production. Cobalt is currently used in a very wide range of industrial processes, such as the production of highly resistant alloys (aluminium-nickel-cobalt) for the production of magnates, cobalt-tungsten mixtures for the manufacture of saw blades, drill and piercing bits, and polishing surgical and dental prostheses. Cobalt salts are used in the preparation of enamels, ceramics and lacquers.

Cobalt is also an essential trace element, a constituent of vitamin B12 or cobalamin. In human beings it must be provided entirely in this physiologically active form. Normal daily cobalt intake has been estimated to be 20-40 µg. Total body cobalt load is approximately 1.50 mg.

METABOLISM

Intestinal cobalt absorption is influenced by nutritional factors, albumin and lactose promoting its absorption. It is absorbed in two stages in the same way as iron: Firstly a mucosal absorption stage followed by a transfer stage, in which 50% of enterocyte cobalt passes into the body. Iron and cobalt absorption appear to antagonise each other, where iron deficiency increases cobalt absorption but does not affect its transfer.

The intestinal absorption of vitamin B12 depends on intrinsic factor, a glycoprotein secreted by gastric parietal cells. Cobalt can also be absorbed through the skin or respiratory tract.

After distribution throughout the body, cobalt is found preferentially in the liver which is believed to store approximately 1/5 of the body's cobalt.

Cobalt is excreted mostly in urine in two stages: 90% is rapidly removed in a few days and the remaining 10% is excreted with a half-life of approximately 2 years.

MECHANISM OF ACTION

Cobalt is the central atom of the cobalamin tetrapyrrole nucleus. The different cobalamins, which are all nutritionally active, are characterised by the group bound to the cobalt atom: Cyanocobalamin, hydroxycobalamin, methylcobalamin and 5-desoxyadenosylcobalamin. Methyl and 5-desoxyadenosylcobalamin are the active coenzymes involved in numerous metabolic processes, particularly as coenzymes in methylmalonylmutase and reductases, involved in DNA synthesis.

The cobalamins are also involved in the conversion of homocysteine into methionine and, as a result, in protein synthesis. They are also involved in detoxification of cyanide ions and in the metabolism of choline and creatine, which forms an energy reserve in the form of creatine phosphate.

SYMPTOMS OF ACUTE POISONING

Symptoms of acute poisoning have been attributed to cobalt ingestion by drinkers of Belgian and Canadian beers (this manufacturing process has now been abandoned), who developed rapid onset right and left side cardiac decompensation with cardiomegaly, pericardial effusion, gallop rhythm, hypotension and shock.

SYMPTOMS OF CHRONIC POISONING

Inhalation of cobalt dust may occur in workers, causing:

– Respiratory symptoms: asthmatiform, dyspnoea which resolves after exposure is discontinued.

– Desquamating alveolitis progressing gradually to diffuse interstitial fibrosis.

– Allergic skin symptoms (contact eczema).

– Haematological signs: Raised red cell count.

– Cardiac symptoms: Cardiomyopathy, with raised myocardial cobalt concentration.

– Dysthyroidism with thyroid hyperplasia, as cobalt inhibits tyrosine iodinase.

INDICATIONS FOR MEASUREMENT

Blood cobalt measurement at the end of shift and at the end of the working week is believed to reflect recent cobalt exposure. There is a good correlation following recent exposure, between plasma cobalt and atmospheric cobalt concentration.

The urine cobalt measurement at the end of day and end of working week appears to be a good reflection of exposure during the previous week provided renal function is normal.

Urine samples produced on the Monday morning appear to reflect long-term exposure.

INFORMATION

■ SAMPLE

3 ml of heparinised whole blood10 ml unacidified urine sample.

Blood and urine samples, obtained at the end of shift and end of working week.

■ QUESTIONS FOR THE PATIENT

Does the patient smoke or drink beer, which can increase urinary cobalt excretion by a factor of 2 to 3.

■ SAMPLE STORAGE AND TRANSPORT

Whole blood and urine samples should be stored and transported to the laboratory at between + 2 and + 8°C.

ASSAY METHODS

Electro-thermal atomisation atomic absorption spectrophotometry (graphite furnace) with Zeeman correction or induction coupled plasma source or mass spectrometry.

REFERENCE VALUES

Reference value in the general population:

– Blood cobalt: < 0.8 µg/l.

– Urine cobalt: < 2.00 µg/g of creatinine (< 2.4 g/l).

For exposure to cobalt and inorganic compounds:

– Blood cobalt = 1.00 µg/l at end of shift and end of week (guideline value in France and in Quebec).

– Urine cobalt = 15.00 µg/l at end of shift and end of week (guideline value in France and in Quebec).

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COCAINE

DEFINITION

Cocaine is an alkaloid extracted from the leaves of four varieties of Erythroxylum including the coco bush, plants which grow mostly on the high Andes plateaus of South America. Different types of preparation result in different various products. At the site of production the freshly gathered leaves are mixed with a strong base such as caustic soda. After being macerated for several days the alkaloids are extracted from the mixture by adding an organic solvent (acetone, kerosene or diesel). The leaves are then removed and sulphuric acid is added to the organic phase to extract the most alkaline alkaloids which are then precipitated with ammonia solution to form the coca paste, popularly known as "**pasta**" or "**bazooka**". This preparation is smoked by local country folk where it is produced.

After treating the paste with potassium permanganate in sulphuric acid some impurities are oxidised (cinnamoylcocaines) and then removed by refining with ether or acetone producing the "***freebase***" (**methylbenzoilecgonine = cocaine base and cocaine sulphate**).

As the "*freebase*" is highly inflammable an additional purification stage is required by dissolving in acetone and adding hydrochloric acid and absolute alcohol. The cocaine base is then precipitated as fine white crystals, **cocaine hydrochloride (methylbenzoilecgonin chloride** or "***snow***", the most widely used compound).

Around 1980, ***crack*** appeared. This is the product of hot precipitation of cocaine hydrochloride by a base. Sodium bicarbonate was mostly used in the French West Indies and ammonia sodium in the United States. The compounds which are produced from this appear to have different psychotropic effects.

The cocaine base forming crack takes the form of a solid bar which is separated into "***stones*** " or "***rocks***" of 50 to 100 mg which are hard and whitish in appearance.

METABOLISM

plasma ½ life	0.5 to 1.5 hours, varying between individuals
Metabolism	Following hepatic hydrolysis, formation of benzoylecgonin (cytotoxic activity) and then the action of hepatic esterases and plasma cholinesterases forming ecgonine methylester. When associated with alcohol: Cocaethylene is formed (which has pharmacological and toxic effects), and is metabolised into norcocaine. Cocaine metabolism is dose-dependent up to approximately 1 µg/ml of plasma cocaine. Specific situation for crack: Formation of the anhydroecgonine methylester which is found in biological fluids and used as a marker of smoked cocaine.

The compounds tested in urine are cocaine hydrochloride (snow) and cocaine base (crack), the main metabolites of which that are found in urine are benzoylecgonine and ecgonine methylester.

INTRODUCTION

METHODS OF USE

The pasta is smoked in the form of large cigars rolled in newspaper (bazookas) or as cigarettes in the producing countries.

Cocaine hydrochloride (ice or snow) was firstly used therapeutically as a local anaesthetic and in the treatment of alcohol or morphine dependency (after being dissolved and used by local application, orally or by intradermal injection). It is currently used by drug addicts as "sniff" (or "snort").

Since 1981, Jamaican rasta communities used the "*freebase*" in smoked form.

Since 1984, crack has been used by inhalation through water pipes after vaporising at around 90°C or from cigarettes after mixing with cannabis ("black joint, wulla").

PHARMACOLOGY

Cocaine acts by stimulating the "reward circuit" located in the cortico-limbic region which controls fundamental behaviours and emotions. This action results in the release of the neuromediators, dopamine and serotonin, which activate the post-synaptic neurones.

Cocaine causes dopamine accumulation and hyperactivation of the reward system which represents the "flash" (high). As feed back mechanisms are disturbed, dopamine depletion or the "come down" follows. A succession of these effects encourages cocaine use and results in dependency. Whilst the physiological and psychological effects of cocaine are the same regardless of the form used, the route of administration influences the extent and duration of these effects. The main effects seen are intellectual hyperactivity and a feeling of euphoria.

TOXICITY

Cocaine poisoning affects several organs:

– Skin: Facial burns from burning solvent residues when smoking the freebase form, scarring from intravenous injection (bruising or ulceration), scleroderma (long-term use, etc.).

– ENT: Damage due to the method of use. Nasal septum perforations in repeated snorting, inflammation or necrosis of the pharynx after using crack.

– Lungs: Cocaine inhalation causes various lesions. Acute dyspnoea, exacerbation of asthma, inhalation pneumonia, oedema and pulmonary haemorrhage. Crack can cause pneumothorax, pneumomediastin or pneumopericardium.

– Liver: Necrotic centrolobular venous damage.

– Gastro-intestinal tract: Colitis and ischaemia, intestinal pain and even necrosis.

– Kidney and muscles: Muscle pain, tingling, muscle weakness or even rhabdomyolysis with secondary acute renal failure. Crack may result in patients requiring dialysis.

– Heart and blood vessels: Chronic cardiomyopathy, atheromatous lesions, thrombosis, hypertension, cardiac conduction abnormalities and increased risk of infarction.

– Central nervous system: Symptoms of paranoid psychosis, seizures, malignant hyperpyrexia, cerebral hypoperfusion, increased risk of cerebral infarction and also cerebral haemorrhage.

INDICATIONS FOR MEASUREMENT

Testing/confirmation of cocaine drug addiction.

Epidemiological monitoring.

INFORMATION

SAMPLE

40 ml urine sample collected at the laboratory preferably into 3 plastic bottles (2 bottles for subsequent tests to be frozen at -20°C). Outside of a medico-legal context a sample taken under the same conditions into a single mid-stream urine container is acceptable.

Ensure the sample has not been tampered with (substitution, addition of water or doctoring agent) by measuring pH, urine density and confirming the sample temperature as soon as possible after it is produced (temperature > 30°C). Close the bottles firmly (seal them when the analysis has been requested in a legal context) and identify them precisely. Record the date, time and place of the collection.

QUESTIONS FOR THE PATIENT

If possible, circumstances of poisoning, route of administration, current treatments or alcohol?

SAMPLE STORAGE AND TRANSPORT

Urine can be stored for 4 weeks at room temperature after adjusting the pH to 5 with ascorbic acid. For longer periods, freeze at + 4°C after adjusting the pH to 5 with ascorbic acid or freeze at – 20°C regardless of pH.

ASSAY METHODS

SCREENING

– Immunochemical (FPIA, EMIT, RIA), etc. The positivity threshold set by the FDA is 300 ng/ml. The limit of detection of these methods is far lower at around 25 ng/ml. NB: Cross-reactions occur between cocaine and cocaethylene and vary depending on the method. Rapid determination methods have also been developed (immunochemical tests which can be used at the patient's bedside) although the limit of detection of these methods is usually > 300 ng/ml and may produce false negative results.

– Thin layer chromatography or high pressure thin layer chromatography.

SPECIFIC CONFIRMATORY METHODS

Gas phase chromatography coupled to mass spectrometry, HPLC coupled to mass spectrometry.

NORMAL EXPECTED VALUES

Negative tests when cocaine substances have not been taken.

INTERPRETATION

The compounds detected in urine are benzoylecgonine and ecgonine methylester. Cocaine compounds can be detected in urine for approximately 2 to 4 days.

It is imperative that if a screening method is positive, a confirmatory and assay method is used particularly to identify and quantitate the anhydroecgonine methylester and to diagnose crack poisoning.

NB: Babies born to mothers using cocaine have high plasma cocaine concentrations for several days after birth. Cocaine also passes into breast milk where it is found at concentrations far higher than those in plasma.

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COENZYME Q10

DEFINITION

Coenzyme Q10 (CoQ10) or ubiquinone is a fat-soluble benzoquinone derivative found in all organs and is both synthesised by the body and obtained from food (meat and fish). Its most important role is as an electron transporter in the mitochondrial respiratory chain, although in its reduced form (ubiquinol or CoQ10H2) and it is a potent lipophilic membrane antioxidant. It is synthesised in the oxidated form (ubiquinone, CoQ10) in the liver and circulates in the reduced form of ubiquinol with the lipoproteins. The lipoproteins which carry coenzyme Q10 in the body are those containing cholesterol, and therefore mostly LDL.

INTRODUCTION

The antioxidant activity of CoQ10 particularly targets lipid fractions. It reduces LDL peroxidation and regenerates vitamin E which like CoQ10 is a fat-soluble antioxidant and with which it acts in synergy. It has been studied in many diseases with known oxidative stress (acute coronary syndrome, neurodegenerative diseases, myopathies, haemodialysis patients, diabetes, etc.). Measurement of CoQ10 may be a useful discriminatory factor to identify patients at risk of developing coronary problems.

INDICATIONS FOR MEASUREMENT

CoQ10 has been described as a sensitive indicator of oxidative stress. The CoQ10/LDL-cholesterol ratio has been described as a cardiovascular risk factor.

INFORMATION

SAMPLE

The blood sample should be taken into a dry tube (without anticoagulant) or into a tube containing EDTA.

QUESTIONS FOR THE PATIENT

Are you being treated with a statin cholesterol lowering agent? Statins inhibit the synthesis of CoQ10.

SAMPLE STORAGE AND TRANSPORT

If the analysis is not being performed within 4 hours, the sample must be centrifuged, separated and frozen within 4 hours of sampling.

ASSAY METHODS

CoQ10 is assayed by high performance liquid chromatography (HPLC) with electrochemical detection.

NORMAL EXPECTED VALUES

Normal plasma concentrations: 0.40 – 1.2 µg/ml (or 0.5 - 2 µmol/l).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Plasma CoQ10 concentrations fall in the elderly and after physical exercise.

PATHOLOGICAL VARIATIONS

Coenzyme Q10 deficiencies may be due to:

– Dietary deficiency (deficient fat intake, fat malabsorption and vegetarian diet).

– Reduced endogenous synthesis (deficiency of precursors such as phenylalanine, pyridoxine, folic acid, vitamin B12, etc. and reduced synthesis).

Low plasma CoQ10 concentrations reflect weak antioxidant activity or high oxidative stress CoQ10 (haemodialysis patients, acute coronary syndromes, diabetes, etc.). Low coenzyme Q10 concentrations have been found in patients with the acute coronary syndrome (0.43 +/0.2 µmol/l). A reduced CoQ10/LDL cholesterol ratio has been described as a cardiovascular risk factor.

In overt deficiency, CoQ10 supplementation results in a rise in its plasma concentration. The effectiveness of treatment can be confirmed by monitoring its concentration.

FOR FURTHER INFORMATION

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COLD AGGLUTININS

DEFINITION

Cold agglutinins are anti-erythrocytic auto-antibodies capable of causing agglutination of red blood cells below 30°C, with maximum reactivity between 0 and 5° C. This phenomenon is reversible after warming again. Cold agglutinins are not necessarily pathogenic: many patients, in fact, have very low levels of natural cold agglutinins in their serum with no in vivo pathological manifestation. On the other hand, certain cold agglutinins have very real pathological implications and can be responsible for "cold antibody" autoimmune haemolytic anaemia (16 to 32% of AIHA's).

CLINICAL MANIFESTATIONS

Cold agglutinins are responsible for a variety of symptoms and may present in the following manner:

– absence of symptoms

– signs of anaemia and/or haemolysis (chronic or acute, intravascular or extravascular)

– cutaneous manifestations due to cold: obstruction of the circulation by cold agglutinated cells resulting acrocyanosis at the extremities (fingers, toes, ears and nose), Raynaud's phenomenon. Possible cold urticaria or livedo reticularis. Very rare distal gangrenes in cases of associated cryoglobulin.

ETIOLOGY

– "Natural" cold agglutinins are present in numerous patients at very low levels (up to 1/32) without clinical significance.

– Acute AIHA's: these generally occur in an infectious context involving young adults, adolescents and children (infections with EBV, CMV, *Mycoplasma pneumoniae*, *influenza*, mumps, etc.). The cold agglutinins in these cases are polyclonal IgM antibodies.

– Chronic AIHA's: these generally occur in a context of auto-immunity or haemopathies (usually lymphoid) in subjects over 50 to 60 years of age. The cold agglutinins in these cases are mainly monoclonal IgM antibodies. The underlying pathology is not always necessarily identified and we speak of chronic idiopathic cold agglutinin disease, but this condition often heralds a pathology, in which it is referred to as pre-neoplasic.

– Paroxysmal cold haemoglobinuria: an exceptional condition, most often linked to the transient presence of a polyclonal IgG which binds when cold and becomes active at 37° C. It causes acute haemolytic episodes after exposure to cold, with dark urine (the colour of "coca cola"). It is most often seen in children 8 to 10 days after a viral infection (rubella, chicken pox or mumps): it is capable of causing severe intravascular haemolytic episodes with sudden death. It lasts for 1 to 3 months and rarely becomes chronic.

– Mixed AIHA's: presence of cold agglutinins at low levels associated with a predominance of warm auto-antibodies.

TESTING INDICATIONS

– Auto-immune haemolytic anaemia analysis: search for cold or warm auto-antibodies

– Dermatological analysis

– Analysis after fortuitous analytical interference with the blood count: see below.

INFORMATION

■ SAMPLE

The sample is collected on EDTA (whole blood) and dry tube (serum). The sample must be collected and kept at 37° C until the serum is decanted (coagulation of blood and centrifuging to take place at 37° C).

■ SAMPLE STORAGE AND TRANSPORT

Store and transport sample at +4° C.

ORIENTATION AND BIOLOGICAL DIAGNOSIS

– ***Full Blood Count:*** The fortuitous presence of cold agglutinins may be suspected because of possible interference with the blood count. In the case of cold agglutinins with significant activity, an agglutination phenomenon occurs in the tube after the blood sample is collected. This interferes with the blood count results: false hyperleukocytosis, false reduction of the number of red cells, false increase of MCV, significant rise in MCHC and rouleaux formation is seen when examining the slide under microscopy. The tube should be kept at 37° C for 1 to 2 hours in order to avoid this interference.

– ***Direct Coombs test:*** Positive C3d test and negative IgG (the antibodies bind when cold but elute at 37° C and therefore remain free in the serum).

– ***Search for cold agglutinins:*** Indirect Coombs test positive at 4° C.

– ***Titration:*** When isolated, only titration above 1/64 at 4° C is significant. In the recent literature, a threshold of 1/256 is proposed to positively affirm the presence of pathogenic cold agglutinins. Very high results up to 500,000 may be encountered. The thermal amplitude, however, is more important than the titration result: in fact, in cases of low measurement at 4° C, if the cold agglutinin is still active at 30-37° C, there may be haemolysis *in vivo*.

– ***Specificity:*** A test for specificity is not systematically performed. It may be indicated if the titre is high but is not of any real interest. The cold agglutinins are mostly of type anti-I or sometimes anti-i. Anti-HI's, anti-MNS's and anti-P's, and rare cold agglutinins of type anti-ABO, also exist.

TREATMENT

– Avoid the cold!

– Transfusion of warmed packed red blood cells

– Corticotherapy, but this is less effective than for warm antibody AIHA's

– Cyclophosphamide

– Fludarabine

– Danazol
– IgIV
– Plasma exchange
– Splenectomy?
– Rituximab® (anti-CD20 monoclonal antibody).

FOR FURTHER INFORMATION

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COMPLEMENT

DEFINITION

The complement system is an integral part of the immune system and represents the main part of the humoral defence system against infectious agents. It is a complex system comprising around twenty circulating proteins, which bind to certain biological membranes. Cascade activation of its proteins triggers various biological reactions resulting in cell, bacterial or viral lysis. The complement system comprises 3 activation pathways, which result in the cleavage of C3 with the formation of the "membrane attack complex" and death of the target cell: the classical pathway, the alternative pathway and the mannan lectin binding pathway.

INTRODUCTION

PHYSIOLOGY OF THE COMPLEMENT SYSTEM

The complement proteins are activated in a cascade which leads to the formation of many enzyme complexes. A component acquires enzymatic activity which induces the activation of the subsequent component which does the same in turn.

- **Classical pathway:** This is generally activated by the binding of C1 to antigen-antibody complexes or IgG1, IgG2, IgG3 or IgM immunoglobulin aggregates in human beings. More rarely it can be activated directly by DNA, C-reactive protein and even some viruses. C1 is the recognition compound for the classical pathway and comprises 3 protein sub-units: C1q, C1r and C1s. After binding to immunoglobulin Fc fragments in the presence of calcium ions, C1q activates C1r through a proteolysis reaction. Once activated, C1r then cleaves C1s which becomes activated C1s. Activated C1s cleaves both C2 and C4 forming activated C4b and C2a fragments. Classical C3 convertase or the C4b2a complex has enzyme activity, which enables it to cleave C3 into 3a and C3b, and also C5 if it is bound to a C3b molecule. The classical pathway is regulated by C1 esterase inhibitor (C1INH) and C4 binding protein (C4bp).
- **Alternative pathway:** This is activated directly by certain polysaccharides and is an immediate anti-infectious defence mechanism. After the activation phase it is accelerated by an amplification phase leading to the formation of alternative C3 convertase. Activation releases C3b, which together with factor B in the presence of magnesium ions, forms a bimolecular complex C3bB. Enzyme D cleaves the factor B of the C3bB complex into Bb resulting in the formation of a new complex C3bBb or alliterative C3 convertase. The C3bBb complex cleaves C3 to form new C3b molecules which in turn produce new alternative C3 convertases following the action of factors B and D. Like classical C3 convertase, alternative C3 convertase binds to a membrane and can also cleave C5. The C3b amplification loop is effectively controlled by two regulatory proteins: proteins H and I. Protein H binds to C3b competitively with factor B resulting in the alternative C3 convertase losing activity. It also facilitates the action of protein I which is to cleave C3b into iC3b (inactive molecule) and then into C3c, C3dg and C3d. Conversely, properdin or factor P acts as a positive physiological control binding to and stabilising the convertases. Other membrane proteins, CR1 and DAF are also involved in the regulation of activation of the alternative and classical pathways.
- **Lectin pathway:** MBP (*mannose binding protein*) originates from blood cells, binds to bacterial mannose or Nacetylglucosamine groups. It binds to the bacterial mannoses and activates 2 serine proteases which cleave and activate C4 and C2 to form the same C3 convertase as in the classical pathway.
- **Lytic complex:** Cytolysis is the end result of action of the lytic complex formed from 5 proteins: C5, C6, C7, C8 and C9. After C5 is cleaved by classical or alternative C5b convertase the C5b fragment forms a stable trimolecular complex with C6 and C7, C5b67 which binds tightly to a membrane. C5b67 then fuses with C8 and C9 to form a new complex, C5b9. This very stable active complex has cytolytic activity. Plasma inhibitors of the lytic complex, vitronectin, exist which binds to the lytic complex and prevents it inserting itself into the membrane and HRF (*Homologous Restriction Factor*) which blocks the binding of C7C8 and protects the cell from lysis by the homologous complement.

BIOLOGICAL ACTIVITIES OF COMPLEMENT

The complement system carries out several essential roles:

– Cytolysis of a cell or pathogenic agent by activating the lytic complex, complement causes lysis of various cells, (red blood cells, platelets and leukocytes) and also bacteria and viruses in their lipoprotein envelope. It has an essential role in anti-infectious defence.

– Opsonisation of some agents allowing them to be phagocytosed, particularly infectious organisms.

– Activation of the immune system by the small pro-inflammatory cleavage fragments, numerous fragments released from the activation of complement proteins such as C3a and C5a (anaphylatoxins) initiating or amplifying the inflammatory reactions. These anaphylatoxins induce histamine release from mastocytes and basophils, producing a rise in vascular permeability and oedema. C5a also has chemotactic activity for neutrophils.

– The complement fractions are involved in the metabolism of circulating immune complexes promoting their dissolution.

INDICATIONS FOR COMPLEMENT INVESTIGATIONS

– Diagnosis of congenital hypocomplementaemia.

– Diagnosis of hypocomplementaemia due to abnormal activation or increased catabolism of complement, explaining whether the abnormality relates to the classical or alternative pathway. This information is an aid to the differential diagnosis of some diseases (infectious diseases, auto-immune diseases, glomerulonephritis, etc.).

INFORMATION

SAMPLE

Serum (usually) or EDTA plasma (C3d). Blood samples should be centrifuged after coagulation at laboratory temperature within 2 hours of sampling.

SAMPLE STORAGE AND TRANSPORT

Complement components are stable for 2 days in serum at +4°C. If testing is to be performed later, the serum should be frozen at -20°C.

Haemolytic assay of CH50 is performed on serum (or aspiration fluid), frozen within an hour of sampling to avoid *in-vitro* consumption of complement proteins.

COMPLEMENT INVESTIGATION METHODS

FUNCTIONAL ASSAYS

Measurement of the complement system haemolytic function:

CH50 test: This measures the functional activity of the classical pathway and proteins of the common lytic pathway. The test is based on the principle of measuring the amount of serum which lyses 50% of a given number of sheep red blood cells sensitised by rabbit anti-sheep red blood cell antibodies. The extent of haemolysis is proportional to the amount of complement present.

AP50 test: AP (*for Alternative Pathway*) measures the functional activity of the alternative pathway using rabbit red blood cells. Similarly, the AP50 unit is defined as the amount of complement required to lyse 50% of the RBC.

Measurement of the activity of each complement component: Functional haemolytic assays can be used to detect native proteins although are difficult to perform and reserved for research purposes.

Functional C1 INH measurement: This measures the inhibitory activity of C1INH on C1s.

MASS ASSAYS

Mass assays of complement components can be performed routinely, particularly measurements of C1q, C2, C3 or C3c, C4, C9, C1 INH and factor B. Immunochemical methods (radial immunodiffusion, immunonephelometry and immunoturbidimetry), and also immunoenzymatic methods (ELISA) are used.

PATHOLOGICAL VARIATIONS

HYPERCOMPLEMENTAEMIA

These are of relatively little interest in pathological terms as it is seen in various inflammatory states, such as with tumours, infectious diseases and some connective tissue disorders. The variations mostly affect total complement and some complement components, particularly C3, C4 and C1INH.

HYPOCOMPLEMENTAEMIA

This is more common and of greater interest as it is due either to defective synthesis in hereditary deficiencies or complement system proteins or due to abnormal activation or increased catabolism of the complement system in secondary or acquired deficiencies.

Inherited deficiencies:

These involve all of the proteins in the complement system: the components themselves (C3, C5, C2, C4, C1q, C1r deficiencies, etc.), or regulatory proteins (C1INH, DAF, HRF, CR3 deficiencies, etc.). Clinically, these result particularly in repeated infections or connective tissue diseases: *cf. table*.

Component or regulatory protein	Related clinical features
C1q	Auto-immune diseases, rheumatic diseases, Systemic lupus erythematosus (SLE)
C1r	SLE, glomerulonephritis
C1s	SLE
C4	SLE, pyogenic infections (*Streptococcus pneumoniae*)
C2	Infections récidivantes à pyogènes, glomérulonéphrites
C3	Infections récidivantes à pyogènes, glomérulonéphrites.
C5	SLE, recurrent *Neisseria* infections
C6	Recurrent *Neisseria* infections
C7	Raynaud's syndrome, *Neisseria* infections
C8	SLE, *Neisseria* infections
C9	Usually asymptomatic
C1-INH	Hereditary angio-oedema
Factor D	Recurrent bacterial infections
CR1	SLE
CR3	Severe recurrent infections
Factor H	Haemolytic – uraemic syndrome
Factor I	Repeated pyogenic infections
Properdin	Recurrent infections

Deficiencies of the initial components of the classical pathway (C1q, C1r, C2, and C4): These have a higher incidence of rheumatic or autoimmune diseases, particularly SLE, than in the normal population. SLE is described particularly for C2 deficiency.

C3 deficiencies are associated with increased sensitivity to infections from Gram + pyogenic bacteria due to loss of the role of C3 in opsonisation and phagocytosis.

C5 deficiencies: Patients with this deficiency occasionally suffer from auto-immune diseases and also recurrent bacterial infections.

C6, C7 and C8 deficiencies: Are also associated with recurrent *Neisseria* infections.

C1INH deficiency: Is responsible for angioneurotic oedema (ANO) or angioedema characterised by attacks of oedema in the skin, gastro-intestinal tract or respiratory tract triggered in response to aggression or various injuries.

Factors H and I deficiency: Is associated with a higher incidence of Gram + bacillary infections, and also glomerulopathies.

Factors B, D and Properdin deficiencies: Are also associated with recurrent bacterial infections.

DAF and HRF inhibitory deficiencies: Are associated with paroxysmal nocturnal haemoglobinuria.

CR3 deficiencies: Are characterised by severe recurrent infections.

Acquired deficiencies:

These are due to *in-vivo* activation of the classical pathway by

circulating immune complexes and also to activation of the alternative pathway in some pathological circumstances: SLE, rheumatoid arthritis, post-streptococcal glomerulonephritis, proliferative membranous glomerulonephritis or AIDS.

FOR FURTHER INFORMATION

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CONSTITUTIONAL KARYOTYPE

DEFINITION

The karyotype is a classification of the chromosomes of a cell undergoing division. The term "constitutional karyotype" is used to define specific, non-acquired features of a person's chromosomes. The constitutional karyotype of a person is identical prenatally and postnatally. The classical distinction between prenatal and postnatal karyotypes is based on the different types of cells which are obtained and analysed before (amniotic fluid, chorionic villous biopsy and foetal blood) and after birth (blood and biopsy of fibroblast rich tissue).

PHYSIOLOGICAL ROLE

The chromosome is a structure containing many proteins and a molecule of DNA. As the support for genetic material, the chromosome undergoes many morphological changes during the cell cycle. During cell division the condensed chromosome becomes visible and can be examined microscopically. Human beings normally have 46 chromosomes. The 22 pairs of autosomes are numbered 1 to 22. The gonosomes are named X and Y and define chromosomal sex.

MAIN INDICATIONS FOR KARYOTYPE

■ INDICATIONS FOR PRENATAL DIAGNOSIS

– Maternal age 38 years old or over.

– Parental chromosomal abnormality.

– Past history in the couple of pregnancy or pregnancies with abnormal karyotype.

– Diagnosis of sex (sex chromosome related diseases).

– Signs on ultrasound.

– Serum trisomy 21 marker, risk score 1/250 or greater.

■ INDICATIONS IN A CHILD

– Neonatal hypotonia.

– Multiple malformation syndrome.

– Facial dysmorphism.

– Ambiguous genitalia.

– Mental retardation.

– Behavioural disorders.

– Growth retardation/assessment of small stature.

– Abnormal development of sexual characteristics.

– Clinical findings suggestive of a known chromosomal syndrome.

■ INDICATIONS IN ADULTS

– Investigation of chromosomal abnormality discovered in a family member.

– Repeated miscarriages.

– Sub-fertility.

– Assessment before Medically Assisted Conception.

– Prolonged amenorrhoea.

– Early menopause.

– Azoospermia or severe oligospermia.

– Transexualism.

INFORMATION

Karyotype profiling is subject to specific regulations in France. Article R162-16 of the Code of Public Health and the decree of 30 September 1997 state that a consultation certificate and signed consent by the pregnant woman must be provided to the laboratory performing the analysis for antenatal diagnosis. For constitutional postnatal diagnosis, decree no. 2000-570 of 23 June 2000 stipulates that the requesting doctor must provide the laboratory with a consultation certificate.

■ SAMPLE

Samples must be obtained sterile: Blood lymphocytes, amniotic fluid cells, chorionic villous sampling, foetal blood, bone marrow and biopsies.

Blood samples must be taken into a heparinised tube.

Biopsy samples must be placed in a culture medium to reduce the risks of culture failure.

■ SAMPLE STORAGE AND TRANSPORT

Samples must be transported as quickly as possible at room temperature to the laboratory performing the karyotype profile.

ANALYTICAL METHOD

The general principle is based on producing a cell culture to obtain dividing cells. The cells are usually blocked in the division stage (metaphase or prometaphase) by adding colchicine at the end of the culture. The cells are then placed in a hypotonic solution and then in a fixation solution containing alcohol and acetic acid. After drying the slides containing the cell preparation, chromosome labelling techniques are used to allow the chromosomes to be identified and analysed. The chromosomes are analysed by the succession of their characteristic bands. The most widely used labelling techniques allow the kariotype to be analysed by the Q, G and R bands. Other additional methods can be used to identify the constituent heterochromatin (C bands) or regions in which the ribosomal RNA genes are active (NOR). Conventional chromosomal analysis may be supplemented by molecular cytogenetics. The most widely used analysis in this case is in-situ hybridisation which provides better definition of some complex chromosomal abnormalities, identification of marker chromosomes and of chromosomal micro-abnormalities.

EXPECTED VALUES

The karyotype normally includes 46 chromosomes in human beings. Autosomal pairs are identical in men and women. The normal female karyotype includes two X chromosomes. The normal male karyotype includes an X chromosome and a Y chromosome.

PATHOPHYSIOLOGICAL VARIATIONS

The chromosomal formula is expressed according to an international nomenclature (ISCN: International System for Human Cytogenetics). The general principle is to express the total number of cell chromosomes, normal gonosomes present and abnormalities or variants present.

■ NORMAL VARIATIONS

Normal variations are seen in the position, size and number of heterochromatin regions and short-arm regions of the acrocentric chromosomes (chromosomes 13, 14, 15, 21, and 22).

■ CHROMOSOMAL ABNORMALITIES

Abnormalities may involve the total number of chromosomes (aneuploidies), autosomes or gonosomes.

A very large number of chromosomal structural abnormalities exist which may affect one or more chromosomes such as addition of material of unknown origin, telomeric association, dicentric chromosome, ring chromosome, marker chromosome, deletion, single product of a translocation involving two chromosomes, duplication, breakage, insertion, inversion, isochromosomes, whole arm translocations, reciprocal translocations and Robertsonian translocation.

In-situ hybridisation techniques with specific probes are particularly useful to identify microdeletions or microduplications. The main microdeletion syndromes for which probes are available are:

– Microdeletion syndrome 1p36.

– Wolf-Hirschhorn syndrome (4p16 deletion).

– Cri-du-Chat syndrome (5p15.2 deletion).

– Williams syndrome (7q11.23 deletion).

– Prader-Willi and Angelman syndrome (15q11.2 deletion).

– Smith-Magenis syndrome (17p11.2 deletion).

– Miller-Dieker syndrome (17p13.3 deletion).

– Di George and VCF syndromes (22q11.2 deletion).

– Kallmann syndrome (Xp22.3 deletion).

FOR FURTHER INFORMATION

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COPPER

DEFINITION

Copper is an essential trace element to life. It is a cofactor for some enzymes involved in erythropoiesis, oxidative metabolism, cell respiration and pigmentation, and it plays a key role in maintaining biological processes. Daily requirements are provided in diet (2 to 5 mg/d). Foods rich in copper are beef liver, raw mushrooms, cashew nuts, almonds, legumes, whole wheat and wholemeal rice.

METABOLISM

Copper is absorbed mostly in the gastro-intestinal tract (stomach, duodenum and jejunum). It crosses the enterocyte bound to a non-specific protein, metallothionein which is responsible for both the active transfer of the metal and its excretion in faeces, following mucosal sloughing. The protein plays a role in copper homeostasis, regulating its transfer through the intestinal wall. Mostly zinc but also calcium phytates and ascorbic acid reduce intestinal copper transfer through a competitive absorption mechanism.

Copper is found in the body in the liver, brain, spleen, heart, kidneys, pancreas, lungs and bones. In the liver, 90% of the copper is bound by an analogous process to that involved in an intestinal absorption to a metallothionein which regulates the distribution of the metal in the hepatic cell: storage and reserves (hepatocuprene), synthesis of copper-dependent enzymes, incorporation into an alpha-2-glycoprotein, apoceruloplasmin, to form ceruloplasmin which passes into the circulation.

Within the circulation, copper is found stored in red blood cells bound to a specific non-diffusing cuproprotein, haemocuprene, and circulating in plasma, 5% is bound to albumin and 95% to ceruloplasmin which is synthesised by the liver.

Copper is excreted mostly in faeces and to a small extent in urine.

INTRODUCTION

Copper is the cofactor for many enzymes which play an important role in the body:

– It is involved in the prevention of anaemia in association with iron.

– It supports the integrity of collagen and elastin (solidity of bone, joints, cartilages, arteries and veins).

– It supports the integrity of myelin in the nervous system.

– It is an excellent antiviral and anti-infectious agent.

– It is involved in the production of superoxide dismutase (SOD), an enzyme which neutralises free radicals.

– It promotes melanin formation.

INFORMATION

SAMPLE

5 ml of whole heparinised blood or 5 ml of plasma: special trace element tube (sodium heparinate) or failing this, dry plastic tube without gel or coagulation activator (beware of vacuum dry tubes as most contain this).

20 ml of a 24 hour, unacidified urine sample. Record urine output.

SAMPLE STORAGE AND TRANSPORT

Blood and urine samples can be stored and transported to the laboratory at ambient temperature or between + 2 and + 8°C.

ASSAY METHODS

Flame atomic absorption spectrophotometry. Induction coupled plasma-mass spectrometry (ICPMS).

REFERENCE VALUES

Reference values in the general population:

Blood copper is low in newborn children because of liver immaturity, which limits the synthesis of ceruloplasmin.

– Newborn children: 90 to 460 µg/l i.e. 1.35 to 6.90 µmol/l.

– From 1 to 3 months old: 300 to 800 µg/l i.e. 4.50 to 12.00 µmol/l.

– From 3 to 6 months old: 600 to 1000 µg/l i.e. 9.00 to 15.00 µmol/l.

– From 6 months old to 15 years old: 700 to 1400 µg/l i.e. 10.50 to 21.00 µmol/l.

In adults: 700 to 1500 µg/l i.e. 10.9 to 23.5 µmol/l.

Urine copper < 150 µg/24H i.e. inf. to 2.4 µmol/24 h.

NON-GENETIC ABNORMALITIES OF COPPER METABOLISM

HYPERCUPRAEMIA

Oestrogens, androgens, progestogens, catecholamines and thyroid hormones raise blood copper, hence the increase in plasma copper in hyperthyroidism, pregnancy and oral contraception, etc.

Blood copper is also increased in:

– Inflammatory and/or infectious disorders.

– Addison's disease (corticosteroids lower blood copper).

– Liver diseases, (hepatitis and cirrhosis).

– Blood dyscrasias, (leukaemia, Hodgkin's disease, lymphoma).

– Haemochromatosis.

– Some cancers (gastro-intestinal system, uterus, breast and bronchi).

HYPOCUPRAEMIA

– Severe nutritional deficiencies (Kwashiorkor).

– Long-term parenteral nutrition without supplementation.

– Gastro-intestinal malabsorption (sprue and coeliac disease).

– Widespread burns.

– Nephrotic syndrome.

– Hypothyroidism.

– Corticosteroid treatment.

GENETIC ABNORMALITIES OF COPPER METABOLISM

■ WILSON'S DISEASE

This is a rare autosomal recessive disorder with a prevalence of 1/30,000 births, generally affecting children during the second decade of life. The clinical features are:

– Hepatic (liver failure, hepatitis, cirrhosis) in 40 to 70% of cases.

– Neuropsychiatric (muscle tone disorders and psychiatric disorders).

– Pigmentory (Kaiser Fleischer rings in the eye, pathognomonic of the disease).

– Haematological (haemolytic jaundice and moderate pancytopaenia) and renal disorders (tubular damage due to copper deposition).

The disease is due to a gene mutation coding for a mutated ATP7B protein belonging to the type P ATPases family required for copper excretion in bile and its incorporation into ceruloplasmin.

Biologically, ceruloplasmin is low or zero and very rarely normal. Blood copper is lowered by 30 to 50% (transient hypercupraemia occurs during haemolytic crises). Urine copper is greatly raised (often 10 times normal) and copper concentrations in the liver are invariably raised.

■ MENKES DISEASE

This is a rare X-linked recessive disorder, the abnormality for which is located in Xq13.3, ATP7A coding for an intracellular copper transport protein. The resultant deficiency in free copper adversely affects the function of copper-dependent enzymes causing symptoms of *in-utero* growth retardation and axial hypotonia in the first months of life with spasticity, seizures and hypothermia. Death generally occurs early in childhood. The diagnosis relies on the measurement of copper in serum (lowered, as is ceruloplasmin) and in skin fibroblasts (raised). Genetic studies can be used to detect carriers and antenatal diagnosis is possible.

COPPER TOXICITY

Rare cases of poisoning:

– Accidental acute ingestion of copper sulphate.

– Ingestion of drinking water containing copper sulphate (algicide in reservoirs).

– Ingestion of acid water dissolving copper in pipe work.

– Excessive trace element supplementation.

– Inhalation of copper-based fungicides.

– Inhalation of wine grower's Bordeaux mixture (CuSO4) copper sulphate.

– Dyes with deodorants or antiseptics.

Symptoms are relatively non-specific with gastro-intestinal features (nausea, vomiting and diarrhoea), headache, dry mouth, neuropsychiatric reactions, acute renal insufficiency with intravascular haemolysis in serious cases.

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CORTICOSTEROID BINDING GLOBULIN (CBG)

DEFINITION

Corticosteroid Binding Globulin (CBG) or transcortin is a 383 amino acid glycosylated α2-globulin. It is part of the serine-protease inhibitor super-family and is synthesised mostly in the liver, although it may be produced in other tissues as small concentrations of mRNA coding for CBG have been found in the endometrium, testes, lung and kidney.

CBG binds mostly to cortisol with a binding constant of $7.6x10^{-7}$ M. A large proportion of cortisol (89.5%) circulating in the blood is therefore bound to this protein. CBG can also bind to other endogenous steroids, such as 11-desoxycortisol (S compound), corticosterone, 17-hydroxyprogesterone, progesterone, desoxycorticosterone (DOC) and aldosterone, but with lower affinity. It does not however bind to synthetic glucocorticoids except for prednisone and prednisolone.

CBG is a high affinity but low capacity cortisol binding system. Its maximum capacity is reached at physiological plasma cortisol concentrations of 600-700 nmol/l or 218-253 ng/ml. As a result, when cortisol concentrations increase above this value, the proportion of the unbound fraction of cortisol, and other steroids (aldosterone and DOC) increases, with respect to the bound steroids.

Synonyms:

CBG = Corticosteroid Binding Globulin = Transcortin.

INTRODUCTION

The physiological role of binding to CBG is unknown. Classically, bound cortisol is a reserve pool as only the unbound fraction can enter target cells and exert its biological activity or be taken up by the liver to be broken down. This circulating buffer system appears to be necessary as there is no storage form in adrenocortical cells.

High affinity CBG specific receptors have also been found on target cell membranes. Only unbound CBG can bind to these receptors although glucocorticoids can bind to the CBG-receptor complex and activate adenyl-cyclase. This mechanism provides a rapid method of action for glucocorticoids when they are present in low concentrations.

From a metabolic perspective, the half-life of CBG is 5 days.

INDICATIONS FOR MEASUREMENT

CBG is measured in order to interpret plasma cortisol concentrations as they enable the unbound and biologically active cortisol to be calculated. The concentration of unbound cortisol is obtained from a mathematical equation including total cortisol, CBG and albumin concentrations. It can also be used to calculate the free cortisol index which is the ratio of total cortisol to CBG concentrations.

INFORMATION

SAMPLE

CBG is assayed in serum. The samples may be taken at any time of the day and any day of the menstrual cycle in women.

QUESTIONS FOR THE PATIENT

Corticosteroid and oestrogen treatments must be reported.

SAMPLE STORAGE AND TRANSPORT

Centrifuge, decant and freeze within 4 hours of sampling.

If transported, serum samples must be sent frozen at – 20°C. They can be stored frozen for 6 months to 1 year.

ASSAY METHODS

CBG is assayed immunologically usually with a radioactive tracer.

USUAL VALUES

Usual values in adults, regardless of sex, are between 30 and 50 ng/ml.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

CBG can be detected in the foetus from the start of pregnancy and concentrations increase regularly to reach approximately half of the values found in adults at term. This explains transplacental transfer of steroids from the foetus to the mother. Concentrations continue to increase during the first 6 weeks after birth.

Concentrations in pre-pubertal children are slightly higher than in adults. They fall slightly with age but do not vary during the 24 hour cycle, between the sexes or during the menstrual cycle.

On the other hand, CBG increases during pregnancy and particularly in the third trimester.

IATROGENIC VARIATIONS

CBG concentrations increase by a factor of 2 to 3 in response to oestrogens administration. The effect is dose-dependent and occurs 2 to 4 days after starting treatment reaching a maximum after 14 days, which is maintained throughout treatment. Concentrations return to normal 7 to 10 days after stopping treatment. Conversely, its production is inhibited by glucocorticoids and interleukin-6. It should be noted that short-term administration of pharmacological doses of glucocorticoids or ACTH has no effect on CBG.

PATHOLOGICAL VARIATIONS

CBG concentrations are low in patients with:

– Cushing's syndrome,

– Polycystic ovarian syndrome,

– Cirrhosis,

– Hypothyroidism,

– Hepatocellular insufficiency,

– Nephrotic syndrome or any other disease associated with protein loss.

CBG concentrations are raised in patients with chronic active hepatitis and in those on anti-epileptic treatments.

Two inherited variants have been described. In one the cortisol binding capacity is reduced and in the other it is increased. Complete CBG deficiency has also been described.

CBG remains normal in adrenal insufficiency.

With all of these variations in CBG, free cortisol concentrations remain within physiological limits whereas total cortisol concentrations follow the variations in CBG in parallel.

FOR FURTHER INFORMATION

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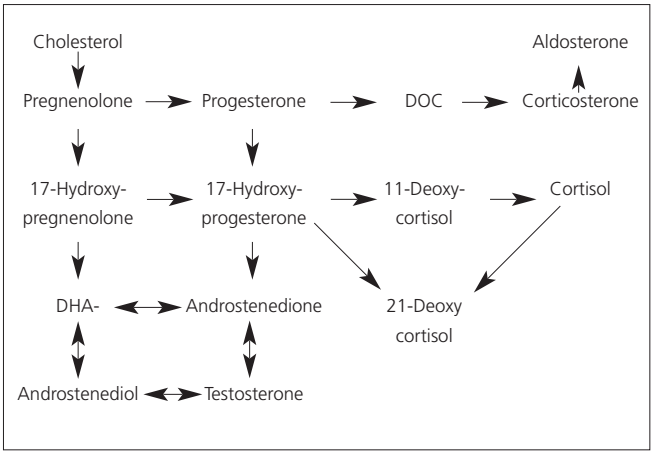
CORTICOSTERONE

DEFINITION

As is the case for all steroids, corticosterone, is derived initially from cholesterol. In the first step, cholesterol is subjected to oxidative cleavage of its side chain to generate pregnenolone. This is then converted to progesterone by 3β-hydroxysteroid dehydrogenase. 21-hydroxylase acts on progesterone to produce 11-deoxycorticosterone (DOC), which is transformed into corticosterone by 11-β-hydroxylase.

Corticosteroid biosynthesis



Corticosterone is synthesised in all three zones of the adrenal cortex; fasciculata, reticulata and glomerulosa. It is the precursor of aldosterone, which is synthesised only in the glomerulosa. Aldosterone synthase (CYP11B2) is involved in hydroxylation of corticosterone at C18 to generate 18-hydroxycorticosterone. Subsequent oxidation of this molecule produces aldosterone.

Much more corticosterone is generated in the zona fasciculata than in the glomerulosa. There is a correlation between the secretion of corticosterone and that of cortisol and both are ACTH dependent.

Most of the circulating corticosterone is bound to transport proteins. Indeed, 77.5% is bound to CBG (corticosteroid binding globulin, or transcortin), 19% to albumin and a tiny amount (0.1%) to SHBG.

Corticosterone has gluco- and mineralo-corticoid properties.

It is metabolised in the liver by successive reduction, firstly at the C3-C4 double bond, then at the C3 ketone residue, thus generating the tetrahydrogenated derivative: tetrahydro-corticosterone (THB). The reduction of the C20 ketone group of this molecule produces the hexahydrated derivative. These metabolites are conjugated with glucuronic acid and are then excreted in the urine.

INDICATIONS FOR MEASUREMENT

The measurement of corticosterone is particularly useful in the investigation of arterial hypertension.

INFORMATION

SAMPLE

Assay for corticosterone can be performed either in serum or in EDTA or heparinised plasma. Lipaemic or haemolysed specimens do not pose a problem in view of the fact that the assay technique involves extraction and chromatography. However, collection should be performed before 10 am because of circadian variation in corticosterone concentration.

NECESSARY INFORMATION

As corticosterone is synthesised in the adrenal cortex, corticosteroid medication (whether systemic, topical or intra-articular) must be recorded. Similarly, it is important to explain if the sample is one of those generated by a Synacthen® stimulation test.

SAMPLE STORAGE AND TRANSPORT

Plasma or decanted serum can be stored and sent to the laboratory at + 4° C.

Frozen specimens can be kept for 6 months to 1 year at – 30° C.

ASSAY METHOD

The radio-immunoassay is usually preceded by a purification stage, in which extraction is followed by chromatography. This is necessary in order to achieve good specificity.

USUAL VALUES

Results are expressed in ng/ml or nmol/l. The conversion from ng/ml to nmol/l necessitates multiplication by a factor of 2.886.

Adult values of corticosterone in the morning range from 2 to 8 ng/ml. The results are similar in men and women, but there is circadian variation in levels, resembling that of cortisol.

DYNAMIC TESTS

Synacthen® stimulation tests

Blood samples are collected at 30 and 60 minutes after intramuscular or intravenous injection of 0.25 mg of Synacthen® (synthetic 1-24 corticotrophin). The serum or plasma corticosterone concentration reaches a maximum of 14.9 to 67.0 ng/ml.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

In children there is no significant alteration with age or gender and values are comparable to those in adults.

PATHOLOGICAL VARIATIONS

Reduction in concentration

<u>Primary adrenocortical insufficiency (Addison's disease)</u>

In primary adrenocortical failure (Addison's disease), all three zones of the adrenal cortex are affected by the disease process. There is, therefore, deficiency of secretion of all the adrenal

steroids (glucocorticoids, mineralocorticoids and androgens). Clinical symptoms do not appear until some 90% of the cortex is destroyed (see cortisol and aldosterone).

Laboratory diagnosis depends on finding low concentrations of all the adrenal steroids (cortisol, corticosterone, aldosterone and DHA sulphate). In contrast, ACTH and renin levels are elevated. The interpretation of cortisol values is not straightforward. Whereas a concentration of less than 30 ng/ml confirms the diagnosis and one of greater than 190 ng/ml rules it out, levels between these limits are more difficult to judge and it is necessary to resort to dynamic testing, in particular to the Synacthen® test.

Secondary adrenocortical insufficiency

In adrenocortical failure secondary to pituitary corticotrophic deficiency (overall anterior pituitary failure or corticotrophic insufficiency secondary to chronic corticosteroid therapy), as corticosterone synthesis is ACTH dependent in the same way as that of cortisol, then the level is reduced in parallel with the values of cortisol and the adrenal androgens. Aldosterone concentration, however, is not altered because it is controlled by the renin-angiotensin system, which is not affected by adrenal cortical dysfunction.

11b-hydroxlase deficiency (see 11-deoxycortisol and DOC)

This enzyme abnormality represents 10 to 15% of known defects of the adrenal synthetic pathways. It affects glucocorticoid and mineralocorticoid pathways but not the pathway for androgens. It results in an increase in adrenal synthesis of androgens and the 11-deoxy- precursors of cortisol and corticosterone: 11-deoxycortisol and 11-deoxycorticosterone (DOC), respectively.

The excessive production of androgen results in virilisation of the female foetus, which can be minor or severe, and which is similar to that produced by 21β-hydroxylase deficiency. What differentiates the two hyperplasias is the presence of hypertension in 11β- hydroxylase deficiency. Indeed, the accumulation of DOC, which has substantial mineralocorticoid activity, explains both the high frequency of hypertension resulting from sodium retention and the fact that a salt-losing syndrome is rare.

Clinically, there are forms which appear early on and those whose presentation is delayed (see 11-deoxycortisol).

Whenever there is evidence of hyperandrogenism in association with arterial hypertension, it is essential to consider the possibility of an 11β-hydroxylase deficiency.

The laboratory diagnosis depends on finding reduced cortisol and corticosterone concentrations in the presence of increased serum or plasma 11-deoxycortisol and DOC. Androgen levels are also increased, except for that of 11β-hydroxyandrostenedione. The response of 11-deoxycortisol and DOC to Synacthen® stimulation is dramatic.

■ **Increased concentration**

CYP11B2 (aldosterone synthase) deficiency (see aldosterone)

This is a rare condition and is an autosomal recessive disorder. Clinically, these children present with severe salt loss associated with retarded growth and development. It is secondary to an enzyme deficiency in the aldosterone synthetic pathway involving aldosterone synthase (CYP11B2). This enzyme has two distinct actions. These are CMO I, corticosterone 18 methyloxidase I, responsible for hydroxylation of corticosterone and CMO II, which converts the 18 hydroxyl group into an aldehyde.

In CMO I deficiency, aldosterone is undetectable and 18-hydroxycorticosterone (18OHB) low, while corticosterone is increased. In CMO II deficiency, 18OHB is highly elevated and aldosterone is detectable.

17α-hydroxylase deficiency

With this enzyme defect, where cortisol synthesis is blocked, ACTH hypersecretion results in increased production of all the precursors above the block. This includes progesterone, pregnenolone and the steroids of the mineralocorticoid pathway (DOC and corticosterone). The defect also affects androgen and oestrogen pathways. This results in primary amenorrhoea in girls and male pseudo-hermaphroditism in boys. In both sexes, there is hypermineralocorticoidism as a consequence of increased production of DOC, corticosterone and their 18 hydroxylated derivatives. Aldosterone production is poor. The clinical picture is one of secondary hypoaldosteronism.

Hypercorticoidism

As is the case for the other corticosteroids, the production of corticosterone is high in all forms of hypercorticoidism. This includes ACTH dependent Cushing's syndrome (Cushing's disease and Cushing's syndrome secondary to ectopic secretion of ACTH) and non-ACTH dependent Cushing's syndromes such as primary adrenocortical hyperactivity due to benign and malignant tumours, or to adrenal hyperplasia or dysplasia.

FOR FURTHER INFORMATION

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CORTISOL

DEFINITION

Cortisol (hydrocortisone) is the principal circulating glucocorticoid. It is secreted by the zona fasciculata of the adrenal cortex. Secretion is stimulated by ACTH from the pituitary and this, in turn, is dependent on hypothalamic secretion of CRH (corticotrophin releasing hormone). The physiological effects of cortisol are seen mainly on carbohydrate metabolism (hyperglycaemia and insulin resistance), protein metabolism (anabolic in the liver and catabolic in skeletal muscle and the skin) and on lipids (lipolysis). Cortisol also possesses anti-inflammatory, immunosuppressive and mineralo-corticoid properties. The latter comprise water and sodium retention and potassium excretion. In the blood, it is 90% bound to transcortin (CBG or cortisol binding globulin), 5% to albumin and 5% to red cells. Oestrogens increase plasma cortisol by stimulating hepatic production of CBG. Cortisol in saliva derives solely from the free fraction of blood cortisol.

INDICATIONS FOR MEASUREMENT

Laboratory investigation for states of cortisol excess (Cushing's syndrome) and deficiency (adrenocortical insufficiency).

INFORMATION

SAMPLE

Serum, heparinised or EDTA plasma. Sample tubes with gel separator are not advised.

Blood sampling: Because of circadian variation in cortisol secretion (maximum at 08.00 hrs and minimum at 24.00 hrs), it is essential to record the time of sampling (usually 08.00 hrs and 16.00 or 20.00 hrs). Any physical effort or mental stress should be avoided before sampling because these affect cortisol levels. It is preferable to take fasting samples.

Consumption of alcohol should be avoided in the week before the specimen is taken.

Saliva sample: Measurement of levels in saliva eliminates the effect of variations in CBG. Saliva is taken into a tube which is specially designed for this purpose (Salivette®). Salivation may be stimulated by chewing a neutral gum for about 5 to 10 minutes. The patient must abstain from food and acidic drinks for a period of at least 30 minutes before the collection of fluid. Neither should the patient brush his or her teeth, so as to avoid contamination of the sample with blood. Collection can also be performed by the patient at home (for samples required between 23.00 hrs and midnight). Such samples can be taken to the laboratory the next day.

ESSENTIAL INFORMATION

Current treatment? Oestrogen treatment (combined contraception or menopausal hormonal replacement treatment), glucocorticoid treatment together with the name of the preparation, the dose, the duration and any date when treatment might have been interrupted (treatment is sometimes interrupted on medical advice before sampling).

Pregnancy?

SAMPLE STORAGE AND TRANSPORT

Plasma or serum: 5 days at + 4° C or at - 20° C for longer periods.

Saliva: Freeze at – 20° C within 4 hours of collection.

ASSAY METHODS

Serum cortisol: Chemiluminescence

Salivary cortisol: Radio-immunoassay.

REFERENCE VALUES

These can vary according to the method used (conversion factor: ng/ml x 2.759 = nmol/l).

For reference: serum or plasma cortisol

Adults			
	Before 09.00 hrs	70 to 250 ng/ml	
	Night	20 to 90 ng/ml	
Children	**Age (years)**	**Boy (ng/ml)**	**Girl (ng/ml)**
	< 6	70 – 170	50 – 110
	6 – 8	70 – 170	50 – 130
	9 - 10	70 – 200	60 – 180
	10- 15	70 – 250	70 - 250

Salivary cortisol

morning	1 à 7,5 ng/ml
Night (from 23.00 to 02.00 hrs)	< 1 ng/ml

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

– Secondary to nutritional state: fasting induces a small increase in level.

– During pregnancy: increase in CBG synthesis results in elevation of blood cortisol (salivary cortisol unchanged).

PATHOLOGICAL VARIATIONS

Cushing's syndrome

The criteria for diagnosis are an increase in free urinary cortisol and disappearance of the circadian rhythm of cortisol and ACTH secretion. Basal cortisol and ACTH levels are sometimes elevated. The short dexamethasone suppression test is usually negative. There can also be non-specific abnormalities, such as hypokalaemia, hyperglycaemia, metabolic alkalosis and hyperleucocytosis. The causes of Cushing's syndrome are as follows:

– Cushing's disease: pituitary micro-adenoma (usually) causing over-production of ACTH and adrenocortical hyperplasia;

– Adrenal: adenoma or malignant tumour;

– Paraneoplastic: ACTH production by a non-pituitary tumour (most often carcinoma of lung).

Secondary hypercortisolaemia

Cortisol secretion is increased physiologically in a variety of situations, such as septicaemia, trauma, surgery, alcohol abuse, hepatic insufficiency, advanced renal failure and some serious psychiatric illnesses.

Hypocortisolaemia

24 hour urinary cortisol excretion, examination of the circadian rhythm of cortisol secretion and ACTH measurement are used for diagnosis when the clinical picture is suggestive. An increase in ACTH suggests primary adrenal insufficiency, whereas normal or reduced ACTH levels are in favour of a pituitary cause. Dynamic testing is sometimes performed when the situation is more obscure.

Addison's disease, or adrenocortical atrophy, is usually of auto-immune origin and, more rarely, tuberculous, inflammatory or due to tumour. In the majority of cases secondary adrenal insuffficiency is the result of prolonged glucocorticoid treatment. Such treatment causes suppression of the hypothalamo-pituitary-adrenal axis. A Synacthen® stimulation test is sometimes necessary to assess adrenocortical reserve. Finally, adrenal insufficiency can result from pituitary disease (atrophy or pituitary tumour).

FOR FURTHER INFORMATION

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■ Schlienger JL., André G., Chabrier G., Reiss D., Simon C., *Explorations fonctionnelles en endocrinologie et métabolisme,* 2e Éd, Paris: Expansion Scientifique française, 1993.

CORTISONE

DEFINITION

Cortisone was the first glucocorticoid isolated from the adrenal gland, although it is in fact an artefact, due to oxidation of the 11β–hydroxy cortisol group during the extraction and purification stages. In addition, once cortisol is finally identified as being the major natural glucocorticoid it was called "hydrocortisone", a name which has remained with it in the pharmacopoeia. Cortisone or compound "E" in the Kendall nomenclature therefore has the structure of cortisol less a hydrogen atom on the 11 functional group (cortisol is compound "F" in the same nomenclature).

INTRODUCTION

Cortisone has negligible intrinsic glucocorticoid activity as it does not bind to the cortisol receptor. It can however be converted into cortisol by the effect of a bidirectional enzyme, 11β-hydroxysteroid dehydrogenase, type I and because of this may indirectly have the physiological effects of cortisol (*see Cortisol*). Conversely, cortisol can be inactivated into cortisone through the effect of 11β-hydroxysteroid dehydrogenase type II.

Cortisone is present both in blood and in urine.

In the blood, like cortisol, cortisone is mostly bound to transcortin, the carrier protein produced by the liver, synthesis of which is sensitive to the action of the sex steroids, being increased by estrogens and reduced by androgens.

INDICATIONS FOR MEASUREMENT

There are no particular indications for cortisone measurement in routine clinical practice as it is only a metabolite of cortisol with no specific physiological activity.

Many studies however have examined pathological variations in 11 β-hydroxysteroid dehydrogenase type II, deficiency of which may be responsible for relative cortisol excess. Cortisol can bind to the mineralocorticoid receptor and a defect in receptor inactivation has been suggested as a possible cause of low renin hypertension with hypokalaemia. Mutations in this enzyme have also recently been described which are responsible for an apparent excess of mineralocorticoids. It is therefore in this limited context that measurement of both cortisone and cortisol may be indicated.

Conversely, significant variations in the cortisol/cortisone ratio have been described with weight although recent findings have been rather contradictory, some suggesting an increase in 11β-hydroxysteroid dehydrogenase type 1 activity in obesity, an increase which is liable to contribute to insulin resistance, and others showing weight loss in obese patients on a calorie restricted diet is associated with a rise in the activity of the enzyme.

INFORMATION

SAMPLE

Measurement is recommended on heparinised plasma, the sample preferably being taken in the morning. Always record the sampling time carefully.

QUESTIONS FOR THE PATIENT

Current treatment, particularly anti-hypertensive?

Record height and weight.

SAMPLE STORAGE AND TRANSPORT

Stable one week at + 4°C.

ASSAY METHOD

This is a physico-chemical assay method using high performance liquid chromatography (HPLC). Results are expressed in ng/ml or in nmol/l. The conversion factor is 2.766: 1 ng/ml + 2.766 nmol/l.

NORMAL EXPECTED VALUES

As an indication: 20 to 45 nmol/l i.e. 7.2 to 16.2 µg/l.

FOR FURTHER INFORMATION

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COTININE

DEFINITION

Nicotine is the major alkaloid in tobacco which is responsible for the dependency, hence the role of its measurement. It is present in cigarette smoke and is rapidly absorbed through the lungs (approximately 70% of inhaled nicotine is absorbed), crosses the pulmonary alveolar capillaries and is then carried to the different organs including the brain (which it takes 7 seconds to reach) and liver before being removed in urine. A smoker absorbs an average of 1 mg of nicotine per cigarette smoked. It is not however a good marker of smoking as its half-life in the circulation is very short (2 hours). Blood nicotine concentrations in a smoker are extremely low and fluctuate, and urinary excretion is short and discontinuous.

Cotinine is a nicotine degradation product produced by the liver. Nicotine is oxidised into cotinine which is found and may be measured in urine (urinary cotinine). Cotinine is currently considered to be the marker of choice for smoking as it has a long half- life (16 to 22 hours), blood concentrations remain stable and its excretion is prolonged.

INTRODUCTION

Urine cotinine is a marker of choice for doctors who manage smoking cessation, which has long been treated empirically. It is useful in conjunction with other factors such as the number of cigarettes smoked daily, the approximately amount of nicotine absorbed, the Fagerström dependency test and measurement of expired carbon monoxide (CO). For the patient, urine cotinine measurements can be used to provide an objective quantification of smoking and therefore to better adjust and monitor cessation with or without treatment. Free cotinine differs from the original nicotine molecule only by the addition of a single oxygen atom and it is an accurate reflection of the persons exposure from smoking.

INDICATIONS FOR MEASUREMENT

In smokers who wish to stop

Urine cotinine measurement helps in the diagnosis, precisely quantifies the patient's exposure from smoking and therefore can be used to help to tailor treatment. When reducing substitution treatment it can be used to adjust the substitution doses and reduce these as exposure falls. It can also be used to correct for possible substitution overdose or under dose to prevent withdrawal effects. Finally it can be used to monitor abstinence from smoking when following up smoking cessation.

In smokers after a clinical event

Smokers who have suffered a coronary event generally manage to stop smoking during the hospitalisation phase although these patients, who are usually highly dependent, start smoking again once they return home. Measurement of urinary cotinine forms part of the management of these patients in the same way as clinical follow-up (improved lifestyle and dietary monitoring) and psychological follow up (cognitive behavioural therapy, monitoring for depression).

In non-smokers

Urine cotinine measurement may be used to measure exposure to tobacco smoke in people exposed to smoke in the home or work environment.

INFORMATION

SAMPLE

Random urine sample (10 ml).

QUESTIONS FOR THE PATIENT

Circumstances in which the measurement is being requested (active smoking, passive smoking or substitution treatment) and concomitant drugs (nicotine patches, varenicline, bupropion hydrochloride, etc.). Active smokers should also be asked how many cigarettes they smoke daily in order to assess the theoretical amount of nicotine absorbed over 24 hours.

SAMPLE STORAGE AND TRANSPORT

Store and transport the sample at +4°C.

ASSAY METHODS

Chromatographic methods: Gas phase chromatography linked to mass spectrometry or high performance liquid chromatography with UV or diode bar detection.

Immunological methods: Isotopic method or using an enzymatic or luminescent marker.

NORMAL EXPECTED VALUES

As an indication:

Non-smokers not exposed to tobacco: Urine cotinine < 10 µg/l

Passive smoking: Urine cotinine < 100 µg/l

Active smoking: Urine cotinine: 200 to 5000 µg/l.

PATHOLOGICAL VARIATIONS

It is recognised that smokers' own estimates of their smoking habit is often incorrect and urine cotinine provides accurate information about their smoking habit. In addition, urine cotinine can clearly distinguish active smokers from passive smokers from non smokers, with concentrations in a smoker being 50 to 100 times higher than in a non-smoker exposed to tobacco smoke (passive smoking).

– In smokers who wish to stop: Urine cotinine concentrations can be used to confirm cessation as they return to the same as those in a non-smoker one week after stopping smoking. Measurement during substitution treatment can be used to confirm absorption of transdermal nicotine and adherence to treatment. When substitution treatment is being reduced, urine cotinine concentrations fall in parallel with the reduction in substitution, returning to zero when substitution is stopped. During bupropion hydrochloride treatment, urine cotinine concentrations are zero if the patients abstain from smoking but remain positive if the patient continues to smoke on treatment.

– In the post-cessation follow up phase, urine cotinine concentrations fall as soon as the person stops smoking and then return to zero although rise again immediately if the person starts smoking again.

– In non-smokers, the presence of cotinine in urine confirms passive smoking. Concentrations found in this situation are usually equivalent to those seen in a smoker smoking 3 to 5 cigarettes daily.

FOR FURTHER INFORMATION

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CREATINE KINASE AND ISOENZYMES

DEFINITION AND SYNONYMS

Creatine kinase (CK) also called creatine phosphokinase (CPK) is an enzyme found in the mitochondrial cell or in the cytosol. It catalyses the phosphorylation reaction of creatine by ATP into creatine phosphate (creatine+ATP↔creatine-phosphate + ADP). It is present in many tissues in the body, mostly in skeletal muscle and cardiac muscle. It is also found in other organs including the brain, kidney and gastro-intestinal tract. CK is a 360 amino acid molecule formed from a 2 sub-unit dimer: M (*muscular*) and B (*brain*). Three CK isoenzymes exist, obtained from combinations of these sub-units: CK-BB (or CK-1), CK-MB (or CK-2) and CK-MM (or CK-3). Macro-CK is another form of isoenzyme said to be "atypical": type 2 macro-CK is the complex formed from CK-BB bound to an IgG unit (or more rarely CK-MM bound to an IgA), the pathological significance of which is poorly understood. The type 2 macro-CK is formed from polymerised mitochondrial CK and is found in some forms of neoplasia.

INTRODUCTION

CK is involved in the muscle contraction by phosphate exchanges in tissues. The tissue localisation of the isoenzymes varies, CK-BB being found in brain and also the gastro-intestinal tract, respiratory system and bladder. It is also found in the foetus. CKMB is found in large proportions in cardiac muscle whereas CK-MM is to a very large extent contained in the skeletal striated muscle. The CK-MM and CK-MB isoenzymes are present physiologically in serum.

Isoenzymes	Serum proportion in a normal person
CK-MM	> 95% of total CK
CK-MB	< 5% of total CK
CK-BB	undetectable

INDICATIONS FOR MEASUREMENT

– Diagnosis and monitoring of myocardial infarction (CK and CK-MB) and myopathy, particularly Duchenne dystrophy (CK and CK-MM).

– Myositis and dermatomyositis (CK and CK-MM).

– Some central nervous system (CNS) disorders with rupture of the blood-brain barrier (CK-BB) and some malignant diseases (CK-BB and macro-CK).

INFORMATION

■ SAMPLE

CK activity is preferably measured on serum although may be measured on heparinised plasma. The tourniquet time must be short and haemolysed specimens must not be used.

CK isoenzymes are separated and measured on serum, avoiding haemolysed samples.

■ QUESTIONS FOR THE PATIENT

Age and sex?

Ethnic origin?

Are you pregnant?

Recent intense physical efforts (less than 3 days)?

Seat of intramuscular drugs which could interfere with CK measurement?

■ SAMPLE STORAGE AND TRANSPORT

CK is difficult to store as the enzyme is unstable and loses its activity very quickly. Samples may be stored for 24 hours at room temperature and approximately 10 days at + 4°C. Isoenzyme stability varies and depends mostly on the methods used.

ASSAY METHODS

Total CK: total serum CK activity is measured by a kinetic enzymatic method. The principle uses dephosphorylation of creatine phosphate linked to other auxiliary reactions resulting in NADPH formation. Measurement of NADPH production per minute is measured spectrophotometrically at 340 nm. The reference method is proposed by the *International Federation of Clinical Chemistry* (IFCC). All commercially available methods are automated and use ready-to-use reagents.

Isoenzyme separation: this is performed by alkaline agarose gel electrophoresis, the fractions being revealed colourimetrically. It can be used to show physiological isoenzyme (CK-MM and CK-MB) and also atypical macro-CK isoenzymes. The type 1 macro CK is identified by its electrophoretic migration between the CKMB and CKMM band, whereas the type 2 macro-CK co-migrates with CK-MM.

Assay of isoenzymes: immuno-inhibition measurement of CKMB is increasingly rarely used as it is not particularly sensitive and impossible to interpret if the serum contains atypical CK forms (BB isoenzymes and macro-CK). It is being replaced by an automatable immunometric mass method, which is far more sensitive and in which CK-MB activity is quantified by mass of protein and expressed in µg/l. CKBB may be assayed immunoradiometrically.

Assay of CK-MB and CK-MM isoforms:

This is performed by electrophoretic separation on high voltage electrophoretic separation on agarose gel (900V) revealed enzymatically and quantified by fluorescence. The result is obtained rapidly in 30 minutes.

NORMAL EXPECTED VALUES

Total CK: the values used vary depending on experimental conditions (as an example):

at 37°C (IFCC method)	
Adult women	60 - 140 U/l
Adult men	80 - 200 U/l

at 30°C	
At birth, cord blood	70 - 580 U/ l
Newborn babies	35 - 145 U/ l
Infants and children < 10 years	20 - 120 U/ l
Adult women	20 - 100 U /l
Adult men	20 - 200 U /l

CK-MB Isoenzyme

CK-MB enzymatic activity	10 –20 U/l
CK-MB immunometric assay	< 2 µg/l

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Age: CK rises in newborn babies up to 1 year old. Total CK is higher in children than in adults as they are often more physically active.

Race: Total CK is higher in black or Caucasian people.

Sex: CK is lower in women than in men associated with the difference in muscle mass.

Physical exercise: This cause a rise in CK concentration which m ay increase to up to 50% of its value returning to normal within 3 days after the exercise. Conversely, CK may fall in people with low muscle mass, such as the elderly or bed bound, cachetic patients and in some incapacitating diseases.

Pregnancy: CK falls during pregnancy.

Drugs: When given intramuscularly these can increase CK concentration.

■ PATHOLOGICAL VARIATIONS

Myocardial infarction (MI): Serum CK rises in acute coronary syndromes, particularly in MI, between 3 and 8 hours after the onset of pain reaching a peak value at between 22 and 26 hours and returning to normal over approximately 72 hours. CK-MB measurement is preferable to total CK because of its higher cardiac specificity. It provides early diagnosis and is used in monitoring the outcome of myocardial necrosis. It is however being less used since the availability of troponin measurements. CK-MB is also increased after cardiac surgery. Measurement of the isoenzymes isoforms is even more sensitive and specific than CK-MB: CK MM3/MM1 and CK MB2/MB1 ratios rise early within 1 to 2 hours after the onset of pain. A rise in the ratio of tissue to serum isoforms also appears to indicate massive rapid myocardial necrosis.

Other diseases: Serum CK is greatly elevated (mostly due to the CK-MM fraction) in some myopathies and in particular in Duchenne dystrophy. It is raised in polymyositis and dermatomyositis, with a predominant CK-MM fraction. Serum CK-MM is raised in all cases of muscle trauma and in hypothyroidism. CK-BB is raised in some CNS diseases (head injury, meningitis and in some neoplastic diseases).

FOR FURTHER INFORMATION

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CREATINE

DEFINITION

Creatine is a natural amino acid present mostly in muscle cells. Serum concentrations depend on the body muscle mass and consumption of creatine by creatine kinase (CK).

A proportion of physiological creatine is provided from the diet and another part from endogenous synthesis. Creatine deficiency does not exist. Metabolic and transport abnormalities exist resulting from genetic mutations which cause neurological and epileptic diseases.

INTRODUCTION

CREATINE: ORIGIN, STORAGE AND CATABOLISM

A 70 kg adult male has approximately 120g of creatine in his body, 95% of which is in the skeletal muscles.

Endogenous synthesis from amino acids provides more than 50% of the creatine in the adult body. Synthesis occurs in the liver, kidneys and pancreas. The other half is provided from the diet (beef, pork and oily fish). Creatine is carried throughout the body by specific transporters.

Creatine update by skeletal muscle is almost irreversible. This is the primary place of creatine storage in the form of phosphocreatine, although creatine is found in other tissues including the brain. It is transported from its sites of synthesis through the blood circulation to muscle and brain.

Degradation of creatine results in the formation of creatinine. Approximately 2 g of creatine is degraded daily in a healthy adult (approximately 1 to 2% of the free creatine in muscle is converted into creatinine). Creatinine is then excreted in urine by glomerular filtration. Conversely, creatine is filtered by the glomerulus and reabsorbed almost entirely by the proximal convoluted tubule.

PROTEIN WITH MUSCLE ACTIVITY

During muscle contraction creatine is phosphorylated into phosphocreatine. Phosphocreatine provides a phosphate molecule to ADP to convert it into the energy molecule ATP.

Creatine carries out several metabolic functions and is an immediate energy reserve, which can be used for muscle contraction (chemical synthesis of ATP leading to mechanical energy). It is therefore essential in the muscle contraction mechanism.

Creatinine is also involved in energy metabolism in the alactic anaerobic pathway. This pathway leads to energy production in the absence of oxygen without the production of lactic acid and can cover the first 20 seconds of intense exercise. The first two minutes use the anaerobic lactic pathway where muscle glycogen is converted into pyruvate and then into lactate without use of oxygen. After the first two minutes, the oxygen using energy pathway takes over, oxidising glucose and fatty acids.

INDICATIONS FOR MEASUREMENT

Creatine may be measured to investigate the presence of muscle disease.

INFORMATION

SAMPLE

Creatine can be measured in serum or a 24 hour urine sample. Urine must be collected from one morning to the following morning. The container must not contain any acid or alkaline preservatives which could convert creatine into creatinine. In view of the variations in urinary creatine excretion, it is recommended that a minimum of two x 24 hour urine samples be collected.

QUESTIONS FOR THE PATIENT

Are you pregnant?

Sports (intense training)?

Are you taking medicines (corticosteroids, etc.)?

Do you have muscle disease?

Do you have kidney, liver or pancreatic disease?

SAMPLE STORAGE AND TRANSPORT

Haemolysed serum samples cannot be interpreted. Samples can be stored for a few days at + 4°C.

ASSAY METHODS

Creatine is measured by the Jaffé method or an enzymatic method before and after hydrolysis into creatinine (the amount of creatine present is obtained from the difference between the two results).

NORMAL EXPECTED VALUES

As an indication:

Serum	< 15 mg/l	< 115 µmol/l
Urine	< 150 mg/24 hours	< 1 150 µmol/24 hours

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

– **Sex:** As creatine is principally concentrated in muscle cells there is a relationship between muscle mass and creatine concentrations (lower muscle mass in women).

– **Age and growth,** influence muscle mass and have a positive impact on serum and urine creatine concentrations.

– **Other variation factors:** Urine and serum creatine concentrations are increased by a meat-rich diet, during pregnancy and when muscle mass is particularly high. Conversely, fasting reduces serum and urinary creatine concentrations.

– **Sports:** Intense training causing increased daily creatine catabolism in sportsmen/women. This catabolism is usually compensated by high muscle mass.

PATHOLOGICAL VARIATIONS

– In muscle disease, an increase in creatine concentrations is seen due to destruction of muscle parenchyma and the release of intracellular creatine into the serum. Myoglobin and creatine kinase may also be measured. Urine creatine is increased in muscle inflammation.

– In metabolic diseases, hormones which activate muscle contraction or endogenous creatine synthesis such as methyltestosterone are released. Hyperthyroidism is associated with an increase in serum creatine concentrations. Conversely, reduced creatine concentrations are seen in hypothyroidism. Serum creatine is increased in diabetic ketoacidosis because of rhabdomyolysis.

– Finally, raised creatine concentrations may be seen in **creatine supplementation** in order to improve sports performance. A rise in urine and serum creatine is seen without any deterioration in renal function.

Disorder	Diseases	Impact on creatine concentration
Muscle disorders	Myonecrosis and rhabomyolysis Myopathies (myositis, poliomyelitis) Dermatomyosis Myasthenia-gravis Muscle trauma	***Rise***
Metabolic disorders	Corticosteroid therapy Hyperthyroidism Diabetic ketoacidosis Creatine supplementation	***Rise***
Muscle disorders	Muscle atrophy	***Fall***
Metabolic disorders	Hypothyroidism	***Fall***

FOR FURTHER INFORMATION

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CREATININE CLEARANCE

DEFINITION – PATHOPHYSIOLOGY

Chronic renal insufficiency (CRI) is a permanent reduction in the glomerular filtration rate (GFR). When it occurs secondary to renal disease, it leads to end-stage renal failure. Early detection of renal disease is essential in order to institute measures to delay the progression of the disease. The GFR, which reflects the fall in the total filtration surface area associated with renal loss, is the most reliable marker of all functions carried out by the kidney, endocrine, homeostatic and waste removal.

The early diagnosis of CRI has long been based on measurement of serum creatinine, although this parameter is less than perfect for several reasons; the relationship between glomerular filtration also depends on muscle mass and therefore a person's height, weight, sex and age. It is also influenced by many factors (meat rich diet, drug interferences, pregnancy and creatinine supplementation). Finally, creatinine is normally filtered by the glomerulus and then secreted by the renal tubule (10 to 40% depending on the person). The proportion of creatinine secreted increases with decreasing glomerular filtration. In renal insufficiency, 90% of the creatinine excreted is due to secretion. In this case it is no longer a good marker of GFR.

GFR cannot be measured directly. It uses the concept of renal clearance, which means that for a substance filtered exclusively and freely by the kidney, the amount excreted in urine (UV: the product of the concentration of the substance in urine U and the urine output V), is equal to the amount filtered by the glomerulus (P.GFR, the product of its plasma concentration P and the GFR).

The GFR can therefore be calculated from plasma and urine measurement of these substances, using the equation GFR = UV/P. The markers of exogenous filtration which provide the best estimates of GFR are inulin or chromium 51-EDTA, although their use is limited in practice as they require complex logistics.

Routinely, GFR is measured from:

– Urine creatinine clearance, calculated from creatinine results in plasma and 24 h urine specimens.

Clcreat = urine creatinine (in µmol/l) x urine output (ml/min)/plasma creatinine (in µmol/l).

It is less likely overall to reflect the actual GFR than estimation methods because of the imprecision of the "complete" 24 hour urine sample collection. It is still useful, however, in some situations (cf "Indications").

– Equations used to estimate the GFR from serum creatinine and simple clinical parameters. The most widely used are the Cockcroft and Gault (recommended by ANAES) and the MDRD (*Modification of Diet in Renal Disease*), adopted by the American Society of Nephrology.

■ The ***Cockcroft and Gault*** equation:

GFR (ml/min)
= [(140–age in years) x weight (in kg)/plasma creatinine in µmol/l] x k
Where k =1.23 for men and 1.04 for women
Or
= [(140–age in years) x weight (in kg)/72 x plasma creatinine in mg/dl
Or

In adults, standardisation for body surface area improves the predictive performance of the equation although requires the patient's height to be known.

GFR (ml/min/1.73m^2) = [GFR [(ml/min) x 1.73] / Sc(m^2)
Where Sc (m^2) = [weight (kg) x height (cm) / 3600]^{0,5}

The Cockcroft and Gault equation is not valid in obese patients (body mass index > 30 kg/m^2), pregnant women or children, in patients over 75 years old and in uncompensated cirrhosis with ascites.

- The MDRD equation (*Modification Diet in Renal Disease*). This equation does not include weight: this can be useful as weight is often difficult to obtain.

MDRD Equation (simplified):

GFR (ml/min/1.73 m^2) =186 x (Plasma creatinine (mg/dl)$^{-1,154}$) x (age)$^{-0,203}$ x (0.742 if female) x (1.210 if African American subject), if creatinine is not standardised;

GFR (ml/min/1.73 m^2) =175 x (Plasma creatinine (mg/dl)$^{-1,154}$) x (age)$^{-0,203}$ x (0.742 if female) x (1.210 if African American subject), if the creatinine is standardised.

Creatinine clearance can be estimated in children from the Schwartz equation:

GFR
= k x height (cm) / plasma creatinine (µmol/l) or plasma creatinine x 8.84 if in mg/l
Where:
k = 29 in premature infants
k = 40 in term infants up to the age of one year old
k = 49 in children between 2 to 12 years old
k = 40 for girls between 13 to 21 years old
k = 62 for boys between 13 to 21 years old

■ COMPARATIVE PERFORMANCE AND MERITS OF THESE EQUATIONS

In general, these equations perform acceptably in people whose muscle creatinine production is close to the population average for the same age, sex and weight. Overall, in the general population, the MDRD equation is more accurate and precise than the Cockcroft and Gault.

The "within 30%" (percentage of patients in the general population +/-15% from their mean GFR) is 36% for the Cockcroft and Gault equation and 56% for the MDRD equation, meaning that for a GFR of 70 ml/min/1.73m^2, only 1 out of 3 patients in the general population would have their GFR estimated by the Cockcroft and Gault equation as being between 60 and 80 ml/min/1.73 m^2 (+/- 15% of 70), compared to 1 out of 2 with the MDRD equation.

■ COMPARATIVE MERITS IN DIFFERENT POPULATION SUBGROUPS

Depending on renal function

At low GFR values, both equations tend to overestimate GFR, particularly when this is less than 20 ml/min. The overestimate is greater with the Cockcroft and Gault equation because of the relative magnitude of tubular creatinine secretion. The MDRD equation is more accurate for these low values, as it was constructed from a population of chronic kidney failure patients.

In obese/thin patients

The estimation of muscle mass by weight fails. The Cockcroft and Gault equation considerably overestimates GFR in obese patients, whereas the MDRD, which is independent of weight, is more accurate. Conversely, in thin patients (body mass index < 18 kg/m^2), the MDRD equation markedly overestimates GFR as the loss of body weight in this situation is accompanied by a parallel reduction in muscle mass.

In diabetic patients

The MDRD equation performs considerably better than the Cockcroft and Gault equation. However at high GFR values the MDRD equation tends to underestimate GFR, whereas the Cockcroft and Gault equation tends to overestimate it (because many of this population are overweight).

In elderly patients

This is the category of patients in which the MDRD equation is particularly useful, performing far better than the Cockcroft and Gault equation. Up to 50 years old, GFR estimates by either of the equations are similar although above this age and particularly above 60 years old the Cockcroft and Gault equation tends to produce a large excess of diagnoses of renal insufficiency because of considerable bias (average bias of 11 and 15 ml/min/1.73 m^2, respectively in women and men over 65 years old with GFR > 60 ml/min/1.73 m^2). The MDRD must always be used in preference in this population.

INDICATIONS FOR MEASUREMENT

GFR estimation from creatinine clearance is recommended (ANAES Recommendations, September 2002):

– In patients with a renal abnormality: proteinuria, haematuria, lithiasis, uropathy, recurrent upper urinary tract infections, known familial or non-familial nephropathy followed by reversible acute renal insufficiency.

– In patients at risk of renal disease: diabetes, hypertension, atheromatous disease, family history of nephropathy, heart failure, liver failure, gout, connective tissue disease with potential renal involvement (lupus, scleroderma, amyloidosis and scarcoidosis), long-term use of nephrotoxic drugs (lithium, ciclosporin, tacrolimus, non-steroidal anti-inflammatory drugs, anti-viral agents, converting enzyme inhibitors, angiotensin II receptor antagonists, diuretics and analgesics).

– In some circumstances: before and after prescription of aminoglycosides, before and after nephrotoxic chemotherapy (cisplatine and related derivatives); for injection of high osmolality iodinated contrast media in the elderly before prescription of drugs removed by the kidney.

– In non-renal clinical or laboratory abnormalities: gastro-intestinal disorders (anorexia, nausea and vomiting), discovery of non-regenerative normochromic normocytic anaemia, clinical phosphate-calcium metabolic abnormalities (vertebral collapse, fractures, bone pain) or laboratory abnormalities (hypo- or hypercalcaemia).

Measurement of GFR (creatinine clearance with measurement of urine creatinine in a 24 hour urine specimen) may be necessary in situations in which the Cockcroft and Gault equation is difficult to interpret:

– When endogenous production of creatinine may be altered: severely malnourished or obese patients or patients receiving parenteral nutrition: if muscle mass is increased or reduced, regardless of cause (skeletal muscle diseases, amputations, para and tetraplegia, corticosteroid therapy); severe hepatocellular failure.

– In the long-term follow up of potentially nephrotoxic drugs.

– To confirm an equivocal diagnosis of renal insufficiency.

– To assess the severity of renal insufficiency.

INFORMATION

■ SAMPLE

Blood sample: serum or heparinised plasma collected during the test. Take the sample in the morning (24 hour variations occur), fasting (not a strict fast as acetoacetate produced in fasting interferes with many assay methods).

Urine: 24 hour urine collection. Patients should be recommended to drink sufficiently, particularly at the start of the test (1/2 litre of water), to avoid tea and coffee and any drug treatment and to rest during the collection period.

■ QUESTIONS FOR THE PATIENT

Height, weight and age?

Are you taking any medical treatment? If yes, which?

In women: are you pregnant?

Record urine output accurately.

■ SAMPLE STORAGE AND TRANSPORT

Whole blood stored for 48 hours at room temperature: plasma stored at room temperature or between 2 and 8°C for 7 days and for several months at -20°C;

Urine stored for 2 - 3 days at room temperature, at least 5 days at + 4°C, possibly with addition of preservatives (thymol).

ASSAY METHODS

Chemical methods (Jaffé, with 3.5-dinitrobenzoic acid); enzymatic methods (amidohydrolase, creatinine deaminase).

The international recommendations (*National Kidney Disease Education Program*) are to use a several point (at least 3) calibration including one point within the normal range (between 50 and 100 µmol/l) and a calibrator standardised by mass spectrometry (now available). In terms of assay imprecision, the American recommendations are that total imprecision including inter-laboratory variation should be < 8% for a creatinine of 88.4 µmol/l with a bias (difference compared to a reference specimen) of < 5%. Enzymatic methods should be used in preference to meet these criteria.

NORMAL EXPECTED VALUES

	ml/min/1.73 m²	ml/sec/1.73 m²
Newborn (< 8 days)	20 - 50	0.33-0.83
Newborn (= 8 days	40 - 65	0.66-1.08
Infants (< 3 months)	50 - 80	0.83-1.33
Infants (3 to 12 months)	65 - 110	1.08-1.83
Children-Adolescents	90 - 140	1.50-2.33
Adult women (20 – 40 years old)	90 - 130	1.50-2.16
Adult men (20 – 40 years old)	100 - 140	1.66-2.33

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Plasma creatinine increases physiologically with age. It varies depending on dietary protein intake, muscle mass and muscle exercise. It is lowered in the initial months of pregnancy and in patients with reduced muscle mass.

■ PATHOLOGICAL VARIATIONS

A classification of renal insufficiency and of the severity of renal disease based on the GFR was proposed in France by ANAES in 2002.

Stage	Definition	GFR (ml/min/1.73 m²)
1	Chronic renal disease*with GFR ≥60	≥ 60
2	Moderate renal insufficiency	30 – 59
3	Severe renal insufficiency	15 – 29
4	End-stage renal insufficiency**	< 15

**Laboratory and/or histological and/or morphological renal abnormalities*

***End-stage renal insufficiency is defined as an estimated creatinine clearance < 15 ml/min/1.73 m2, independently of whether renal replacement treatment has started (dialysis or transplantation).*

– Patients with GFR < 60 ml/min/1.73m² have unequivocal renal insufficiency whether or not concomitant markers of renal disease are present.

– Patients whose GFR is between 60 and 90 ml/min/1.73 m², who have markers of renal disease which have persisted for more than 3 months are deemed to have chronic renal disease. In the absence of markers of renal disease their renal function and markers of renal disease should be monitored (inadequate information to diagnose renal insufficiency or chronic renal disease).

The markers of renal disease are:

– Microalbuminuria: 20 to 200 µg/min or 30 to 300 mg/24 hours or urine albumin/creatinine ratio >2mg/mmol

– Proteinuria: > 300 mg/24 hours or proteinuria /creatininuria > 200mg/g

– Haematuria > 10 red blood cells/mm³ or >10000/ml

– Leukocyturia > 10 white cells/mm³ or >10000/ml

– Ultrasound: size asymmetry, irregular outline, small or large polycystic kidneys, nephrocalcinosis, calculus and hydronephrosis.

– In children over 2 months old, creatinine clearance calculated by the Schwartz equation < 60–70 ml/min/1.73m² represents early renal insufficiency.

The following should be performed if renal insufficiency is discovered:

– Confirm the actual abnormality, looking for reasons for changes in plasma creatinine: interferences from other substances whether or not drug related, drugs affecting tubular creatinine secretion (cimetidine and trimethoprim), or assay circumstances. If uncertain, a second GFR measurement is recommended (same assay method if possible, in the same laboratory). If doubt persists, GFR measurement is recommended.

– Exclude acute renal insufficiency and confirm that the renal insufficiency is chronic. Consider drug-induced causes of acute renal insufficiency (iodinated contract media, aminoglycosides, non-steroiodal anti-inflammatory agents, converting enzyme inhibitors, angiotensin II receptor antagonists etc.). Renal insufficiency is said to be chronic when it has persisted for at least 3 months and is irreversible.

– Establish the aetiological diagnosis based on the clinical history, clinical examination and paraclinical investigations.

FOR FURTHER INFORMATION

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CRYOGLOBULIN

DEFINITION

The cryoglobulins are immunoglobulins which precipitate or form a gel at a temperature below + 37°C and dissolve again reversibly with warming. The temperature at which they precipitate varies, from + 4°C to + 36°C. This explains why the results of some blood tests are altered if the tests are not performed at 37° C.

Immunochemical analysis shows that there are three types of cryoglobulin.

Classification of cryoglobulins *(according to Lecomte C et al, Spectra Biologie 2008)*

Type	Composition
Type I monoclonal	A single monoclonal isotype
Type IIa mixed	A monoclonal isotype combined with one or more polyclonal isotypes
Type IIb mixed	One or more oligoclonal isotypes combined with one or more polyclonal isotypes
Type III mixed	Combination of several polyclonal isotypes

INTRODUCTION

Types I and II cryoglobulins are usually associated with a lymphoid blood disorder, such as myeloma, Waldenström's macroglobulinaemia or chronic lymphatic leukaemia. Types II and III cryoglobulins are seen during certain infectious, inflammatory or auto-immune diseases.

They should be looked for:

– When there are suggestive symptoms. These include cutaneous and vasomotor signs which are often precipitated by the cold, such as vascular purpura, necrosis of extremities, urticaria, livedo and Raynaud's syndrome. The kidneys may be affected by acute or sub-acute glomerulonephritis and there may be neurological problems (peripheral neuropathy), arthralgia and haemorrhagic syndromes.

– When there are biological abnormalities, notably when there is precipitation in the syringe used to take blood. Variations in tests according to the temperature at which they are carried out, sedimentation rate, white cell count, platelet counts and gamma-globulin levels. There may also be unexplained variations in serum protein measurements.

INFORMATION

The accuracy of the analysis depends on strictly observing the conditions on collection and clotting.

■ SAMPLE

Equipment for sampling should be pre-heated to + 37° C. Blood is collected into a dry tube (without gel) for serum, with the patient fasting. Allow the sample to clot for 1 hour at + 37° C. The serum is separated after passive sedimentation of cells or immediately after rapid centrifugation in a centrifuge at + 37° C (carrier pre-heated to + 37° C) for 10 minutes at 3,000 rpm.

■ QUESTIONS FOR THE PATIENT

Clinical and laboratory test picture (see request form)?

Current anticoagulant therapy? Heparin treatment prevents proper clotting *in-vitro*, which can interfere with the measurement of cryoglobulin.

■ SAMPLE STORAGE AND TRANSPORT

If the blood tube has to be transported from the place of sampling to the laboratory, it should be placed immediately in an isothermal box or in a thermos flask containing water at a temperature close to + 37° C.

AVAILABLE ASSAY METHODS

Measurement of cryoglobulins necessitates sampling under strictly controlled conditions (see above). Once it has been separated, the serum should be poured into a long narrow tube, which is the most suitable type for the observation of precipitation. A drop of an antibacterial agent can be added. The tube should be corked and left for a minimum of 8 days at + 4° C. It should be checked daily or, at least, at 24 hours, 48 hours and 8 days, and even at 1 month.

The protein is identified using immunochemical techniques (immunofixation, immunoelectrophoresis or tissue blot immunoassay).

Quantification of the cryoglobulin can be performed by various techniques: Cryocrit or measurement of proteins in the cryoprecipitate.

INTERPRETATION

■ CRYOGLOBULIN DETECTION

The test is negative if there is no precipitate or gel in the tube maintained at + 4° C for at least 8 days. If the clinical picture is strongly suggestive, the tube should be left at + 4° C for a further week (or even a month) before completion. In addition the phenomenon of cryoprecipitation, it can, though rarely, sometimes be intermittent, so it can be useful to repeat the test at intervals of several days.

The test is considered positive if a white precipitate is seen, producing an appearance of "spirals of smoke" when it is suspended again in the tube. More rarely, a gel forms and when this is the case, it can sometimes involve the whole mass of the serum. The precipitate or the gel re-dissolves with warming. Most frequently it goes back into solution at + 37° C, though sometimes a higher temperature is required (+ 40 – 45° C). Type I cryoglobulins (present in substantial amounts) often appear rapidly (48 hours) but types II or III, which present in smaller quantities, sometimes can only be detected after 3 weeks at + 4° C.

■ IDENTIFICATION

An asymptomatic mixed cryoglobulin at concentration < 0.01 g/l is present in 2 to 3% of "healthy" individuals aged over 60 years.

Monoclonal cryoglobulins are found with lymphatic blood dyscrasias (5 to 10% of monoclonal immunoglobulins in myeloma or Waldenström's macroglobulinaemia cryoprecipitate).

Mixed or polyclonal cryoglobulins appear in:

– Autoimmune diseases, such as systemic lupus erythematosus, Sjögren's syndrome, polymyositis and, more rarely, rheumatoid arthritis and polyarteritis nodosa.

– Viral diseases, such as hepatitis C (the prevalence of mixed cryoglobulinaemia in Hepatitis C infection varies between 40 and 80%), hepatitis B, infectious mononucleosis, HIV infection, cytomegalovirus and EB virus.

– Bacterial and parasitic diseases, such as sub-acute endocarditis, syphilis, Q-fever, Lyme disease, malaria, toxoplasmosis and schistosomiasis. The presence of cryoglobulins is usually transient.

Sometimes no associated condition is found and the cryoglobulinaemia is considered to be essential or idiopathic.

■ PRINCIPAL BLOOD ANALYSES ALTERED BY THE PRESENCE OF CRYOGLOBULIN

Sedimentation rate is increased at + 37° C and reduced at + 20° C.

White cell and platelet counts: Pseudo-leucocytosis and pseudo-thrombocytosis.

Serum protein levels show unexplained variations.

Serum protein electrophoresis shows hypogammaglobulinaemia, disappearance of a peak.

Detection of auto-antibodies.

These tests must be performed at a temperature close to + 37° C.

FOR FURTHER INFORMATION

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CRYPTOCOCCUS NEOFORMANS

DEFINITION

Cryptococcus neoformans is a round or oval capsulated yeast responsible for Cryptococcosis, which is an opportunistic disease occurring in immunosuppressed patients, particularly those suffering from AIDS with advanced immunosuppression. The disease is most commonly meningeal, resulting in a subacute, very severely progressive meningoencephalitis. Two varieties of *C. neoformans*, the organism most commonly incriminated in human disease, can be distinguished. The neoformans variety, serotypes A and D, which are cosmopolitan and the *gattii* variety, serotypes B and C which are absent from our temporate regions. The diagnosis is relatively straightforward in a suggestive clinical situation and is based mostly on identifying and isolating the capsulated yeasts from the cerebrospinal fluid (CSF) and on testing for circulating antigens in the CSF and serum.

Synonym: Cryptococcus.

INTRODUCTION

CLINICAL EPIDEMIOLOGY

The *neoformans* variety largely predominates in our countries in immunosuppressed patients. Infection is usually contracted after inhaling the dust of pigeon or bat droppings containing the spores of the fungus. Infection may also occur through broken skin lesions when handling contaminated earth. The *gattii* variety is present in the faeces of the koala bear which feeds on eucalyptus leaves in tropical and subtropical forests.

Neuromeningeal cryptococcosis affects 80% of HIV seropositive patients who have a very low CD4 lymphocyte count. It is the commonest systemic fungal infection in AIDS and the third leading opportunistic central nervous system infection after toxoplasmosis and cytomegalovirus or HIV viral encephalitis in these patients. The new anti-retroviral treatments being used are now delaying or even stopping the progress of the disease.

Other patients liable to develop neuromeningeal cryptococcosis are those suffering from malignant (leukaemias, lymphomas, solid tumours, etc.) or auto-immune (sarcoidosis, etc.) diseases or transplant patients treated long-term with oral corticosteroid therapy. Occasionally no predisposing factor is found.

Clinically the disease is characterised principally in immunosuppressed patients by neurological signs such as headache and occasionally behavioural disorders, fever and meningism, etc. Other infection sites are also seen, particularly the lungs (main portal of entry) and skin (lesions with the appearance of umbilicated pustules suggestive of *Molluscum contagiosum*). After the Cryptococci spread into the blood, bone, eye, cardiac and prostate disease may be found.

SEARCH INDICATIONS

Suspected cryptococcosis in an immunosuppressed patient, usually in an HIV seropositive patient with advanced immunosuppression *(cf. suggestive clinical signs above)*.

INFORMATION

SAMPLE

Cerebrospinal fluid for direct diagnosis, culture and antigen testing.

Serum for the testing of Cryptococcus antigen.

Urine and broncho-alveolar lavage fluid.

QUESTIONS FOR THE PATIENT

Are you HIV seropositive?

Have you been treated with long-term oral immuno-suppressants or corticosteroids?

SAMPLE STORAGE AND TRANSPORT

CSF and serum must be stored and transport at + 4°C (urgent investigation to be processed as soon as possible).

DIAGNOSTIC METHODS

– Direct microscopic examination of the CSF centrifugation pellet between slide and coverslip in a drop of China ink diluted 1/3 or 1/5. Other samples can be used to test for an isolate of the yeast, such as blood, urine, broncho-alveolar lavage fluid, pus from skin lesions, biopsies, etc.

– Culture on simple Sabouraud or Sabouraud-chloramphenicol medium without cycloheximide, at + 37°C.

– Testing for Cryptococcus antigen in CSF and serum (or other samples) by latex agglutination method on particles sensitised by polyclonal antibodies specific for the *C. neoformans* capsule polysaccharide.

INTERPRETATION OF RESULTS

The diagnosis is based on finding the yeast on direct examination, isolating it by culture or by a positive Cryptococcus antigen in CSF and/or serum. In practice, the diagnosis of cryptococcosis is made and treatment is started if at least one of these three investigations is positive.

DIRECT DIAGNOSIS

After staining with China ink, *C. neoformans* usually appears as a budding yeast within a capsule forming a clear halo, doubling or even tripling the diameter of the yeast. Note, however, that the capsule can be greatly reduced in a number of AIDS patients or may even be absent. This investigation provides the diagnosis in 90% of cases.

CULTURE

C. neoformans grows in 2 to 4 days, as white concave smooth shiny colonies with clear edges, flowing more or less depending on serotype. They become more cream-fawn or ocre over time. The *gattii* variety grows better at + 30°C. Growth is occasionally slower and a month should be left before reporting a culture as negative.

Microscopic examination of the colonies reveals more or less budding yeasts but the capsules may be visible or absent.

Apart from their morphological appearance, the diagnosis of the *C. neoforman* species is based on:

– Urea hydrolysis at + 37°C in 1 to 4 hours (this is not a sufficient feature as other species hydrolyse urea within 4 hours including Trichosporon and some Cryptococci do not exhibit this property);

–Cycloheximide sensitivity (no growth in the presence of cycloheximide);

– Sugar assimilation in an oxidising medium: no growth with lactose, glycerol, xylitol or melibiose; variable growth with arabinose, cellobiose, ribose and erythritol and positive with inositol;

– Positive phenoloxydase activity (Auxacolor® gallery Biorad), specific to *C. neoformans*.

The two varieties of *C. neoformans* can be distinguished by serotype (in a specialist laboratory) or by their D-proline assimilation: negative for *neoformans* and positive for gattii.

■ TESTING FOR CIRCULATING ANTIGENS (CSF, SERUM, ETC.)

Agglutination which is visible to the naked eye offers good sensitivity for the diagnosis of Cryptococcus meningo-encephalitis and can also be used to monitor the response to treatment and to test for possible relapses. Beware of false negatives (early infection or isolated skin disease) and false positives in serum of patients suffering from autoimmune diseases (systemic lupus erythematosus, sarcoidosis, scleroderma, etc.) or those who have received infusions of macroglobulins and the presence of other micro-organisms *(Trichosporon, Klebsiella, and Pseudomonas)* because of common antigens.

■ NON-SPECIFIC LABORATORY FEATURES

Cryptococcosis is occasionally associated with increased cerebrospinal fluid protein, decreased cerebrospinal fluid glucose and in fewer than 50% of cases with raised cerebrospinal fluid leukocyte count. Concomitant hyponatraemia is considered to be a negative prognostic indicator.

TREATMENT

This involves an association of amphotericin B and fluocytosine for approximately 2 weeks followed by a switch to fluconazole for approximately 8 weeks at a dose of 400 mg as a single daily dose in adults. Maintenance treatment with 200 mg/d of fluconazole is then recommended.

The effectiveness of treatment is monitored by clinical examination and regular testing for the yeast by culture. Monitoring relies on systematic testing for the Cryptococcus antigen to provide an early diagnosis of recurrence in immunosuppressed patients.

FOR FURTHER INFORMATION

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CRYPTOSPORIDIA

DEFINITION

Cryptosporidium is a cosmopolitan protozoon belonging to the Coccidia family. The parasitic cycle of this organism may occur in a large number of animal species (mammals, reptiles, birds and fish). It is responsible for cryptosporidiosis, a well known intestinal protozoosis in veterinary medicine but which was only described in human beings in 1976. The main pathogenic species to human beings is *C. parvum*, initially described as the cause of short episodes of diarrhoea mostly in children. It has been known particularly since the emergence of AIDS causing serious cholera-like diarrhoeal illnesses in immunosuppressed patients.

Synonyms: species known in human beings: *Cryptosporidium parvum* and *C. muris*.

INTRODUCTION

Human beings are infested by contact with animals or infected people or by ingesting the protozoa oocysts after consuming water or foods which have been soiled by the faeces of an infested host. Nosocomial transmission has also been described. The life-cycle of the parasite takes place in the host enterocytes and lasts an average of 3 days when the different stages of parasites succeed each other, the sexual phase resulting in the birth of new spore-forming resistant oocysts which are excreted in faeces.

The prevalence of cryptosporidiosis is approximately 2% in industrialised countries and 6% in developing countries, although it is notably higher in immunosuppressed patients, particularly patients infected with the human immunodeficiency virus (HIV) in whom the prevalence is as high as 14 to 24 % in the absence of anti-retroviral treatment.

In immunocompetent people the disease mostly affects children under two years old (nursery epidemics), farmers, veterinary practitioners or hospital staff and travellers returning from a low level hygiene country. It is asymptomatic or after an incubation period of 5 to 21 days causes gastroenteritis which resolves spontaneously over 1 to 2 weeks. It may cause growth or psychomotor development retardation in infants.

In the immunosuppressed, cryptosporidiosis causes aqueous cholera-like diarrhoea of 10 to 20 bowel movements per day, although this may alternate with periods of normal bowel transit or even constipation. Daily faecal volume is generally very high, with up to 10 litres/day or even more. The diarrhoea may last for several weeks although generally persists for several months resulting in the patient dying in a clinical picture of water and electrolyte disturbance and severe cachexia. Biliary and respiratory damages have also been described.

SEARCH INDICATIONS

– Acute or chronic diarrhoea

Immunosuppressed patients infected with HIV with a lymphocyte count of less than 200 CD4/mm^3, oncology and haematology patients.

Immunocompetent patients, such as children in communities, people in contact with animals, hospital staff, and travellers or in the case of epidemics of diarrhoea, particularly hospital-acquired (nosocomial) infection. Testing must always be preceded by 3 stool parasitology tests at 2 to 3 day intervals. If the 3 parasitology tests are negative and symptoms persist, Cryptosporidia should be tested for amongst others.

– Pulmonary or hepatobiliary damage damage in an immunosuppressed person particularly HIV positive patients with a CD4 count less than 50 /mm^3; or testing for asymptomatic carrier status in the close contacts of an affected person.

INFORMATION

■ SAMPLE

Fresh stool sample must be collected. Oocysts are excreted intermittently; therefore it is recommended that the test be performed on at least 3 samples at intervals of 2-3 days.

– Duodenal or intestinal biopsies.

– Broncho-alveolar lavage fluids and samples of bile, etc.

■ QUESTIONS FOR THE PATIENT

History of immunosuppression (HIV infection, current treatment with an immunosuppressant or anti-cancer agent)?

Past history of standard stool parasitology tests?

■ SAMPLE STORAGE AND TRANSPORT

Stool samples to be stored and transported at + 4°C until analysis. Because of the significant risk of infestation, specific precautions should be taken (mix the sample with 10% formaldehyde, wear gloves, mask and goggles when handling).

DIAGNOSTIC METHODS

– Direct parasitology examination of a stool smear revealing parasite oocysts. Stool concentration (Ritchie technique) is recommended particularly when testing for a healthy carrier in a person in contact with an infected patient.

NB: Cryptosporidia are not revealed by conventional stool parasitology methods, therefore specific testing must be requested by the requesting physician in order for appropriate methods to be used.

The staining method which produces the best results for specific Cryptosporidia testing in the modified Ziehl-Nielsen method with formol-ether concentration (staining with phenic fuchsin and counterstaining with malachite green). Other stains can be used, particularly Heine staining. Reagents containing a fluorescent monoclonal antibody against the oocyst wall, which can be read by fluorescence microscopy may also be used.

– Antigen detection kits are available. Testing is more expensive but may be performed by staff untrained in microscopic parasitology diagnosis.

– Molecular biology techniques for the identification of the parasite DNA by PCR; provides a species diagnosis but is not currently used routinely.

NB: Serology is of no diagnostic use. It is used for epidemiological studies.

INTERPRETATION OF RESULTS

The diagnosis relies principally on finding the parasite on the direct examination of a stool smear (either with or without concentration). As the oocysts are excreted discontinuously a result cannot be reported as negative until three samples have been tested. NB: *C. parvum* may be found in diarrhoea stool or formed stools. On direct examination the oocysts are rounded, refringent and have no characteristic features enabling a specific diagnosis of *C. parvum*.

After staining with the modified Ziehl-Nielsen technique the cryptosporidia oocysts stain red on a blue-green background. They can be detected from an x 20 lens upwards (5-8 µm). At high magnification (immersion) the residual body (black), nuclei of the sporozoites (black) and a vacuole within a granular cytoplasm can be distinguished. The specific species diagnosis can be made by gene amplification techniques.

Morphological differential diagnosis with other enteropathogens described in a similar clinical context *(modified Ziehl Nielsen staining, immersion examination).*

	Size (microns)	Ziehl-Nielsen features
Cryptosporidium parvum *oocysts*	5 to 7	Red staining; Residual body and nuclei of sporozoites (black), vacuole, granular cytoplasm
Cyclospora cayetanensis *oocysts*	8 to 10	Red staining although heterogeneously within the same smear
Isospora Belli oocysts	20 to 33 x 10 to 19	Red staining; oval shape, obtuse, large.
Yeasts	2 to 10	Green staining
Fat globules	Very variable	Pink staining with no internal organisation

Isolation of *C. parvum* in a patient with gastro-intestinal symptoms (gastroenteritis and diarrhoea), provides a diagnosis of cryptosporidiosis. In immunosuppressed patients the gastro-intestinal diseases sometimes associated with pulmonary or biliary involvement (*C. parvum* can then be found in bile).

Asymptomatic carrier status may last for several years after recovery, or with recurrent infestations.

Treatment – Prevention of infections

Treatment is above all symptomatic with rehydration in serious cases. Anti-retroviral treatment in HIV+ patients restores immunity, helps prevention and controls the cryptosporidiosis. Macrolides (azithromycin, clarithromycin and spiramycin) can be used.

In a hospital environment, prevention of human cryptosporidiosis relies on specific measures (careful hand washing, wearing gloves, treating effluent, etc.). The main recommendations for immunosuppressed patients and travellers from areas with poor hygiene, is to drink bottled mineral water or boiled or filtered water (0.2 microns) and to increase hand hygiene.

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■ Paugam A., Tourte-Schaefer C., *Commentaire sur les frottis de selles contenant Cryptosporidium sp.* In: Annales du contrôle national de qualité n°1. Agence du médicament, Saint-Denis, 1995: 48-50.

CYCLIC AMP

DEFINITION

Cyclic AMP (cAMP) or cyclic adenosine 3', 5'monophosphate is an important intracellular second messenger, regulating many of the cell's activities by activating protein kinases to transduce the biological effects of certain hormones. cAMP is generated inside the cell from ATP (adenosine triphosphate) by adenylyl cyclase which is activated by the binding of these hormones to its specific membrane-bound receptor. Adenylyl cyclase activity is dependent on G proteins, which may be stimulatory (Gs) or inhibitory (Gi), respectively increasing and decreasing the intracellular cAMP concentration. Hormones which exert their effects by raising cAMP levels are glucagon, oxytocin, vasoactive intestinal peptide (VIP), prostaglandin, histamine, LH, FSH, TSH, ACTH, PTH, adrenaline (β–receptors), corticotropin releasing hormone (CRH), growth-hormone releasing hormone (GRH) and serotonin (S2 receptors). Hormones which act by lowering cAMP are somatostatin, adrenaline (receptor 2), dopamine and serotonin (S1 receptors).

cAMP activates protein kinases which catalyse the phosphorylation of intracellular proteins which either turn certain genes on or off, to mediate the hormone's physiological effects.

cAMP is rapidly broken down by phosphodiesterases.

INTRODUCTION

cAMP concentrations in the plasma and urine provide an indirect indication of intracellular concentrations. cAMP in the urine comes from two sources:

- One fraction which comes from glomerular filtration and is not re-absorbed in the tubule.
- The rest is synthesised in the kidney after induction by parathyroid hormone or parathyroid hormone-related peptide (PTHrP).

INDICATIONS FOR MEASUREMENT

A cAMP assay used to be ordered when hyperparathyroidism was suspected but this test has been superseded by assays for intact parathyroid hormone in kit form.

Measuring urinary cAMP or its nephrogenic fraction gives a measure of the tubular response to PTH or PTHrP. Nephrogenic cAMP—which corresponds to total urinary cAMP minus the fraction filtered from the plasma—is calculated using the following equation which takes the concentrations of creatinine in the plasma and urine into account:

- Nephrogenic cAMP =
  [urinary cAMP x (plasma creatinine/urinary creatinine)] - plasma Camp

This parameter makes it possible to diagnose different forms of pseudohypoparathyroidism.

INFORMATION

SAMPLE

cAMP is assayed in plasma from a blood sample drawn into EDTA in a pre-cooled tube. Neither serum nor heparinised plasma can be used. The plasma should be separated off by centrifugation at +4°C and frozen at –20°C within an hour of blood drawing.

QUESTIONS FOR THE PATIENT

In addition to the subject's age and gender, the blood calcium concentration should be stipulated as well as details of any medications that might affect calcium and phosphorus metabolism.

SAMPLE STORAGE AND TRANSPORT

Urine should be stored at +4°C during collection (24 hours), and then pooled and frozen. Since cAMP is extremely labile, both urine and plasma samples should be sent to the laboratory frozen.

ASSAY METHODS

cAMP is assayed using a radioimmunological method. The cAMP in the sample competes with [I^{25}]-labelled cAMP for binding to a specific antiserum. Values for unknown samples are read off a standard curve plotted in parallel.

NORMAL EXPECTED VALUES

Plasma: < 25 nmol/l

Urine: < 500 nmol/mmol creatinine

Nephrogenic cAMP: < 20 nmol/l glomerular filtrate.

PATHOLOGICAL VARIATIONS

ELEVATED NEPHROGENIC CAMP

cAMP rises in primary hyperparathyroidism but since the arrival of highly specific and sensitive assays for intact and biologically active parathyroid hormone (PTH), assaying cAMP has lost its relevance in this pathological context. In contrast, an increase in cAMP confirms a diagnosis of neoplastic hypercalcaemia because PTH levels are low due to the secretion of PTH related peptide (PTHrP) by the tumour.

DIMINISHED NEPHROGENIC CAMP

cAMP levels drop together with those of PTH in malignant osteolysis. cAMP levels also drop in hypoparathyroidism and pseudohypoparathyroidism, whereas PTH levels are low only in true hypoparathyroidism.

PTH deficiency can be differentially diagnosed by a PTH infusion, in response to which cAMP levels and phosphaturia rise in true hypoparathyroidism. In contrast, in Type I pseudohypoparathyroidism, neither cAMP nor phosphaturia change, and in Type II pseudohypoparathyroidism, there is a cAMP response but no change in the phosphaturia.

FOR FURTHER INFORMATION

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CYCLOSPORIN A

DEFINITION

Cyclosporin A is an 11 amino acid cyclical polypeptide. It is an immunosuppressant which inhibits calcineurin, used in the preventative or curative treatment of organ transplant rejection, the treatment of autoimmune and inflammatory diseases (rheumatoid arthritis, psoriasis, atopic dermatitis, corticosteroid-dependent and corticosteroid-resistant nephrotic syndrome and severe non-infectious uveitis) and acquired severe bone marrow aplasia.

PHARMACOKINETICS

Cyclosporin A is marketed in different galenic forms (soft capsules, oral solutions or injectable form) under two proprietary names with different dosages. The soft capsule and oral solution forms are bioequivalent. Steady state is achieved in 1 to 4 days.

■ **Methods of administration in organ and tissue transplantation**

Average oral dosage: initial doses are 6 to 15 mg/kg/day then progressively decreasing to maintenance doses of between 2 and 8 mg/kg/day. Cyclosporin A is usually administered as two daily doses.

Injectable route dosage: administration by continuous intravenous infusion for at least the first 30 minutes and then at frequent intervals: 2 to 5 mg/kg/day, switching as soon as possible to oral administration.

INDICATIONS FOR MEASUREMENT

Regular blood cyclosporin measurements are required to monitor treatment in organ and tissue transplantation and acquired severe bone marrow aplasia for the following reasons:

■ Cyclosporin has a narrow therapeutic range (low safety margin between the therapeutic range and toxic range),

■ Intra and inter-individual variability in blood concentrations (variable bioavailability and metabolism),

■ The correlation demonstrated between blood concentration and therapeutic effect and between blood concentration and toxicity (particularly concentration-dependent nephrotoxicity),

■ Numerous therapeutic interactions changing the metabolism of cyclosporin.

Regular measurements during treatment allow dosages to be adjusted in order to ensure treatment is effective, at the same time preventing toxic reactions. Measurements are particularly useful in certain circumstances:

■ Immediately post-transplantation,

■ When the proprietary product is changed as the two medicinal products containing cyclosporin which are currently marketed are not bioequivalent. The first measurement must be performed 4 to 7 days after switching,

■ In patients with severe hepatic dysfunction (hepatic metabolism),

■ In suspected rejection or in adverse effects (particularly a rise in plasma creatinine),

■ If concomitant treatment is changed, particularly if the drug concerned causes pharmacokinetic interactions with cyclosporin *(cf. below)*.

INFORMATION

■ SAMPLE

Whole blood sample into an EDTA tube without separator gel. Heparinised tubes must not be used.

Take the sample at steady state, after treatment for 1 to 4 days. Two types of sample may be used:

■ A sample for measurement of trough cyclosporin A concentrations (C0) immediately before the next dose.

■ A sample 2 hours (+/-10 minutes) after the dose (C2): the post-dose concentration is currently recognised to be a good index of exposure assessing the extent of absorption and estimating the area under the curve (AUC), which varies greatly between patients.

■ Repeated samples are sometimes taken to estimate the AUC 0-4h or AUC 0-12h (pharmacokinetic studies). A relationship has been established between risk of rejection and cyclosporin AUC 0-12h.

■ ESSENTIAL INFORMATION

Any request for drug measurement must include the reasons for the request (testing for efficacy or toxicity), the sample time, date treatment was started and/or any change in dosage, dosage information (amount administered, frequency and route of administration), and the age, height and weight of the subject whenever possible.

State the reason for the measurement (for transplantation state the type of transplant and the time from the transplant together with the date treatment was started).

Renal function (plasma creatinine).

Current treatment, which may interfere with cyclosporin metabolism.

■ ***Medicines (or substances) which increase blood cyclosporin concentration:***

– Macrolides and related substances, such as erythromycin, josamycin, pristinamycin, roxithromycin, midecamycin and clarithromycin.

– Azole anti-fungal agents, such as ketoconazole, itraconazole and fluconazole.

– Calcium inhibitors, such as nicardipine, diltiazem and verapamil.

– Corticosteroids, particularly when administered IV.

– Oral contraceptives.

– HIV anti-proteases, such as ritonavir, nelfinavir, indinavir, saquinavir, amprenavir and lopinavir.

– Others: amiodarone, methotrexate, cimetidine (≥800 mg/d) and sirolimus.

– Danazol.

– Grapefruit juice (because of inhibition of Cytochrome P450 3A4).

■ ***Drugs which reduce blood cyclosporin concentrations***

– Anti-epileptics, such as carbamazepine, phenobarbital, phenytoin and primidone.

– Rifampicin and rifabutin.

– Non-nucleoside anti-retroviral agents, such as efavirenz and nevirapine.

– Trimethoprime IV and clindamycin.

– Orlistat.

– Octreotide and lanreotide.

– St. John's Wort.

■ SAMPLE STORAGE AND TRANSPORT

Samples can be stored for 8 to 12 days at room temperature, 8 to 12 days between + 2° and 8 °C and for at least 3 months at -20°C.

Transport at room temperature or at + 4°C.

ASSAY METHODS

Chromatographic or immunological methods.

NORMAL EXPECTED VALUES

The therapeutic ranges for C0 and C2 vary depending on the type of transplant, time since transplantation and assay method.

Results must be interpreted taking account of clinical situations or co-prescriptions of drugs which alter blood cyclosporin concentrations.

Therapeutic ranges for trough cyclosporin concentrations (C0) depending on indication and time since transplantation *(as an indication)**:***

Type of transplant	Time since transplant (months)	Concentration range (ng/ml)
Renal	0 - 1	150 - 300
	1 - 3	100 - 200
	3 - 6	60 - 150
Hepatic	0 - 1	250 - 350
	1 - 3	200 - 300
	3 - 6	150 - 200
Bone marrow	1 - 6	100 - 200
Cardiac	< 3	200 - 300
	> 3	150 - 250

Therapeutic ranges for cyclosporin 2h concentrations (C2) depending on indication and time since transplantation *(as an indication: these are values produced in the USA; values for French treatment regimens have not been established)*

Type of transplant	Time since transplant (months)	Concentration interval (ng/ml)
Hepatic	0 - 6	800 - 1 200
	6 - 12	640 - 960
	> 12	480 - 720
Renal	1	1360 - 2040
	2	1200 - 1800
	3	1040 - 1560
	4 - 6	880 - 1320
	7-12	720 - 1080
	> 12	640 - 966

FOR FURTHER INFORMATION

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CYFRA 21.1

DEFINITION

The cytokeratins are proteins expressed specifically by epithelial cells and are components of intermediary cytoskeletal filaments. Around twenty of these proteins have been identified. Cyfra 21.1 is a fragment of cytokeratin 19 (CYFRA for CYtokeratin FRAgment), a major constituent of simple epithelium. The cytokeratins are relatively insoluble, although their fragments can be found in serum and because of the specific distribution they may be used as tumour markers.

INDICATIONS FOR MEASUREMENT

Cyfra 21.1 is the serum marker of choice for monitoring treatment and early detection of recurrences of non-small cell lung cancer, particularly squamous cell cancer. It is also used as a laboratory marker of bladder cancers.

Measurement in pleural fluid is sometimes useful to distinguish between benign and malignant effusions.

INFORMATION

■ SAMPLE

Preferably serum. Uncitrated plasma may be accepted depending on the methods. Refer to the manufacturer's recommendations.

Haemolysis or hyperlipidemia can interfere with some assay methods.

It may also be measured on aspiration fluids (cyst, ascites, etc.).

No circadian rhythm is described. A fasting sample is not required.

■ QUESTIONS FOR THE PATIENT

Chemotherapy, radiotherapy or surgery? Types and dates of treatment?

■ SAMPLE STORAGE AND TRANSPORT

5 days at + 4°C; – 20°C beyond this time. Transport the sample to the referral laboratory frozen at -20°C.

ASSAY METHODS

Immunometric method.

NORMAL EXPECTED VALUES

As an indication, usual serum values are < 3.40 ng/ml.

Values may vary depending on the method used.

PATHOLOGICAL VARIATIONS

Increases in Cyfra 21.1 in non-small cell lung cancers

At the time of diagnosis: Cyfra 21.1 measurement may be used as a reference value. Its diagnostic sensitivity (approximately 60%) is better in squamous cell cancer than in adenocarcinoma or large cell cancer. In the latter two situations, combination with CEA measurement increases the diagnostic sensitivity of Cyfra 21.1.

– The serum Cyfra 21.1 concentration correlates with tumour mass: high values at the time of diagnosis require a thorough staging assessment to investigate for unrecognised metastases. Large overlaps however exist between Cyfra 21.1 values in different patient subgroups and as such it cannot be used as a factor determining operability.

– It is also believed to have independent prognostic value from other parameters such as disease stage.

In treatment monitoring: its serum concentration correlates with clinical response. Like the other tumour markers it returns to normal with effective treatment. A recrudescence in values suggests relapse or metastasis.

Increase in other diseases

Moderate increases (< 10 ng/ml) in serum Cyfra 21.1 concentrations have been described in the following situations:

– Benign bronchopulmonary diseases: pneumonia, sarcoidosis, tuberculosis, chronic bronchitis, asthma and emphysema.

– Benign gastro-intestinal diseases: cirrhosis and pancreatitis.

– Acute or chronic renal insufficiency.

– Cancers (other than non small cell lung cancers or bladder cancers): uterine cancers, head and neck cancers.

FOR FURTHER INFORMATION

■ Nonnenmacher L., *Cyfra 21.1*, Encycl Méd. Biol., 2003; 18 (5): 289-97.

CYSTATIN

DEFINITION

Cystatin C is a small molecule belonging to the cystatin super-family (S, SN and SA) which are protein inhibitors of the cysteine proteases. Cystatin C is produced by all nucleated cells in the body at a constant rate and is freely filtered by the renal glomerulus. It is then reabsorbed and catabolised by the proximal tubular cells. Its plasma and serum concentrations only depend on glomerular filtration rate and, unlike creatinine, it is not influenced by sex, age, muscle mass or diet.

Synonym: post-gamma globulin (former name).

INTRODUCTION

Cystatin C inhibits cystein-proteases, particularly cathepsins B, H and L. It is involved in protein homeostasis particularly in the brain.

The glomerular filtration rate (GFR) is defined as the clearance of a substance transported in plasma, only metabolised in the kidney and filtered freely through the glomerular membrane. Whilst inulin clearance is the reference method to estimate GFR renal function is in practice usually assessed by measuring the plasma creatinine, which is far easier to perform, and by estimating its clearance. Creatinine however is partly secreted by renal tubular cells resulting in an overestimate of the GFR. It is also influenced by muscle mass and varies with age and sex and is subject to numerous assay interferences, particularly from drugs.

Cystatin C has been proposed for a few years as an alternative marker to creatinine to estimate GFR. It has the advantage of being independent of sex and age (between 1 and 50 years old), diet and muscle mass and appears to be a more sensitive marker to detect an early decline in renal function. Its plasma/serum concentration correlates better with GFR values estimated from inulin clearance and its plasma renal clearance is identical to that of ^{51}CrEDTA, the reference marker. In practice, its performance is similar to that of creatinine clearance (not straightforward to perform as it requires a 24 hour urine collection) and better than that of serum creatinine: it detects falls in GFR < 72 ml/min/1,73 m^2, whereas serum creatinine may remain within normal limits at GFRs between 70 and 40 ml/min/1,73 m^2.

Finally, as it is to a large extent catabolised by proximal renal tubular cells, concentrations in urine are believed to be a better marker of tubular function than beta-2 microglobulin or alpha-1microglobulin.

INDICATIONS FOR MEASUREMENT

Assessment of glomerular filtration rate to detect renal insufficiency. As it is more expensive than creatinine, measurements are only used second line mostly in the following situations:

– When a detailed estimate of renal function is needed (testing for early decline, for example in diabetes), in paediatrics, pregnant women or after renal transplantation.

– In people with reduced muscle mass, such as children, patients with cirrhosis, the elderly and/or malnourished.

– When interferences occur with creatinine measurement (suspected or recognised).

As a marker of proximal renal tubular function: potential use of urinary cystatin C measurement in tubule diseases.

INFORMATION

SAMPLE

Serum or plasma collected into lithium heparin.

A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Are you taking oral corticosteroids? Corticosteroid treatment in transplant and asthma patients produces a dose-dependent increase in plasma/serum cystatin C.

SAMPLE STORAGE AND TRANSPORT

Store at + 4°C for 5 days; beyond this time, store at – 20°C.

Transport at + 4°C or in at -20°C if the sample has already been frozen.

ASSAY METHODS

Immunonephelometry and immunoturbidimetry.

NORMAL EXPECTED VALUES

These may vary slightly depending on assay methods and laboratories.

As an indication: between 1 and 50 years old: 0.5 to 1 mg/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Plasma/serum cystatin C concentrations are higher in children under 1 year old and increase slightly in the elderly.

PATHOLOGICAL VARIATIONS

In renal insufficiency:

Plasma/serum cystatin C concentration is a better marker than creatinine in the diagnosis of renal insufficiency *(cf. table)*. It increases in renal insufficiency and then returns to normal when renal function improves.

Comparative performance of creatinine and cystatin C in the diagnosis of renal insufficiency

	Creatinine	Cystatin C
Sensitivity	83%	97%
Specificity	89%	65%
Negative predictive value	87%	96%
Pathophysiological variations	Muscle mass, diet, age	Corticosteroids, melanoma, HIV
Analytical interferences	Bilirubin, haemolysis, lipaemia, drugs	Rheumatoid factor
Reference values	Depend on sex and age	Independent of sex and age between 1 and 50 years old..

- **After renal transplantation:**

  Plasma/serum cystatin C concentrations fall faster than creatinine and reflect the return of renal function; however they do not always rise early in rejection. Transplant patients continue to have higher long-term plasma/serum cystatin C levels than non-transplant patients, even when the transplant is successful (possibly related to glucocorticoid treatment).

- **Other pathological situations:**

  Plasma/serum cystatin C concentrations are raised in malignant melanoma and human immunodeficiency virus (HIV) infection.

FOR FURTHER INFORMATION

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CYSTIC FIBROSIS

DEFINITION

Mucoviscidosis or cystic fibrosis of the pancreas (or CF for *Cystic Fibrosis*) is one of the commonest inherited diseases in the Caucasian population, and is autosomal recessive in transmission. The disease manifests in the neonatal period or in early childhood and in its classical form is an association of obstructive lung disease and gastro-intestinal problems. Since the CFTR *(cystic fibrosis transmembrane conductance regulator)* gene was cloned in 1989, more than 1000 mutations have been identified, some of which are responsible for the severe phenotype, whereas others are associated with latent or late onset forms of the disease. Considerable variability in phenotypic expression, age of presentation and severity of the disease are therefore seen.

Synonyms: Cystic fibrosis of the pancreas; *Fibrocystic disease of pancreas; Mucoviscidosis; Pancreas Fibrocystic Disease; CFTR (Cystic Fibrosis Transmembrane Conductance Regulator); CF (Cystic Fibrosis).*

INTRODUCTION

Cystic fibrosis is due to dysfunction of the CFTR protein involved in transepithelial chloride and water transport. The CFTR protein is formed from two transmembrane domains (TM1, TM2), two adenosine triphosphate binding domains (NFB1, NFB2 - NBF n*ucleotide binding fold*) and a central regulating domain (R) *(figure 1)*. The CFTR protein is a chloride channel, activation of which results in Cl^- ions leaving the epithelial cell, although the channel also indirectly controls sodium, potassium and some anion transport. Dysfunction of the CFTR channel results in Cl^- ion retention in the cell, preventing passive water loss. This results in thickening of secretions and mucus responsible for the obstructive effects seen in the lungs and pancreas. The direction of ion flows in the sweat glands is reversed, explaining the high Cl^- ion concentration in the sweat of affected people.

Symptoms

Respiratory disease

– Chronic infection/colonisation by typical pathogenic agents: *Staphylococcus aureus, Haemophilus influenzae, Pseudomonas aeruginosa and Burkholderia cepacia.*

– Chronic bronchitis.

– Lung radiograph abnormalities such as bronchectasis, atelectasis, infiltrates and emphysema.

– Respiratory tract obstruction characterised by wheezing and expiratory dyspnoea.

– Naso-sinus polyposis.

– Finger clubbing.

Gastro-intestinal disease and nutritional problems

– Bowel: Meconium ileus (in 15% of cases) and rectal prolapse.

– Pancreas: Exocrine pancreatic failure (in 85% of cases), abdominal pain, steatorrhoea and chronic pancreatitis.

– Liver: Chronic liver disease – focal or multilobular biliary cirrhosis.

– Growth retardation with hypoproteinaemia and complications of fat soluble vitamin deficiency.

Salt loss syndrome

– Increased sensitivity to dehydration and metabolic alkalosis.

Male infertility due to bilateral absence of the vas deferens (BAVD)

EPIDEMIOLOGY

Cystic fibrosis affects one out of 2500 newborn babies in the Caucasian population. It is autosomal recessive in transmission and only homozygotes are affected. Heterozygotes (or healthy carriers) are phenotypically normal and make up approximately 4% of the population. The disease has full penetrance in homozygous carriers of severe mutations. Almost 1000 different molecular abnormalities have been found on the CFTR gene. Mutation ΔF508 (a deletion of 3 base pairs located on exon 10 resulting in loss of a phenylalanine in position 508 in the mutated protein) is the main mutation found in the white European population with a decreasing gradient from North West to South. The frequency of the ΔF508 mutation in France is between 60% (Languedoc-Roussillon) and 80% (Brittany) of affected cases.

The prevalence of the other mutations in France is no more than 4%. These are the G542X (3.4%), G551D (2.4%), W1282X (2.1%), N1302K (1.8%), R553X (3.4%) and 1 717-1 G→A (1.1%) mutations. These uncommon mutations reflect the patient's ethnic origin. The G551D mutation has a prevalence of approximately 5% in Celtic (Ireland, Scotland, Brittany) and Central European populations and the G542X mutation is relatively prevalent in Mediterranean Basin countries. The W1282X mutation is found more often in Ashkenazi Jews.

THE GENE

The gene responsible, which is located on the long arm of chromosome 7 in position 7q3.1, was cloned in 1989. The gene contains 27 exons extending over 230000 pb (230Kb) and codes for a 1480 amino acid protein called CFTR for *Cystic Fibrosis Transmembrane Conductance Regulator,* related to the ABC *(ATP-binding cassette)* glycoprotein family involved in ion and metabolite transport.

The CFTR gene may contain numerous molecular abnormalities (missense mutations, nonsense mutations, insertions-deletions and splicing mutations). Molecular abnormalities are not consistently dispersed, the commonest being found on exons 9, 10, 11, 12 and 19, 20, 21, 22, 23, which code for the first and second NFB domains respectively (NFB1, NFB2). A 5 category classification of the mutations has been proposed:

– Mutations affecting production of the protein (nonsense mutations W1282X, G542X, splicing mutations, frameshift mutations).

– ***Mutations affecting maturation of the protein*** (ΔF508, ΔI507, N1303K).

– ***Mutations affecting regulation of the chloride channel*** (missense mutations in the ATP NFB1 and NFB2, G551D binding sites).

– ***Mutations causing conduction dysfunction*** (R117H and R334W).

– ***Mutations affecting expression of the protein*** (IVS8-5T, 3849 + 10Kb C > T).

– ***Mutations affecting the stability of the protein*** (truncation of the C-terminal part).

The large variability in the clinical expression of the disease is therefore explained by the diverse range of CFTR gene mutations and their consequences on the synthesis and/or functioning of the protein.

SEARCH INDICATIONS

In newborn babies with meconium ileus.

In infants, with fatty diarrhoea (steatorrhoea), rectal prolapse, height and weight growth retardation, repeated bronchitis, pneumonia and chronic rhinosinusitis.

In children, adolescents or adults: suggestive diagnosis from a clinical picture of gastro-intestinal and/or respiratory features suspected following an abnormal sweat test (> 60 mEq/l chloride).

In pregnant women following indicative ultrasound signs (foetal intestinal hyperechogenecity) and in the partner.

Couples undergoing medically assisted pregnancy in which the man is infertile due to bilateral vas deferens agenesis (BAVD).

Family screening (family studies in relatives and partners of a homozygote or compound heterozygote index case).

INFORMATION

■ SAMPLE

5 ml of whole blood drawn into EDTA.

■ QUESTIONS FOR THE PATIENT

In France, certification of genetic consultation **must** accompany the request from the prescribing physician (or failing this, request from the prescribing physician accompanied by the patient's informed consent) in order to take the sample and enable the test to be performed. The result and report are sent only to the requesting physician.

The request form should be completed, to include the reason for the genetic test (relevant clinical and laboratory information suggesting cystic fibrosis, including the sweat test results using the reference method (pilocarpine stimulation)), the family tree for an identified index case, geographical origin of the person and date of start of pregnancy for pregnant women.

■ SAMPLE STORAGE AND TRANSPORT

At room temperature.

DIAGNOSTIC METHODS

Neonatal screening in France uses measurement of immunoreactive trypsin (IRT) on an eluate of blood taken on the 3rd day after birth at the same time as screening for phenylketonuria, hypothyroidism and congenital adrenal hyperplasia. A raised IRT concentration on D3 (> 50 µg/l) must be repeated 3 weeks (D21) after birth. If the day 21 IRT is still raised (> 60 µg/l), testing is performed for the main CF gene mutations with the parents' consent.

The diagnosis of cystic fibrosis is made from the ***sweat test*** which measures chloride concentration in a sample of at least 100 mg of sweat obtained by pilocarpine iontophoresis (Gibson and Cooke method). Normally, sweat contains less than 40 mEq/l of chloride and the test is pathological at values above 60 mEq/l. Between 40 and 60 mEq/l, the result is deemed to be equivocal and the test must be repeated. Two positive sweat tests are required to confirm the diagnosis and trigger molecular investigation of the CFTR gene. This test is difficult to interpret before the age of 1 month. The sweat test can therefore confirm a diagnosis of cystic fibrosis based on the identification of 2 mutations by molecular CFTR gene analysis. This test is particularly important to diagnose atypical forms of the disease in both children and adults. It is a necessary prerequisite before requesting testing for rare mutations.

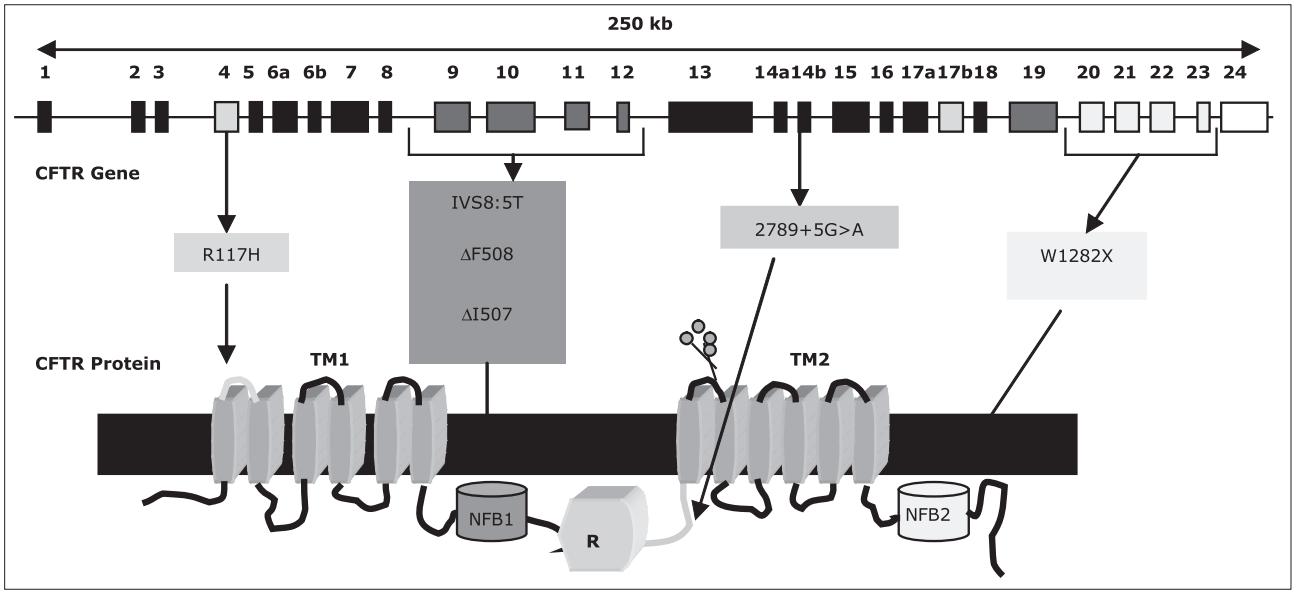
Genetic studies are indicated for antenatal diagnosis in heterozygote couples, couples in which one of the people has the disease, couples requesting medically assisted pregnancy, particularly those in which the man is sterile because of bilateral vas deferens agenesis or in suspected antenatal situations from ultrasound findings (hyperechogenic abdominal appearance suggesting bowel obstruction).

First line genetic tests

Targeted test for 30 to 36 mutations using PCR multiplex-ARMS *(PCR-amplification-refractory mutation system)*, PCR-OLA *(PCR-oligo ligation assay)*, PCR multiplex and reverse hybridization assay *(LiPA or Line Probe Assay)* methods which identify 75 to 90% of mutated alleles depending on the region.

Second line genetic tests

Testing for other mutations (rare mutations or those not detected by commercial kits) by analysing the 27 exons of the gene and their flanking intron regions (method: denaturating agent gradient electrophoresis or DGGE, denaturating high pressure chromatography or DHPLC).



TREATMENT

The management of affected people is with antibiotic therapy for respiratory infections, respiratory physiotherapy and dietary measures (high calorie normal fat diet supplemented with vitamins and pancreatic extracts).

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CYSTICERCOSIS

DEFINITION

Cysticercosis is a parasitosis due to accidental development of a larval form of *Taenia solium and Cysticercus cellulosae*, in human beings. The intermediary host in the life-cycle is the pig although human beings substitute and then develop the disease. Larvae in the central nervous system (CNS), is a particularly worrying feature and is responsible for severe cysticercosis.

Taenia solium (Ts), still called armed Taenia or armed tapeworm, is a flatworm which belongs to the Plathelminth branch, Cestodes class Cyclophyllides order and *Taeniidae* family.

The adult measures 1 to 3 metres long and has a scolex equipped with suction pads and hooks. It has a small number of thick uterine branches.

The larva or *cysticercis* is an ovoid vesicle containing fluid measuring 5 to 15 mm long and 4 to 12 mm wide with a protoscolex (the scolex of the future adult *Taenia*).

INTRODUCTION

EPIDEMIOLOGY

It is a cosmopolitan worm, with infestation occurring from faecal contamination and also from proximity between human beings and pigs, particularly in farming regions.

Cysticercosis is mostly contracted by ingesting the *Taenia solium* embryophores contaminating food or drinking water, and far more rarely from self-infestation.

It is common on Central America (Mexico), Asia (China, India and Thailand), Central Europe, Central and South Africa and in Madagascar.

It has almost disappeared from La Réunion Island and the Iberian peninsular.

LIFE-CYCLE

The adult worm is found in the small intestine in human beings and the terminal rings are excreted in faeces. The eggs excreted into the external environment are ingested by the intermediary host, pig (or accidentally, human beings) and then rupture in the stomach releasing embryons into the gastro-intestinal tract. These cross the intestinal wall and once in circulation reach muscles, subcutaneous tissue and the CNS. The embryos then transform into larvae known as cysticerci.

Human beings become infested by eating poorly cooked pork, from water or from raw vegetables. The Taenia grows in the intestine and becomes adult in 3 months and then begins to release the rings.

SYMPTOMS

The clinical symptoms are directly related to the location of the cysticerci and their anatomical consequences. The most common sites are the nervous system, eye, and subcutaneous tissue.

- **Cerebral sites:**

  Cysticercosis particularly affects the CNS causing epileptiform attacks, seizures, raised intracranial pressure, various neurological deficits and even a picture of dementia.

- **Ocular sites:**

  Damage of the globe of the eye is the most common although the larvae can also reach the annex tissue such as the orbit, eyelid, conjunctiva, the interior of the vitreous and the retina, causing major visual disorders.

- **Muscle and subcutaneous sites:**

  Muscle involvement is usually asymptomatic or causes mild myalgia or cramps. Subcutaneous involvement is revealed by the presence of small nodules, particularly on the chest and back.

SEARCH INDICATIONS

Diagnosis of cysticercosis in a person with suggestive epidemiological and/or clinical and/or radiological evidence.

Diagnosis of cysticercosis in a person with a past history *T. solium* taeniasis.

Differential diagnosis from nematode infestation in anatomopathological examination of an excision section.

INFORMATION

SAMPLE

Serum, cerebrospinal fluid (CSF) and other biological fluids (aqueous humour) for immunological diagnosis.

Subcutaneous nodule or intramuscular biopsy for anatomopathological examination.

QUESTIONS FOR THE PATIENT

Clinical symptoms (systemic or local signs)?

History of a stay in an at risk area?

Past history of T. solium taeniasis (self-infestation)?

Current antihelminth treatment?

SAMPLE STORAGE AND TRANSPORT

Serum or CSF samples should be stored at + 4°C for up to a week and frozen beyond this time.

DIAGNOSTIC METHODS

NON-SPECIFIC DIAGNOSIS

Blood hypereosinophilia: Fickle, raised in the initial months.

Raised cerebrospinal fluid protein in neurocysticercosis.

SPECIFIC DIRECT DIAGNOSIS

It is very difficult to diagnose cysticercosis with certainty as the parasite has reached the end of its cycle and it is not therefore exteriorised.

Only macroscopic and microscopic examination of a biopsy sample can reveal the cysticerci or some of their parts (scolex or hooks).

Anatomopathological examination of subcutaneous nodules or intramuscular biopsies in the investigation of choice to diagnose extra-cerebral forms of cysticercosis.

■ IMMUNOLOGICAL DIAGNOSIS

This involves testing for antibodies against *Cysticercus cellulosae* antigens in serum, CSF, or more rarely in other biological fluids.

The methods available (Ouchterlony agar, double diffusion, indirect immunofluorescence, ELISA and immunoelectrophoresis) use a wide variety of antigens from various sources, with very different sensitivity and specificity. Numerous common antigens with other cestodoses exist, particularly with hydatid disease, alveolar ecchinococcosis and cenurosis and also with various nematode and distomatode infestations.

In practice, ELISA is now used for screening (sensitivity 80% and specificity 75%) and any positive result is confirmed by Western blot (specificity > 99%).

INTERPRETATION

The diagnosis of cysticercosis is rarely straightforward and is usually made from a series of epidemiological, clinical, radiological (a cerebral CT and MRI scans are essential) and laboratory evidence.

Serology is useful in neurocysticercosis when simultaneous analysis of serum and CSF can guide the diagnosis.

Ocular involvement is characterised by the production of *in-situ* antibodies (aqueous or vitreous humour).

TREATMENT

Cestocide treatment has improved the outcome of cysticercosis, particularly with cerebral damage. The treatment is the same as for taeniasis although at higher doses for longer periods of time, to allow the treatment to diffuse into tissue cysticercis sites.

■ DRUG TREATMENT

Praziquantel or Albendazole.

Treatment varies depending on the site of the larvae:

– In neurocysticercosis, cestocidal treatment is combined with corticosteroid therapy and sometimes followed by surgery.

– In ocular cysticercosis, surgery and laser are often combined with medical treatment.

– Other sites involve mostly drug treatment.

■ PROPHYLAXIS

General: Combating human faecal contamination, increased water and food hygiene measures, monitoring pork meat in abattoirs, testing pig herds and epidemiological surveillance.

Individual: Adequate cooking or freezing of pork meat and treatment of taeniasis.

FOR FURTHER INFORMATION

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CYTOMEGALOVIRUS

DEFINITION

Cytomegaloviruses are members of the *Herpesviridae* family, *Betaherpesvirinae* sub-family, which have narrow species specificity. Human cytomegalovirus, CMVH or human Herpes virus 5 (HHV5) is not morphologically different from the other herpes viruses. The virions measure approximately 150 nm and are enveloped. The genome is a large linear double chain DNA and viral replication is intranuclear. After primary infection, CMVH enters the latent phase like all of the *Herpesviridae*. The latent sites are still poorly understood and are probably multiple (peripheral leukocytes, bone marrow, macrophages, etc.). Reactivation occurs following lowered cellular immunity. The presence of antibodies does not prevent reactivation or reinfection with a new strain of CMVH.

INTRODUCTION

EPIDEMIOLOGY

The virus reservoir is strictly human and in France, approximately 50% of adults have antibodies against CMVH. The virus is present in circulating leukocytes in infected people. It may be excreted asymptomatically and for long periods of time in saliva, urine, genital secretions and breast milk. CMVH is relatively fragile in the external environment. Infection occurs through direct contact via respiratory (aerosol droplets) secretions, sexual contact, transfusion of non-leukocyte depleted blood or organ transplantation. Materno-foetal transmission can occur regardless of the term of the pregnancy.

SYMPTOMS

Clinical expression depends on the infected person's immune status.

Primary infection in immunocompetent people

Cytomegalovirus infection is asymptomatic in the great majority of cases. It may however present with a picture of prolonged fever with a mononucleosis-like syndrome, often accompanied by laboratory changes in liver function tests and numerous transient immunological abnormalities (rheumatoid factor and anti-organ antibodies). Polyradiculoneuritis has been described but is rare.

The post-transfusion mononucleosis syndrome beginning 3 to 5 weeks after massive transfusion has become extremely rare since the use of leukocyte depleted blood products.

Materno-foetal transmission and perinatal infection

CMVH infection is probably the commonest of the materno-foetal infections throughout the world. It is estimated to have an incidence of 0.5 to 3% at birth. The virus crosses the placenta during the maternal viraemia which accompanies active cytomegalovirus infection.

It is high risk following primary infection, which is usually silent occurring in the first trimester of pregnancy, when the infection is transmitted to the foetus in 1/3 of cases and may result in in-utero death. When the pregnancy has progressed to term, fewer than 10% of newborn babies display symptoms. Generalised cytomegalovirus inclusion disease, which is a very severe septicaemic form, is often associated with encephalitis. However, it is fortunately rare but fatal in 30% of cases. Most surviving children have severe complications, particularly neurological (microcephalus, hydrocephalus, intracranial calcifications and chorioretinitis). More than 10% of asymptomatic children at birth develop neurosensory and/or psychological complications in the first two years of life.

Foetal transmission following maternal cytomegalovirus reactivation is far less common common (less than 5%) and usually has no clinical consequences. It does however carry a significant risk of neurosensory abnormalities (deafness) occurring in the first two years of life.

Finally, maternal reinfection by a new strain of virus, which is antigenically different to the endogenous virus is rare but can cause severe congenital infection.

Perinatal infection occurs as a result of infection of the newborn child either during childbirth or from breast-feeding or maternal baby care. This is usually benign.

Transmission following organ or bone marrow allotransplantation

As CMVH can persist in the latent state in donor cells and organs, recipients can become infected. In addition, this is predisposed to by immunosuppressant treatment. Without prevention, cytomegalovirus infection can occur in more than half of transplant patients. The insidious development of a flu-like febrile syndrome is a warning sign. Primary infection can have very serious consequences in CMVH-seronegative recipients, (interstitial pneumonia after bone marrow transplantation, often fatal if not treated). Cytomegalovirus infection can also predispose to rejection and increase the risk of superinfection.

Cytomegalovirus infection and AIDS

This is a major complication of the immunosuppression produced by HIV infection (retinitis, gastro-intestinal ulceration and neurological disease) but the risk of cytomegalovirus infection has fallen considerably since the introduction of highly active antiretroviral treatments.

SEARCH INDICATIONS

Aetiological diagnosis of a mononucleosis-like syndrome.

Investigation of fever in a transplant patient or pregnant woman.

Investigation of neurological syndrome, pneumonia or gastro-intestinal symptoms in the immunosuppressed.

Monitoring pregnant women.

Investigation of foetal abnormalities.

Aetiological diagnosis of congenital disease (septicaemic form and localised form).

INFORMATION

SAMPLE

Depending on the symptoms, various different samples may be taken. Whole blood taken into EDTA or citrate, urine, CSF, aqueous humour, bronchial secretions, genital secretions, broncho-alveolar lavage specimens, bone marrow or liver biopsy. Amniotic fluid must not be taken before the 21st week of pregnancy and must be taken at least six weeks after the date of maternal seroconversion if this can be assessed.

Antibody testing is performed on non-haemolysed serum kept at +4°C. Testing for IgM antibodies can be performed on foetal blood obtained by cordocentesis after the 17th week of pregnancy.

QUESTIONS FOR THE PATIENT

Immune status, possible HIV seropositivity, pregnancy status, ultrasound abnormalities, clinical signs, type and onset of symptoms.

SAMPLE STORAGE AND TRANSPORT

As CMVH is reputed to be relatively unresistant in the external environment it is recommended that samples be promptly transported refrigerated to the laboratory (in a few hours if possible and in less than 3 days). Swab samples (eye, throat and cervix) must be placed in a transport medium. It is recommended that CSF be frozen and that serum be kept at 4°C if the laboratory is distant to the sampling site.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

Histo-cytological examination

This involves staining to identify large cells (cytomegaly) with nuclear inclusions ("fish eye" cells). It may be useful to examine tissue biopsies.

Detection of viral antigens in the sample cells

This is performed by immunofluorescence or possibly immunoperoxidase, on for example, cells obtained from broncho-alveolar lavage and on histological sections. Monoclonal antibodies against antigens which develop early in the CMVH replication cycle are often used. Antigenaemia can be quantified by counting the percentage of polynuclear cells containing pUL83 protein (pp65) by labelling with a specific monoclonal antibody. This is a rapid method but must be performed within a few hours after the sample is obtained in order to be reliable.

Detection of the viral genome

This is usually performed by PCR on circulating blood leukocytes and possibly on plasma or serum, CSF or tissue biopsies. Detection of cytomegalous DNA in amniotic fluid indicates materno-foetal infection. Quantification of the circulating viral load (DNAaemia) by real time PCR can be used to assess the potential progression of the infection and to monitor the effectiveness of treatment. NASBA technology has been proposed to detect late messenger RNA, the presence of which is a marker of active infection.

Isolation of the virus

CMVH can only be cultured on human embryonic fibroblasts, and the cell lesions develop slowly (10 to 30 days). The test can be accelerated by centrifuging the sample on a cell monolayer and then revealing the CMVH with labelled monoclonal antibody (fluorescence or peroxidase) against very early antigens after 48 to 72 hours.

This method is normally used to test for CMVH in urine. It has limited use for viraemia because the inoculum is frequently toxic to the cell monolayer. In this case, measurement of pp65 antigenaemia or viral load is preferred.

INDIRECT DIAGNOSIS

Testing for total antibodies or IgG antibodies may be performed by passive agglutination and by ELISA. Seroconversion indicates the existence of primary infection. The presence of stable antibody titres indicates prior contact with CMVH meaning that the person is carrying the virus in the latent stage. Detection of IgM antibodies preferably using the immuno-capture method which is reputed to be less subject to interferences is an indicator of recent primary infection or active infection. It is essential however, to be aware of the existence of non-specific reactions (EBV infections, for example) particularly with low values. The findings of IgM antibodies in foetal blood obtained by cordocentesis are a good indicator of *in-utero* cytomegalovirus infection.

Measurement of the antibody avidity index involves measuring IgG antibodies before and after treating the antigen-antibody complex with a concentrated urea solution. A high avidity index excludes recent primary cytomegalovirus infection.

INTERPRETATION

DIAGNOSIS OF PRIMARY CYTOMEGALOVIRUS INFECTION

The best criterion is seroconversion. IgM antibodies are a warning sign although can be found in secondary infection. Possible non-specific positive reactions must also be considered. Measurement of antibody avidity may help in some cases to differentiate primary infection from reactivation. This test is particularly useful in pregnant women in which case serum from as early as possible after the start of the pregnancy should be used.

DIAGNOSIS OF ACTIVE INFECTION

Serology is generally not particularly contributory, particularly in immunosuppressed patients, and testing for markers of viral replication is required (particularly antigenaemia and DNAaemia). Systematic monitoring must be started in allotransplant patients after transplantation to detect early cytomegalous infection as early as possible.

The finding of the virus or its components in an anatomical site or in a biological fluid must always be interpreted with caution and considered in the context of the clinical picture and patient's immune status.

■ DIAGNOSIS OF MATERNO-FOETAL INFECTION

In suspected primary cytomegalovirus infection during pregnancy or if ultrasound abnormalities (cerebral ventricles and intestinal loops) are found, testing for the virus and particularly for CMVH DNA by PCR in amniotic fluid is indicated. The sample must not be taken before the 21st week of pregnancy and at least 6 weeks after the date of maternal primary infection if this can be established. Quantification of amniotic fluid viral load can provide an aid to help assess the potential severity of the foetal disease.

Anti-CMVH IgM can be found in foetal blood from the 17th week of pregnancy and can be trigger testing for the virus in amniotic fluid.

In newborn babies, isolation of CMVH in urine during the first two weeks of life indicates acquired in-utero infection. Beyond 3 weeks after birth it may indicate perinatal infection.

■ DETERMINATION OF CMVH SEROLOGICAL STATUS

The isolated finding of IgG antibodies indicates prior contact with the virus. When antibodies are absent, CMVH-negative pregnant women and transplant recipients who are therefore at risk of primary infection can be identified in order to take appropriate prophylactic measures. CMVH-seronegative blood donors can also be selected for transfusions in seronegative at risk patients (transplant patients, pregnant women and newborn children).

Measurement of serological status is not currently recommended systematically for pregnant women.

TREATMENT

■ CURATIVE

Three antiviral agents can currently be used: Ganciclovir, cidofovir and foscarnet (Foscavir®) are only virostatic and have no effect on latent CMVH. As a result, a 3 week loading treatment must be started, followed by prolonged maintenance treatment. Because of their toxicity (haematological for ganciclovir and renal for the other two), their use must be restricted to severe infections in the immunosuppressed, which is proven to be due to cytomegalovirus. Mutant viruses resistant to these drugs have been described.

Administration of high dose gamma globulins can reduce the severity of symptoms.

■ PREVENTATIVE

There is as yet no vaccine against cytomegalovirus.

■ DURING PREGNANCY

As CMVH is commonly excreted in the urine and saliva of young children (crèches, nurseries, paediatric departments, etc,) for long periods of time, pregnant women who do not have anti-CMVH antibodies who come into contact with young children must take special precautions, washing their hands frequently after each nappy change, not sharing cutlery, not tasting from the feeding bottle, not sharing toiletry items or linen and not kissing the child on the mouth. These precautions must be followed until delivery.

■ BLOOD OR LABILE BLOOD PRODUCT TRANSMISSION

These products are filtered systematically to remove leukocytes. Filtration is combined with selection of CMVH-seronegative donors for transfusion in people at risk of developing severe primary cytomegalovirus infection. It would be desirable but not practically realistic to select CMVH seronegative organ transplant donors.

■ POST ALLOTRANSPLANTATION PREVENTION

Valaciclovir, ganciclovir or one of its pro-drugs, valganciclovir, can be used, as can gamma globulins.

FOR FURTHER INFORMATION

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D-DIMERS

DEFINITION

D-dimers are specific degradation products of fibrin. During fibrinolysis, plasma degrades fibrinogen and soluble fibrin into fragments X, Y, D and E. Whereas stabilised fibrin (by factor XIIIa) is degraded into smaller variable fragments called X oligo-dimers, which themselves are degraded into D-dimers (end products of clot lysis). The molecular structure of the D-dimers is heterogeneous, partly explaining problems in standardising the assay, particularly due to the use of antibodies against different epitopes.

INTRODUCTION

Blood coagulation results in the formation of a clot and vascular extension of which, is controlled physiologically by the fibrinolysis system. The key enzyme in this system, plasmin, cleaves fibrin which has been stabilised by factor XIIIa and releases fibrin degradation products, the D-dimers.

INDICATIONS FOR MEASUREMENT

The main use of D-dimer measurement is in the exclusion of recent deep vein thrombosis (DVT) or pulmonary embolism (PE) (less than 10 days old). The aim of measurement (combined with clinical probability) is to identify patients in whom anticoagulation treatment can be avoided entirely safely, faster at lower cost and non-invasively.

D-dimer measurement now forms part of a non-invasive, diagnostic strategy for thromboembolic events combined with Doppler ultrasound, pulmonary CT angiography and/or ventilation-perfusion scintigraphy.

D-dimers are an indicator of coagulation activation. Because of this, their measurement is useful in the diagnosis and monitoring of disseminated intravascular coagulation (DIC) and primary fibrinolysis, alongside other laboratory markers (platelet count, measurement of fibrinogen and other coagulation factors).

Recent studies have shown that D-dimers are useful in identifying patients at risk of recurrence after stopping anti-vitamin K treatment. If the D-dimers are negative 3 to 4 weeks after stopping treatment, the likelihood that a patient will suffer a recurrent venous thromboembolic event is lower than if the D-dimer level is raised. Because of this, measurements may help the decision to continue or stop oral anticoagulation.

INFORMATION

SAMPLE

0.109 M citrated plasma, 1/10 (0.5 ml per 4.5 ml of blood). 0.129 M citrated tubes are also acceptable.

The person does not need to be fasting. A light fat-free snack is permitted but coffee, smoking and alcohol must be avoided in the hour before the sample.

For further information refer to the chapter *"General haemostasis pre-analytical conditions"*.

QUESTIONS FOR THE PATIENT

Current treatment?

– Anti-vitamin K drugs: D-dimers are reduced on anti-vitamin K treatment.

– Thrombolytic treatments: Streptokinase, urokinase, rt-PA, tenecteplase. These treatments increase plasma D-dimer concentrations.

Patient age?

Outpatient or hospitalised patient (recent surgery etc.)?

Intercurrent disease (infection, cancer, etc)?

Are you pregnant?

SAMPLE STORAGE AND TRANSPORT

The sample is stable for 24 h at ambient temperature and then for weeks at -20°C and for several months at –70°C.

The sample is sent frozen within 4 hours after sampling. It is recommended that it be thawed rapidly in a water bath at 37°C.

ASSAY METHODS

Many commercially-available assay methods are now available. Some methods provide a qualitative result, others a semi-quantitative result and others a quantitative result.

The major problem is a lack of method standardisation. These may differ depending on the method used (agglutination of various types of particles, ELISA), type of antibody used (monoclonal or polyclonal) and the epitopes recognised by the antibodies.

The oldest techniques are the sensitised latex particle agglutination methods. These give semi-quantitative results and have a limit of detection of 500 ng/ml. They are not sufficiently sensitive to exclude DVT (66 to 96%). Their use nowadays is reserved for the diagnosis or monitoring of DIC.

More recent techniques are immunoenzymatic and quantitative latex methods.

1st generation ELISA tests are 10 times more sensitive than the semi-quantitative latex tests (sensitivity 89 to 100%). These were the methods which demonstrated the utility of D-dimer measurement in excluding DVT although they are lengthy to perform and not suitable for routine use.

Rapid automated D-dimer measurement tests have more recently been marketed. These are either automated single sample ELISA methods (2nd generation), or so-called "quantitative latex" methods which can be adapted to automated coagulation instruments. Overall, these recent methods are highly sensitive (approximately 100%) enabling them to be used in the exclusion diagnosis of DVT or recent PE.

UNITS AND REFERENCE VALUES

UNITS

No international standard exists. The concentrations measured by different methods are not therefore comparable, particularly as some results are expressed in fibrinogen

equivalent units (FEU), whereas others are expressed as D-dimer units (DDU), 1 µg/ml FEU is equivalent to 0.5 µg/ml DDU. Efforts have been made since 1993 to standardise the D-dimer measurement unit in fibrinogen equivalent units.

NORMAL EXPECTED VALUES

Reference values vary between reagents. This must be taken into account when results are interpreted. Nevertheless, a number of published validated methods, particularly the quantitative ELISA methods, have quoted reference values of less than 500 ng/ml (i.e. 0.5 µg/ml).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

D-dimers increase with age and are raised in pregnancy.

PATHOLOGICAL VARIATIONS

A plasma D-dimer concentration below a given threshold for a method can exclude the diagnosis of venous thromboembolic disease with a sensitivity of almost 100% provided that the threshold value has been validated in clinical studies conducted using this method. D-dimers however are increased in many non-specific situations explaining their poor sensitivity to exclude thrombosis.

There are various non-specific causes of raised D-dimers: Pregnant women, patients over 80 years old, hospitalised patients, patients suffering from cancer, infection or inflammation and after surgery or recent injury.

Raised D-dimer concentrations cannot in any situation be used to diagnose DVT or PE *(cf. above)*.

There are no validated studies at present establishing a relationship between D-dimer concentrations and the presence of thrombosis, or in thrombosis, between plasma D-dimer concentrations and the site or extent of the thrombosis, or the future risk of recurrence. Diagnosis can only be made with certainty by imaging (Doppler ultrasound, helicoidal CT scan, phlebography, scintigraphy, etc.).

Investigations for possible DIC should be performed if D-dimer values are very raised.

D-dimer concentrations < 500 ng/ml, 3 to 4 weeks after stopping anti-vitamin K treatment may be an argument to support stopping anticoagulation.

FOR FURTHER INFORMATION

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D-XYLOSE TEST

DEFINITION

D-xylose is a monosaccharide, which is not present in human beings. Concentrations are measured in serum and urine, after swallowing a defined dose of D-xylose. This test is called the "D-Xylose test" or "D-xylose tolerance test" and is used to investigate proximal small bowel absorption capacity.

INTRODUCTION

D-xylose is 50% absorbed in the small bowel. The molecule is not metabolised and 100% is removed in urine. Dietary carbohydrates are hydrolysed by salivary and pancreatic amylases into disaccharides. Only the monosaccharides can be directly absorbed by enterocytes through active transport. In order to facilitate absorption, enzymes hydrolyse the disaccharides in the enterocyte apical membranes. Any damage to the enterocytes reduces carbohydrate absorption and produces a change in the amount of D-xylose absorbed.

INDICATIONS FOR MEASUREMENT

The D-xylose tolerance test is useful to assess sugar absorption by the intestinal mucosa in malabsorption syndromes and in chronic diarrhoea.

INFORMATION

SAMPLING PROTOCOL

– Subjects should be fasting for 12 hours, resting, lying down or seated throughout the time of the test and should not eat or drink during the test.

– At "T0" the patient should be asked to pass urine and the urine should then be discarded. A blood sample is withdrawn into a dry tube or fluoride tube for xylose measurement (baseline value).

– Adults should swallow 25 g of D-xylose dissolved in 500 ml of water.

– Children should swallow 0.7 g of D-xylose per kg body weight, dissolved in 100 to 200 ml of water.

– At "T+1 hour" for children and "T+2 hours" for adults, a measurement of serum xylose (dry tube or fluoride tube) should be performed.

– Collect urine for the 5 hours after taking the xylose in children and adults for measurement of urinary xylose (although this is almost never now performed routinely). Record the urine volume.

The patient must be monitored throughout the test because of the risk of vomiting and diarrhoea making the test uninterpretable.

QUESTIONS FOR THE PATIENT

Before starting the test the patient should be checked to ensure he/she is not suffering from renal insufficiency and is well hydrated. Reduced renal clearance may bias the test result. Patients must also be asked about whether they have taken aspirin, atropine or indomethacin, which may interfere with the test.

SAMPLE STORAGE AND TRANSPORT

Centrifuge blood samples and store plasma or serum at - 20°C. Transport frozen at - 20°C.

Urine must be stored in a refrigerator at + 2°C to + 8°C throughout the test collection. Transport at + 4°C.

ASSAY METHODS

Colourimetric (p-bromoaniline, thiourea in acetic acid).

NORMAL EXPECTED VALUES

For reference:

	Serum at 60 minutes	Serum at 120 minutes	Urine
Children	150 - 200 mg/l 0.98 – 1.3 mmol/l	> 200 mg/l > 1.3 mmol/l	> 4 g/ 5h (26 mmol/ 5h) 16 – 32% dose
Adults	200 - 570 mg/l 1.3 – 3.7 mmol/l	300 - 580 mg/l 1.95 – 3.77 mmol/l	> 4 g/ 5h (26 mmol/ 5h) > 14% dose

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Physical activity can increase D-xylose absorption.

PATHOLOGICAL VARIATIONS

Changes in D-xylose tolerance (reduced absorption) are due either to a defect of the digestive system or to gastro-intestinal infection.

Digestive system	Mucosal abnormality	Sprue
		Enteritis
		Crohn's Disease
		Whipple's Disease
		Short small bowel syndrome
		Coeliac disease
Infection	Viral	Viral gastroenteritis
	Bacterial	Growth of bacteria in the small intestine
	Parasitic	Giardia lamblia infection
		Ascaridiosis
	HIV	Malabsorption syndrome

Xylose absorption is unchanged in renal insufficiency, although its removal is delayed and serum concentrations remain elevated for longer (at 3 and 5 hours if further samples are taken; 2 hour values are unchanged).

FOR FURTHER INFORMATION

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DECARBOXYPROTHROMBIN

DEFINITION - INTRODUCTION

Prothrombin or coagulation factor II is a vitamin K dependent protein. It is synthesised in the presence of vitamin K and contains a carboxyl group which is necessary for the action of the protein. Defective carboxylation either secondary to vitamin K deficiency or a post-translational defect (independently of vitamin K deficiency) as is seen in hepatocellular carcinoma results in an increase in plasma decarboxyprothrombin (DCP) concentrations.

Synonym: Des-gamma-carboxyprothrombin (DCP), *Protein induced by vitamin K absence or antagonist II* (PIVKA II).

INDICATIONS FOR MEASUREMENT

Detection of hepatocellular carcinoma (HCC) in at risk patients; prognostic indicator and monitoring treatment of recurrent HCC. Measurements are used widely in Asia but are not greatly used in France.

INFORMATION

■ SAMPLE

Discard the first millilitre of whole blood, taken into a citrate tube. Citrate concentration 3.2 % (0.109 M) 1/10 (0.5 ml per 4.5 ml of blood). 3.8% citrated tubes (0.129 M) are also acceptable.

A fasting sample is not required. Patients may take a light fat-free snack.

■ QUESTIONS FOR THE PATIENT

Are you taking anti-vitamin K treatment? These medicines cause an increase in plasma DCP concentrations.

Current cancer treatment: Chemotherapy, radiotherapy, surgery (methods and date of treatment).

■ SAMPLE STORAGE AND TRANSPORT

Centrifuge for 10 minutes at 2500 g, separate and freeze at –20°C within 4 hours of sampling.

Transport: Frozen at – 20°C.

ASSAY METHODS

Radioimmunological assay or immunoenzymatic assay.

NORMAL EXPECTED VALUES

As an indication: < 2 ng/ml.

PATHOLOGICAL VARIATIONS

Increased plasma DCP concentrations in HCC

Decarboxyprothrombin (DCP) is a more specific marker of HCC than AFP, plasma concentrations being little if at all increased in other liver disease (hepatitis, cirrhosis, liver metastases, etc.). On the other hand it is less sensitive. It is however raised in approximately 70% of HCC (some of which have a normal AFP). Because of this the combination of both markers is believed to increase their sensitivity to diagnose and monitor HCC.

A recent Japanese study in patients with recurrent HCC after liver resection has shown that AFP and DCP are both significant prognostic indicators of recurrent HCC and that measurement during follow up can improve the prognosis of these patients. DCP was reported to have better diagnostic precision for invasive HCC compared to AFP, particularly in chronic HBs antigen carriers with intra-hepatic tumour.

Non-specific rises in DCP

Anti-vitamin K treatments.

Avitaminosis K: Deficient intake or usually malabsorption.

FOR FURTHER INFORMATION

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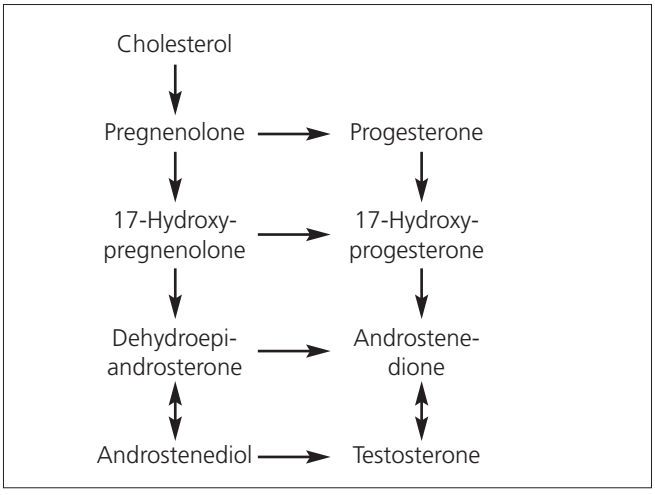
DELTA 4-ANDROSTENEDIONE

DEFINITION

Androstenedione is synthesised in the gonads, adrenal glands and peripheral tissues, where it is the immediate precursor of testosterone. As applies to the other steroids, it is biosynthesised from cholesterol, the side chain of which is cleaved to convert it into pregnenolone. Androstenedione is formed from pregnenolone by two pathways, the 4-ene (or Δ4) pathway which involves the sequence progesterone, 17-hydroxyprogesterone and androstenedione and the 5-ene (or Δ5) pathway, with the sequence 17-hydroxypregnenolone and dehydroepiandrosterone. Shifts between pathways may occur at any level under the action of 3β-hydroxysteroid dehydrogenase and an isomerase.

Androstenedione exists in equilibrium with testosterone both in steroid-producing tissues, in the liver and in peripheral tissues, particularly adipose tissue.

Biosynthesis of androgens



Androstenedione is therefore partly secreted by the gonads and adrenals and partly formed in peripheral tissues from precursors. The contributions of glandular secretion and peripheral formation to total androstenedione production vary depending on sex and age.

Origin of circulating androstenedione

In women (follicular phase)		
Ovarian secretion	Adrenal secretion	Peripheral Conversion
45%	45%	10% (DHA)*
In post-menopausal women		
Ovarian secretion	Adrenal secretion	Peripheral Conversion
20%	70%	10% (DHA)*
In men		
Testicular secretion	Adrenal secretion	Peripheral Conversion
40-50%	40-50%	5% (T)* 15% (DHA)

** precursor*

In women during the active ovarian time of life, androstenedione is secreted by the adrenal gland and ovary, particularly the ovary carrying the pre-ovulatory follicle and then the corpus luteum. The contribution of ovarian secretion to total production varies during the cycle from 45% at the start of the cycle to almost 60% in the pre-ovulatory phase, returning to 45% in the middle of the luteal phase. Conversely, extragonadal conversion of dehydroepiandrosterone into androstenedione makes up approximately 10% of total production regardless of the phase of the cycle.

In post-menopausal women ovarian secretion falls greatly, contributing no more than 20% to total production, the contribution from adrenal secretion making up 70%. The contribution of peripheral conversion remains identical at 10% of total production.

In men, the testes and adrenal cortex contribute similar amounts, ranging between 40 and 0%, peripheral conversion contributing around 20%.

Androstenedione is not bound to SHBG in the blood and circulates in the unbound form or bound to albumin. Its metabolic clearance does not therefore vary and any overproduction inevitably results in raised plasma concentrations.

Metabolically, androstenedione is inter-converted into testosterone in the liver and in peripheral tissues. It is metabolised through two pathways in the liver: one of these pathways leads to the production of androsterone and etiocholanolone; the 17-ketosteroids, which are sulpho- and glucurono-conjugated before being removed in urine. The other pathway leads to the formation of androstanediol which is excreted in urine as the glucurono-conjugate.

Androstenedione can be aromatised into estrone, both in the liver and in peripheral tissues and in particular in adipose tissue. Conversion increases with age both in women and in men.

Synonyms: Androstenedione = delta-4-androstenedione = delta-4 A.

INDICATIONS FOR MEASUREMENT

Androstenedione is the female androgen. Measurement forms part of the investigation of hirsutism and virilism in women or girls in order to establish a diagnosis of excess androgen production, to establish its cause and to monitor treatment. Similarly, measurement is indicated in all cases of ambiguous genitalia and precocious sexual development. On the other hand, androstenedione has limited use in men outside of adrenal disease.

INFORMATION

■ SAMPLE

Androstenedione can be measured both in serum and in plasma (collected into EDTA or heparin). Haemolysed and lipaemic samples must be avoided. In addition the sample must be taken in the morning because of the presence of a 24 hour cycle similar to that of cortisol, and during the early follicular phase in women.

FURTHER INFORMATION

In addition to age and sex, any corticosteroid or oestrogen-progestogen treatments being taken must be reported because of the combined adrenal and ovarian origin of androstenedione.

SAMPLE STORAGE AND TRANSPORT

Separated plasma or serum should be stored at + 4°C and transported at this temperature to the laboratory. Samples may be stored for 6 months to 1 year frozen at –30°C.

ASSAY METHODS

The methods used are immunoassays, generally with an isotopic label. The anti-sera now available are sufficiently specific to allow assay without chromatography, although pre-extraction of plasma or serum is required for some kits.

UNITS

Results can be expressed either as ng/ml or nmol/l. Ng/ml are converted to nmol/l by multiplying ng/ml by 3.492 and nmol/l to ng/ml by multiplying nmol/l by 0.286.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Androstenedione concentrations are higher at birth in boys than in girls and then fall rapidly reaching very low values at the end of the first year of life in both sexes. They remain at this level until puberty.

In adulthood, concentrations are higher in women than in men and do not vary significantly during the menstrual cycle.

Women in active ovarian phase	0.7 – 3.5 ng/ml
Men	0.5 – 2.2 ng/ml

Concentrations fall markedly after the menopause and continue to fall with age.

Age (years)	**< 60**	**60 - 70**	**> 70**
Androstenedione (ng/ml)	0.3 – 1.4	0.2 – 1.3	0.2 – 1.3

PATHOLOGICAL VARIATIONS

In women

Excess androgen production with high concentrations of androstenedione can be seen in various pathological conditions:

Ovarian or adrenal tumours

Testosterone and androstenedione are greatly elevated in ovarian tumours. DHA sulphate is also greatly elevated in adrenal tumours.

Congenital adrenal hyperplasia

In 21-hydroxylase deficiency, measurement of 17-hydroxyprogesterone is used to diagnose in basal conditions for the complete form or after a Synacthen® stimulation test in incomplete forms. In 11β-hydroxylase deficiency, the diagnosis is made using 11-deoxycortisol.

Polycystic ovarian syndrome

Increased circulating androgen concentrations are accompanied by increased LH concentrations.

In children

In ambiguous genitalia due to 17ketoreductase deficiency (the enzyme converting androstenedione into testosterone), the diagnosis is made by measurement of androstenedione.

Investigation of precocious sexual development in women should include the measurement of androstenedione.

In men

Androstenedione is increased in adrenal diseases.

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DELTA AMINOLEVULINIC ACID DEHYDRATASE

DEFINITION

Delta-aminolevulinic acid dehydratase (ALAD) is a cytosolic enzyme that participates in the haem synthesis pathway, catalysing the biosynthesis of porphobilinogen (PBG) from two molecules of δ-aminolevulinic acid (ALA), the starting point for porphyrin synthesis.

INTRODUCTION

Lead poisoning (saturnism) interferes with the activity of many cytosolic and membrane-bound proteins as a result of the metal reversibly binding to thiol groups. Among the enzymes inhibited are those involved in the haem biosynthesis pathway, including aminolevulinic acid dehydratase (ALAD) and ferrochelatase. Inhibiting ALAD leads to a rise in the concentration of ALA in the urine, and inhibiting ferrochelatase leads to a build-up in free erythrocytic protoporphyrin. The result is haem deficiency which compromises haemoglobin production and certain intracellular processes, notably mitochondrial respiratory activity and oxidative metabolism.

INDICATIONS FOR MEASUREMENT

ALAD is the first physiological parameter affected in Lead poisoning. A specific, sensitive assay of erythrocytic ALAD has been proposed for the biological monitoring of saturnism although this test has certain disadvantages:

– There is major variability from one patient to another due to genetic polymorphism in this enzyme which affects the kinetics of lead toxicity.

– Photodegradation.

– Imperfect correlation with exposure levels.

If lead poisoning has been diagnosed, the most suitable tests are those that either directly assess exposure (such as the Lead concentration in the blood, urine or the bone tissue) or measure impact in the body (ALA in the urine or the level of protoporphyrins in erythrocytes, in particular zinc protoporphyrins).

Congenital ALAD deficiency (Doss porphyria), very rare and is cause by an autosomal recessive gene (locus 9q34).

INFORMATION

SAMPLE

Take 5 ml whole blood on EDTA or heparin and keep sample at 4°C in the dark.

SAMPLE STORAGE AND TRANSPORT

The sample should be transported and tested as quickly as possible to avoid haemolysis.

ASSAY METHODS

Colourimetric enzymatic assay.

REFERENCE VALUES

For reference: over 12 µmol ALA/min per litre of erythrocytes.

INTERPRETATION

In Lead poisoning, ALAD is inhibited at a very early stage with a reduction in its activity detectable at a blood Lead concentration below 100 µg/l. In a subject with congenital heterozygous deficiency, ALAD activity is reduced by over 90%.

FOR FURTHER INFORMATION

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DELTA-AMINOLEVULINIC ACID

DEFINITION

Delta-aminolevulinic acid (ALA) is a precursor of protoporphyrin IX, an intermediate in the synthesis of haem (which is important in haemoglobin and a variety of haemoproteins involved in redox reactions). Mitochondrial ALA synthase combines succinyl CoA with glycine to generate ALA; the activity of this enzyme is regulated by the end-product haem in a negative feedback loop. ALA is converted to porphobilinogen by the cytosolic enzyme delta-aminolevulinate dehydrogenase; this enzyme carries a thiol group and can therefore be inhibited by Lead.

Most haem is synthesised at two sites, the bone marrow and the liver. Haem synthesis can be perturbed by an enzyme deficiency, either congenital or acquired.

INTRODUCTION

The main acquired deficiency is due to the inhibition of thiol-containing enzymes by Lead. Thus ALA dehydrogenase Lead inhibition results in a build-up of ALA which is then excreted in the urine. However, the blood Lead concentration is the most reliable indicator of exposure to Lead. The urine concentration of ALA measures impregnation over the weeks preceding collection of the sample. Inherited deficiencies—the various forms of porphyria—may manifest as problems with erythropoiesis or in the liver. Hepatic forms of porphyria include porphyria cutanea tarda (PCT) and three acute forms, namely acute intermittent porphyria, hereditary coproporphyria and variegate porphyria. Acute intermittent porphyria is the most commonly observed form in France, affecting one in every ten-thousand people. Asymptomatic forms account for 90% of cases.

In the acute phase of porphyria, ALA synthase is stimulated (stimulation by fat-soluble medicinal products, steroids and drugs that induce cytochrome P450). This inhibits haem production and the associated feedback mechanism leading to a build-up in porphyrin precursors (ALA and porphobilinogen [PBG]). There is a very rare autosomal recessive deficiency in ALA dehydrogenase which causes acute hepatitis. Erythropoietic porphyria and porphyria cutanea tarda are not associated with the secretion of high levels of ALA and PBG. Urine ALA concentrations are normal in asymptomatic patients and during periods of remission from hepatic porphyria in symptomatic patients.

INDICATIONS FOR MEASUREMENT

Test to evaluate effects on the body after recent exposure to Lead: Ideal in occupational health in the event of brief or accidental exposure.

A complementary diagnostic test in the acute phase of hepatic porphyria in a patient with unexplained problems such as neurological disorders or abdominal pain. Also in subjects with a family history of porphyria.

INFORMATION

SAMPLE, STORAGE AND TRANSPORT

Morning urine or a urine sample at the end of the work shift. Keep in the dark at a temperature of between +2 and +8°C.

QUESTIONS FOR THE PATIENT

Investigate the possibility of Lead poisoning (paint, contacts, recent redecoration, etc).

Investigate the possibility of hereditary porphyria—ask about close relations.

For the acute phase of porphyria, ask about the recent taking of medicinal products that can induce ALA synthase, i.e. barbiturates, phenytoin, oestrogen-progestogen combinations and steroids. It is recommended to allow a therapeutic window of 2-4 weeks before the test. Attacks may also be triggered by alcohol and low calorie dieting.

ASSAY METHODS

Colourimetric reaction after separation by column chromatography. Certain medicinal products can interfere with the assay method, notably sulfamethoxazole and tetracycline.

High performance liquid chromatography.

NORMAL EXPECTED VALUES

General population: < 4 mg/g creatinine.

Values presented in the official French occupational diseases register:

– Symptomatic Lead poisoning: blood Lead concentration > 80 µg/100 ml and ALA in the urine > 15 mg/g creatinine.

– Acute and sub-acute manifestations: blood Lead concentration > 40 µg/100 ml and ALA > 15 mg/g creatinine.

PATHOLOGICAL VARIATIONS

Increased ALA excretion in the urine is an early sign of Lead poisoning. It rises as of the second week of exposure and correlates well with the blood Lead concentration once the latter reaches 60 µg/100 ml. The test is moderately sensitive, only giving a positive result once the blood Lead concentration has reached 40 µg/100 ml. The result returns to normal within fifteen days of the last exposure. A rise in ALA excretion in the urine is a complementary indicator in the acute phase of porphyria.

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DEMODEX FOLLICULORUM

DEFINITION

Demodex are small common cosmopolitan parasites belonging to the arthropod branch, arachnid class, acarid sub-class and Democides family. They are present in all mammals with species specificity: *D. folliculorum* and *D. brevis* are the two species found in human beings.

INTRODUCTION

EPIDEMIOLOGY – SYMPTOMS

Demodex folliculorum is present in the hair follicles (each may contain up to 200) at the base of the eyelashes, nose (particularly the nasal furrow) and forehead. Locally the females lay fusiform eggs producing larvae which after several mutations migrate to the surface of the skin and become adult in 28 days. Once fertilised the female hollows a furrow in the skin at around 6 weeks in which it becomes established to lay its eggs. The adult male lives for 2 months and the female for 3 months.

The pathogenicity of *D. folliculorum* is disputed as its presence is generally not associated with any symptoms. It is however considered to be responsible for blepharitis by irritating the eyelashes.

D. folliculorum and *D. brevis* can also be found deep in sebaceous glands particularly on the side of the nose associated with a comedo (blackhead). Large numbers present have been associated with facial acne, peri-oral dermatitis, rosacea and folliculitis, particularly in the immunosuppressed.

SEARCH INDICATIONS

Main indication: Blepharitis for persistent problems of unknown origin.

Secondary infections: Folliculitis, rosacea, etc.

INFORMATION

SAMPLE

A few eyelashes (or hairs) torn out with the bulb using forceps.

Contents of one (or more) blackheads collected completely using a blackhead remover.

Skin sample by scraping with a scraper at the base of hairs and eyelashes.

SAMPLE STORAGE AND TRANSPORT

Stored at ambient temperature; slide cover-slipped and sent to the laboratory within 24hrs.

DIAGNOSTIC METHODS

Direct microscopic examination of skin scales, hairs, eyelashes or contents of blackheads placed in a drop of physiological saline (or oil) between slide and cover slip.

INTERPRETATION

Demodex folliculorum is confirmed by finding the parasite on microscopic examination. It is usually live, allowing the movements of its legs to be clearly seen. Morphologically it is shaped like a long worm, 400 microns in length and approximately 40 microns in width with 4 pairs of short legs emerging from the thorax. *D. brevis* is seen more rarely and is smaller.

Histological examination of a skin biopsy, which is occasionally performed, confirms the diagnosis and can be used to assess a number of Demodex present, helping to determine whether it may be pathogenic. In the absence of supporting evidence, caution should be taken in attributing the parasite as being responsible for the clinical symptoms.

Local treatment is recommended for troublesome disorders using 1% mercury oxide applied twice daily for 3 weeks for blepharitis or metronidazole (1 application/day for 6 weeks) for rosacea or foliculitis.

FOR FURTHER INFORMATION

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www. Sante.gouv.fr/htm/dossiers/preanalytique/parasitologie.

DENGUE
(VIRUS)

DEFINITION

Dengue is the most widely distributed and commonest arbovirosis in the world affecting more than 100 million people annually and is a real public health problem. The virus belongs to the ***Flaviviridae*** family and ***Flavivirus*** genus which also contains the yellow fever virus, Japanese encephalitis virus, tick borne encephalitis virus and other less well known viruses. The dengue virus has 4 serotypes (1, 2, 3 and 4).

It is a single chain enveloped, positive polarity RNA virus measuring between 40 and 60 nm in diameter.

INTRODUCTION

EPIDEMIOLOGY

Dengue is ***endemic*** or ***endemo-epidemic*** in transmission in intertropical areas of Asia, Oceania, America and Africa. It is endemic in South-East Asia and epidemic in places which have large population movements, such as the Caribbean, the Pacific Islands, Indian Ocean and East Africa.

The 4 serotypes are responsible for sever forms of the disease. Dengue is said to be "primary" when primary infection with one of the 4 serotypes is present or "secondary" if the infection develops in a patient who has already produced antibodies to one of the other 3 viruses.

The reservoir is mostly human and the virus is transmitted by a mosquito belonging to the *Aedes* genus, particularly *Aedes aegypti*. People become infected from bites from infected mosquitoes.

PATHOPHYSIOLOGY AND SYMPTOMS

The pathophysiology of the virus is poorly understood and the virus receptor is not known.

It is believed to multiply firstly at the inoculation site in reticulo-endothelial and/or fibroblast cells, before reaching the lymph nodes. From there it is believe to spread towards other organs such as the liver and bone marrow. Viraemia occurs quickly in the region of 3 to 5 days. Many asymptomatic forms of the disease exist and there is a very large variety of clinical features in the symptomatic forms.

- **Classical symptomatic disease or DF *(dengue fever)*** is characterised by a flu-like syndrome with high sudden onset of fever, rigors, headaches, arthralgia, lymphadenopathy, myalgia, mucocutaneous rash on the trunk and limbs and occasionally haemorrhage.

  It generally follows a benign course resolving spontaneously in one to two weeks with a long convalescent period.

- **Haemorrhagic dengue or HD** is the severe form of the disease. It develops 3 or 4 days after the episode of fever and presents with purpura accompanied by mucosal and gastro-intestinal haemorrhage. At this stage, thrombocytopaenia and disseminated intravascular coagulation (DIC) can be seen in the laboratory investigations. It may resolve or progress to hypovolaemic shock with tissue anoxia, metabolic acidosis and fatal cardiovascular collapse.

  Other severe forms of dengue are seen with hepatic, neurological, pulmonary and cardiac disease.

  The conditions under which these severe signs develop are not understood.

SEARCH INDICATIONS

Diagnosis of a syndrome of fever and pain accompanied by purpura and/or haemorrhage in a person returning from an endemic area.

Differential diagnosis with malaria and other tropical fevers with a rash.

INFORMATION

SAMPLE

Peripheral blood to detect the virus in the acute phase.

Serum for serological diagnosis.

CSF in encephalitis for culture or serology.

Post-mortem biopsy samples.

QUESTIONS FOR THE PATIENT

Have you returned from an endemic area?

Clinical symptoms?

SAMPLE STORAGE AND TRANSPORT

Serum (Dry Tube). Store the separated sample at + 4°C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

- **Cell culture:**

  This is the method of choice to isolate the virus and is performed on serum and also on leukocytes or homogenates of biopsy tissues on a mammal cell line (Vero E6) or continuous mosquito cell line (C6/36, AP61, Tra284), which are more sensitive. More rarely, an intracerebral inoculation is administered to newborn mice. The virus can be isolated from peripheral blood between days 1 and 6 after onset of the fever.

- **Molecular biology:**

  RT-PCR (polymerised chain reaction preceded by reverse transcription) has become the method of choice for the early diagnosis of the infection. The method is optimised on serum but can also be performed on plasma, mosquitoes, infected cells and biopsies.

- **Detection of serum viral antigen NS1:**

  Glycoprotein NS1 is a viral glycoprotein expressed by the dengue virus, the role of which has not been clearly established. Several tests are commercially available, such as ELISA and immunochromatographic methods. These provide an early diagnosis of infection in serum as soon as clinical signs develop.

■ INDIRECT DIAGNOSIS

Several methods are available. IHA and complement binding reactions are being replaced by immunoenzymatic methods with testing for specific IgM by immunocapture.

IgM develops at around day 5 or day 6 after the sudden onset of fever, increases rapidly and generally persists for between 3 and 6 months.

IgG develops at the end of the first week in primary dengue, increasing slowly to reach a peak 2 to 3 weeks after the onset of infection, whereas they precede the IgM in secondary infection. For this reason, combined testing for IgG and IgM is required to interpret serological testing.

The seroneutralisation test is the most sensitive and specific of the serological methods to diagnose dengue. It is used for sero-epidemiological investigations.

■ NON-SPECIFIC DIAGNOSIS

Leukopaenia with atypical lymphocytes, thrombocytopaenia, haemoconcentration or hepatitic laboratory changes may help with the diagnosis.

■ NEW APPROACHES TO THE LABORATORY DIAGNOSIS OF DENGUE VIRUS

New diagnostic approaches developed are the early rapid detection of viral NS1 antigen by immunocapture, using capillary blood as an alternative to venous blood, IgG avidity to discriminate primary infection from reinfection, and the use of recombinant proteins obtained from the virus envelope protein to improve the sensitivity and specificity of immuno-enzymatic methods.

INTERPRETATION

Cell culture is reserved for specialist reference laboratories and RT-PCR only provides a diagnosis in the acute viraemic phase. For this reason, serology is the most widely used routine diagnostic method. A second serum sample should be taken 8 days after the first and any cross-reactions with antibodies against other Flavivirus (particularly after yellow fever vaccination) should be taken into account.

TREATMENT

At present there is no vaccine available for dengue.

Treatment is entirely symptomatic and involves monitoring affected patients in order to hospitalise them at the first sign of any progression to severe disease.

Prophylaxis against dengue involves combating vectors and monitoring endemic outbreaks.

FOR FURTHER INFORMATION

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■ Matheus S, Lacoste V, Labeau B, *et al. Techniques de routine et approches innovantes pour le diagnostic biologique de la dengue*. Revue Francophone des laboratoires, Dec 2009;417: 49-57.

DEOXYCORTICOSTERONE (11-)

DEFINITION

11-deoxycorticosterone (DOC) is synthesised from progesterone by the action of 21-hydroxylase. It then undergoes 11β hydroxylation to be converted into corticosterone. DOC and corticosterone are produced in three areas of the adrenal cortex, the zona fasciculata, zona reticulosa and zona glomerulosa. It is only in the latter two of these, however, that the two steroids act as precursors for aldosterone. This conversion involves an 18-hydroxylase, with the formation of 18-hydroxydeoxycorticosterone and 18-hydroxycorticosterone. All of these steroids, DOC, corticosterone and their derivatives have mineralocorticoid activity but far less than that of aldosterone.

Quantitatively, DOC and corticosterone are produced above all by the zona fasciculata and zona reticulosa and their secretion which is ACTH-dependent, is parallel to that of cortisol.

DOC circulates in plasma mostly bound to plasma proteins, 36% to CBG (Corticosteroid Binding Globulin or transcortin) and 60% to albumin.

As applies to all of the other steroids, DOC is metabolised in the liver by successive reductions to the tetrahydro derivative: THDOC which is then glucuronide-conjugated before being removed in urine.

INDICATIONS FOR MEASUREMENT

DOC measurement is useful in investigating hypertension, particularly hypertension associated with hypokalaemia when diuretics have been stopped for at least two weeks and the patient is following a normal sodium diet. It is also recommended for use in female children with sexual differentiation abnormalities associated with hypertension. Finally, DOC is measured in all cases of hypercorticism regardless of origin.

INFORMATION

■ SAMPLE

DOC is measured in serum or plasma. Anticoagulant does not interfere although the blood sample is taken in the morning in view of the circadian secretion-cycle and without stress.

■ NECESSARY INFORMATION

As DOC is produced by the adrenal glands, any corticosteroid treatments (systemic, local or intra-articular) must be reported. Similarly, information must be provided as to whether measurement is part of a Synacthen® stimulation test or metyrapone (Métopirone®) test.

■ SAMPLE STORAGE AND TRANSPORT

Separated plasma or serum should be stored at +4°C and transported at this temperature to the laboratory. Samples can be stored for 6 months to 1 year frozen at –30°C.

ASSAY METHODS

DOC is assayed generally by radioimmunoassay with a pre-purification stage involving extraction followed by chromatography. This is required in order to achieve good specificity.

It can also be measured by high performance liquid chromatography (HPLC) coupled with mass spectrometry.

NORMAL EXPECTED VALUES

The results are generally expressed in pg/ml or ng/l. pmol/l is converted to pg/ml by multiplying pg/ml by 3.026.

Usual morning plasma DOC concentrations in adults measured radioimmunologically after chromatography are between 40 and 140 pg/ml.

No sex-related differences are found although concentrations vary during the 24 hour period following a cycle similar to that of cortisol.

Serum and plasma concentrations are high in both sexes in children during the first year of life. They subsequently fall, becoming similar to those in adults from the second year of life in boys although this effect is only seen close to the end of puberty in girls.

DYNAMIC TESTS

Dynamic investigation of DOC uses the Synacthen® test in particular.

In the metyrapone test, 11-deoxycortisol is the preferred measurement to assess the integrity of the hypothalamo-pituitary-adrenal axis.

– Synacten® test

Stimulation of the adrenal cortex with Synacthen® (0.25 mg) causes a rise in DOC to a peak of 600 pg/ml, 30 to 60 minutes after administration.

– Metyrapone Test (Métopirone®)

Metyrapone is an 11β-hydroxylase inhibitor, administration of which causes a large fall in cortisol resulting in increased secretion of ACTH, with increased 11-deoxycortisol and DOC secretion by feedback control. The metyrapone test is used to assess the integrity of the hypothalamo-pituitary-adrenal axis. Refer to the 11-deoxycortisol for proposed protocol for this test.

PATHOLOGICAL VARIATIONS

■ 11 β-HYDROXYLASE DEFICIENCY

This deficiency makes up 5 to 8% of known cases of adrenal blockade. It affects both the gluco- and mineralocorticoid pathways, whereas the androgen pathway is not affected. This results in increased adrenal secretion of androgens and the 11-deoxycortisol precursors of cortisol and aldosterone, 11-deoxycortisol and 11deoxycorticosterone (DOC) respectively.

From a pathophysiological perspective, accumulation of DOC which has potent mineralocorticoid action explains firstly the common findings of hypertension due to salt retention and secondly the rarity of salt losing syndromes.

Clinically, early and late presenting forms are seen.

Early presenting forms

These are characterised by hypertension and sexual differentiation abnormalities in female children. *In-utero* androgen exposure at birth may be responsible for external genital organ malformations with the risk of the child being declared male at birth. The internal female genital organs are always normal. The diagnosis must be made promptly in order to avoid symptoms of virilisation worsening and the development of early pseudo-puberty with pubic and axillary hair growth.

The hyperandrogenism does not cause clinical abnormalities at birth in boys.

Late presenting forms

The symptoms of late presenting forms are hyperandrogenism developing in a female adolescent or young adult woman, presenting with severe acne, hirsutism and alopecia.

11-hydroxylase blockade should be considered as a priority diagnosis if hyperadrogenism is found associated with hypertension.

Laboratory diagnosis

This is based on increased 11-deoxycortisol and DOC concentrations in serum or plasma. Androgens are also increased except of course for 11β-hydroxyandrostenedione.

The 11-deoxycortisol and DOC response to Synacthen® is greatly exaggerated.

17 α-HYDROXYLASE (CYP17) DEFICIENCY

17α-hydroxylase deficiency is a rare cause of congenital adrenal hyperplasia. This deficiency is diagnosed at the expected age of puberty from hypertension, hypokalaemia and hypogonadism. The hypogonadism presents in females with primary amenorrhoea and absence of secondary sexual characteristics.

Males present with male pseudohermaphrodism with female external genital organs but no uterus or fallopian tubes. The testes are generally intra-abdominal and contain Leydig cell hyperplasia. Regardless of sex, most patients have hypertension associated with hypokalaemia, which may be mild.

Pathophysiologically, CYP17 codes for an enzyme which has both 17 α-hydroxylase and 17,20-lyase activity. In the same way as cortisol, androgen and oestrogen synthesis rely on 17 α-hydroxylase and is blocked. Androgen and oestrogen synthesis are also blocked as these depend on 17,20-lyase. The deficiency may involve the 17 α-hydroxylase or the 17,20-lyase or both of these enzyme activities. Regardless of the deficient enzyme activity, however, the mineralocorticoid pathway is not affected as DOC and corticosterone accumulate, together with their 18-hydroxylated derivatives. Active renin is inhibited and aldosterone concentrations are low. As a result, CYP17 deficiency causes a hyper-mineralocorticoid state with secondary hypoaldosteronism.

The laboratory diagnosis is based on increased concentrations of non-C17 hydroxylated steroids, particularly DOC and corticosterone, together with a fall in cortisol, androgens and oestrogens.

OTHER ADRENAL DISEASES

Plasma DOC concentrations are extremely high in Cushing's syndromes regardless of cause and in Cushing's disease. The same applies to adrenocorticalomas and particularly benign or malignant DOC secreting tumours. DOC is also raised together with aldosterone in Conn's syndrome. Conversely, DOC is reduced in hypocorticism, regardless of cause, whether endogenous or iatrogenic.

FOR FURTHER INFORMATION

■ Vexiau P., *Déficits enzymatiques corticosurrénaliens*, Encycl. Méd. Chir (Paris-France), Endocrinologie-Nutrition, 10015-B-20, 1992, 6p.

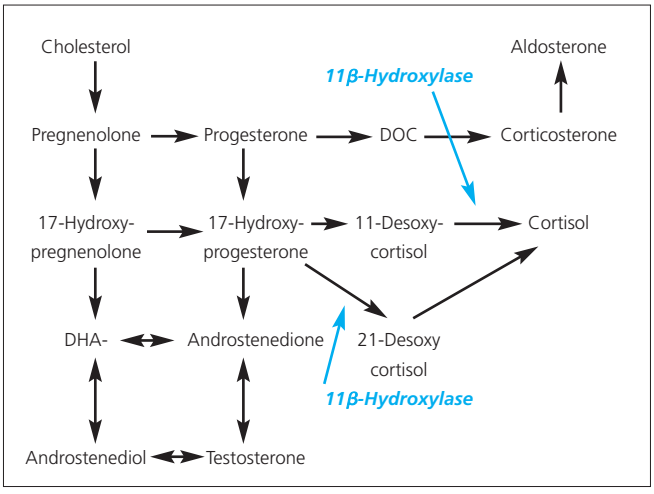
■ Orth D.N., Kovacs W.J., *The adrenal cortex*. In: Wilson J.D., Foster D.W., Kronenberg H.M., Larsen P.R., eds. Williams Textbook of Endocrinology, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, 1998: 517-664.

DEOXYCORTISOL (11-)

DEFINITION

11-Deoxycortisol is one of the two immediate precursors of cortisol, from which it differs only by the absence of an 11 hydroxyl group, as indicated by its name *(cf. biosynthesis diagram)*. It is formed in the adrenal glands from 17-hydroxyprogesterone due to the action of 21-hydroxylase.

Biosynthesis of the corticosteroids



A large proportion of circulating 11-deoxycortisol is bound to CBG and albumin. It is metabolised in the liver by successive reductions to 11-tetrahydrodeoxycortisol or THS, which is glucurono-conjugated before being removed in urine.

Synonyms: 11-Deoxycortisol = 11DF.

INDICATIONS FOR MEASUREMENT

Measurement of 11-Deoxycortisol is indicated in particular in the investigation of hirsutism and virilisim in girls and women and is an aid to the aetiological diagnosis.

INFORMATION

SAMPLE

11-Deoxycortisol is measured either in serum or in EDTA plasma. No interference occurs with the anticoagulant. Similarly, lipaemic or haemolysed samples do not pose problems as the method used involves extraction and chromatography. The sample must be taken, however, in the morning before 10:00 in view of the circadian variations in concentration.

ESSENTIAL INFORMATION

As 11-Deoxycortisol is produced by the adrenal glands, any corticosteroid treatments (systemic, local or intra-articular) must be reported. Similarly, information must be provided as to whether measurement is part of a Synacthen® stimulation test of the adrenal cortex or by metyrapone (Métopirone®).

SAMPLE STORAGE AND TRANSPORT

Separated plasma or serum should be stored at +4°C and transported at this temperature to the laboratory.

Samples can be stored for 6 months to 1 year frozen at –30°C.

ASSAY METHODS

11-Deoxycortisol is assayed by radioimmunoassay with a pre-purification stage involving extraction followed by chromatography. This is required in order to achieve good specificity.

USUAL VALUES

Morning 11-Deoxycortisol concentrations vary between 0.20 and 1.10 ng/ml. There are no sex-related differences although concentrations vary over the 24 hour period in a cycle similar to that of cortisol.

No significant differences are seen in children by age or sex and concentrations are similar to those in adults.

Results can be expressed either in ng/ml or nmol/l. Ng/ml are converted to nmol/l by multiplying ng/ml by 2.890.

DYNAMIC TESTS

SYNACTHEN® TEST

11-Deoxycortisol rises to a peak of 2.6 ng/ml in blood samples taken at 30 and 60 minutes after intramuscular or intravenous injection of 0.25 mg of Synacthen® (synthetic 1-24 corticotropin).

METYRAPONE® TEST

Metyrapone is an 11β-hydroxylase inhibitor. The administration of which, causes a large fall in cortisol resulting in oversecretion of ACTH and increased 11-Deoxycortisol secretion by feedback control. The metyrapone test is used to assess the integrity of the hypothalamo-pituitary-adrenal axis and is used in the aetiological diagnosis of Cushing's Syndromes.

Of the many protocols proposed the most widely used are the short and long tests.

Short test

Metyrapone is administered orally as a single midnight dose of 30 mg/kg. 11-Deoxycortisol, and possibly cortisol and ACTH, are measured in samples taken on the day before and on the next day at 8 hours after the metyrapone is taken. Plasma 11-Deoxycortisol concentrations must rise to over 70 ng/ml.

Long test

This test is generally less well tolerated than the short test and may cause malaise, headache, hypotension and dizziness. It involves administering 750 mg of metyrapone every 4 hours for 24 hours beginning at 8 hours. The response is assessed from plasma 11-Deoxycortisol the following day at 8 hours, concentrations of which should exceed 150 ng/ml.

Interpretation

When negative, these tests provide a diagnosis of occult adrenal insufficiency. The ACTH response is used to distinguish peripheral from central adrenal insufficiency.

In Cushing's disease the response to the metyrapone test is increased, whereas a zero response is seen in Cushing's syndrome due to an adrenal tumour.

PATHOLOGICAL VARIATIONS

11β-HYDROXYLASE DEFICIENCY

This deficiency makes up 10 to 15% of known cases of adrenal blockade. It affects both the gluco- and mineralocorticoid pathways, whereas the androgen pathway is not affected. This results in increased adrenal secretion of androgens and the 11-Deoxy- precursors of cortisol and aldosterone, 11-Deoxycortisol and 11-Deoxycorticosterone (DOC) respectively.

From a pathophysiological perspective, accumulation of DOC which has potent mineralocorticoid action explains firstly the common findings of hypertension due to salt retention and secondly the rarity of salt losing syndromes.

Clinically, early and late presenting forms are seen.

Early presenting forms

These are characterised by hypertension and sexual differentiation abnormalities in female children. *In-utero* androgen exposure at birth may be responsible for external genital organ malformations with the risk of the child being declared male at birth. The internal female genital organs are always normal. The diagnosis must be made promptly in order to avoid symptoms of virilisation worsening and the development of early pseudo-puberty with pubic and axillary hair growth.

The hyperandrogenism does not cause clinical abnormalities at birth in boys.

Late presenting forms

The symptoms of late presenting forms are hyperandrogenism developing in a female adolescent or young adult woman, presenting with severe acne, hirsutism and alopecia.

11-hydroxylase deficiency should be considered as a priority diagnosis if hyperadrogenism is found associated with hypertension.

Laboratory diagnosis

This is based on increased 11-Deoxycortisol and DOC concentrations in serum or plasma. Androgens are also increased except of course for 11β-hydroxyandrostenedione.

The 11-Deoxycortisol and DOC response to Synacthen® is greatly exaggerated.

OTHER ADRENAL DISEASES

11-Deoxycortisol increase in Cushing's disease, ectopic ACTH secretion and adrenal cortex adenomas. Conversely, concentrations are reduced in hypocorticism regardless of cause either endogenous or iatrogenic.

FOR FURTHER INFORMATION

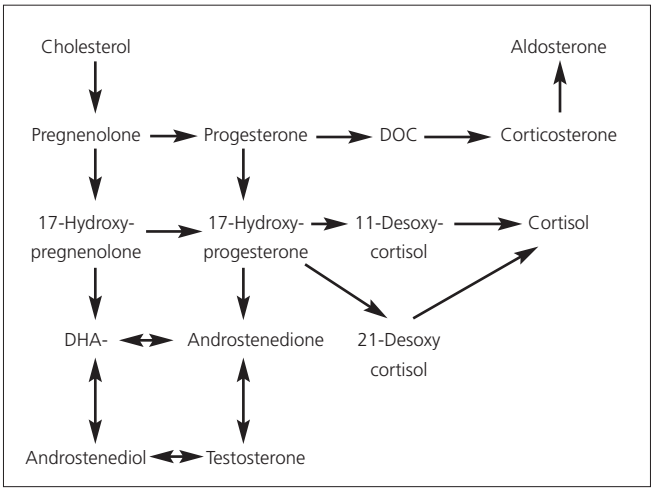
- Tabarin A., Corcuff J.B., Roger P., *Physiologie et exploration des secretions de cortisol et d'androgènes par la glande corticosurrénale*. In: Encycl. Méd. Chir (Paris-France), Endocrinologie-Nutrition, 10-014-B-10, 1993; 9p.
- Vexiau P., *Déficits enzymatiques corticosurrénaliens*. In: Encycl. Méd. Chir (Paris-France), Endocrinologie-Nutrition, 10-015-B-20, 1992; 6p.
- Orth D.N., Kovacs W.J., *The adrenal cortex*. In: Wilson J.D., Foster D.W., Kronenberg H.M., Larsen P.R., eds. Williams Textbook of Endocrinology, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, 1998: 517-664.

DEOXYCORTISOL (21-)

DEFINITION

21-deoxycortisol (21DF) is one of the two immediate precursors of cortisol, produced from 17-hydroxyprogesterone by the action of 11β-hydroxylase *(cf. biosynthesis diagram)*.

Biosynthesis of the corticosteroids



The pathway passing through 21DF is an accessory one, most cortisol being formed from 11-deoxycortisol. This however becomes a predominant pathway in 21-hydroxylase deficiency.

21DF is metabolised in the liver by successive reductions to pregnanetriolone and pregnanetetrol.

Synonyms: 21-Deoxycortisol = 21DF.

USE OF MEASUREMENT

21DF measurement is recommended in the investigation of hyperandrogenism in women to investigate for incomplete 21-hydroxylase deficiency.

INFORMATION

■ SAMPLE

21DF is measured either in serum or in EDTA or heparinised plasma. No interference occurs with the anticoagulant. Similarly, lipaemic or haemolysed samples do not pose problems as the method used involves extraction and chromatography. The sample must be taken, however, in the morning before 10:00 am in view of the circadian variations in 21DF concentrations.

■ NECESSARY INFORMATION

As 21DF originates from the adrenal glands, any corticosteroid treatments (systemic, local or intra-articular) must be reported.

Similarly, information must be provided as to whether measurement is for a Synacthen® stimulation test of the adrenal cortex.

■ SAMPLE STORAGE AND TRANSPORT

Separated plasma or serum can be stored and transported to the laboratory at +4°C. Samples can be stored for 6 months to 1 year frozen at –30°C.

ASSAY METHOD

21DF is assayed by radio-immunoassay with a pre-purification stage involving extraction followed by chromatography. This is required in order to achieve good specificity.

USUAL VALUES

Results are expressed in ng/ml and are converted into nmol/l by multiplying ng/ml by 2.886. 21DF concentrations in the morning vary in young men between 0.10 and 0.30 ng/ml and in young women between < 0.05 and 0.25 ng/ml.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Serum 21DF concentrations vary greatly during the 24 hour cycle, completely in parallel with cortisol. No variations are seen however during the menstrual cycle.

■ PHARMACOLOGICAL VARIATIONS

Administration of dexamethasone or metyrapone causes a large fall in concentrations, whereas administration of ACTH increases concentrations greatly. 21DF concentrations in normal people do not exceed 0.70 ng after Synacthen®. Conversely, neither oestrogen-progestogens nor hCG change circulating 21DF concentrations.

■ PATHOLOGICAL VARIATIONS

The biosynthesis of cortisol is partly or completely blocked in congenital adrenal hyperplasia due to 21-hydroxylase deficiency, depending on whether the enzyme deficiency itself is partial or complete. ACTH secretion therefore increases leading to adrenal stimulation and overproduction of the 21-deoxylated precursors of cortisol, 21-deoxycortisol and 17-hydroxyprogesterone (17-OHP).

17-OHP however is produced by two sources, the adrenal glands and the gonads, whereas 21DF only comes from the adrenal gland. 21DF is therefore considered to be a more sensitive marker than 17OHP to diagnose not only homozygotes but also heterozygotes for 21-hydroxylase deficiency. This has been shown in the basal state and particularly after adrenocortical stimulation with tetracosactide (Synacthen®).() Most people with late onset 21-hydroxylase deficiency have high concentrations of 17OHP and 21DF and the 21DF response to Synacthen® is far greater than 17OHP. In addition, there is no overlap between the 21DF response in heterozygotes and normal people. 21DF measurement can therefore be used to test for heterozygotes with a sensitivity of more than 90% compared to only 20% for 17OHP measurement.

In monitoring treatment of hyperplasia due to 21-hydroxylase deficiency, 21DF offers similar utility to 17OHP and either may therefore be used.

FOR FURTHER INFORMATION

■ Fiet J., Gosling J.P., Soliman H. et *al. Hirsutism and acne in women: coordinated radioimmunoassays for eight relevant plasma steroids*. Clin Chem 1994; 40: 2296-2305.

DEOXYPYRIDINOLINE

DEFINITION

The pyridinolines (Pyr) and deoxypyridinolines (D-Pyr) are bridging molecules which provide cohesion between collagen molecules and for elastin molecules (prolene and hydroxyproline cross links). Pyr and D-pyr are degradation products of type 1 collagen and elastin which are removed in urine. D-Pyr are specific to bone, whereas Pyr also come from tendons, cartilage and the aorta. Only D-pyr is currently measured and is a bone tissue degradation marker.

INTRODUCTION – INDICATIONS FOR MEASUREMENT

All situations which promote increased bone remodelling are accompanied by increased Pyr and D-Pyr. High level bone remodelling or chronic/prolonged imbalance in favour of osteolysis, are factors promoting bone tissue fragility. Oestrogen deficiency in the menopause is associated with a relative increase in bone destruction, a phenomenon which is particularly significant during the 5 years prior to the menopause.

■ OSTEOPOROSIS (MAIN INDICATION)

– Resorption markers are not of diagnostic value as urinary D-Pyr concentrations and bone densitometry (BDM) correlate poorly.

– Prognostic value: Increased resorption markers in menopausal women are a predictive factor for rapid bone loss.

– At the time of diagnosis, a rise in resorption markers appears to be an aid in choosing treatment, arguing in favour of the use of a resorption inhibitor (bisphosphonate, and oestrogens).

– Bone remodelling markers can be used to monitor the effectiveness of resorption inhibitor therapy. A fall in D-Pyr concentration of approximately 20 to 30%, 3 to 6 months after starting treatment is an indicator of efficacy and predicts a gain in BDM (+ 3%). The simplicity and testing interval for this assessment could help to improve patient adherence, as the expected effect can only be assessed by bone densitometry after treatment for approximately 2 years.

■ PAGET'S DISEASE

A significant fall in urine D-Pyr excretion in the days after starting pamidronate therapy (bisphosphonate or bone resorption inhibitor) confirms effective treatment.

■ OTHERS

Hyperparathyroidism and identification of an osteolytic process.

INFORMATION

■ SAMPLE

The following recommendations should be observed:

Take the urine sample fasting in the morning before 0900 hours, if possible at the same time particularly when repeated measurements are intended: 5 ml from the 2nd morning urine pass.

■ QUESTIONS FOR THE PATIENT

Treatment with bisphosphonates, oestrogens, androgens, rH-PTH, vitamin D, corticosteroids or heparin?

Vitamin D deficiency, recent fracture or renal insufficiency?

Intercurrent diseases (recent prolonged bed-bound period)?

ASSAY METHODS

LIA or Chemoluminescence kit.

NORMAL EXPECTED VALUES

Urinary deoxypyridinoline (chemoluminescence):

Men: 2.3 – 5.4 nmol/mmol of creatinine

Non-menopausal women: 3 – 7.4 nmol/mmol of creatinine. Menopausal women should be kept within the normal range established before the menopause.

INTERPRETATION

■ **Results must be interpreted taking account of circumstances which increase resorption markers:**

– Being bed-bound for more than 2 days.

– Being within 6 months of the fracture.

– Receiving corticotherapy or heparin treatment.

– Being vitamin D deficient.

– Having bone metastases, Paget's disease or multiple myeloma.

■ **Intra-individual variability factors:**

Intra-individual variation can be estimated for individual patients. The main factors responsible for variation are:

– 24 hour cycle (samples should preferably be taken in the morning at the same time).

– Dietary status (samples should preferably be taken fasting).

– Extent of physical activity (avoid taking the sample when the patient has been bed-bound for more than 2 days before measurement).

– Excessive intake of alcohol or tobacco.

■ **Indications and use in primary osteoporosis:**

Samples should be taken at a specific time (cf. variability factors) to estimate intra-individual variability.

– No established diagnostic value.

– Prognostic use, correlates with T score.

– Use in monitoring bone resorption inhibitor therapy (observe a minimum of three months after starting treatment before measuring).

■ **Increases are seen in the following diseases:**
– Paget's disease in the resorption phase;
– Hyperthyroidism.

■ **Assessment of the efficacy of bone resorption inhibitor therapy:**
A significant fall on bone resorption inhibitor therapy is defined as > 20 – 30% over a 3 month interval.

FOR FURTHER INFORMATION

■ Cormier C, Kindermans C et Souberbielle JC. *Marqueurs du remodelage osseux : données récentes sur leur intérêt et leurs limites dans l'exploration des ostéoporoses*. Ann Biol Clin, 2001, 60:343-346.

■ Delmas P. *Utilisation clinique des marqueurs du remodelage osseux dans l'ostéoporose post-ménopausique*. Ann Biol Clin, 2001, 59:299-308.

■ Garnero P et Delmas P. *Les marqueurs biochimiques du remodelage osseux : application à l'exploration des ostéoporoses*. Ann Biol clin, 1999, 57:137-148.

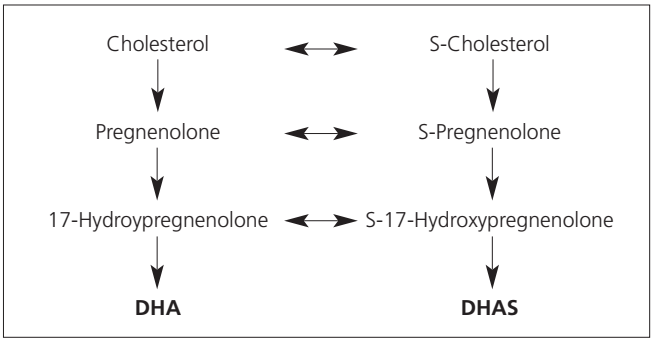
■ Bellik S et Houzé P. *Le métabolisme osseux : physiologie, méthodes d'exploration, intérêt des nouveaux marqueurs*. Feuillets de Biologie, 1999, XXXIX :45-59.

DHA AND DHAS

DEFINITION

Dehydroepiandrosterone (DHA) is synthesised in all steroid-producing tissues, the ovaries, testes and adrenal glands (zona reticulata) from cholesterol following the pathway shown diagrammatically on the figure below and passing through pregnenolone and 17-hydroxypregnenolone. DHA can be converted into DHA sulphate (DHAS) in the adrenal gland and in peripheral tissues, primarily the liver.

Biosynthesis of DHAS in the adrenal cortex



DHAS can also be synthesised through another pathway, the sulphate pathway, from cholesterol sulphate, in which all of the intermediary products are sulphates.

Whilst DHA is secreted mainly by the adrenal cortex and partially by the gonads, DHAS is only secreted by the adrenal cortex. Regardless, DHA and its sulphate are inter-converted in peripheral tissues, mostly the liver.

In adults, regardless of sex, adrenocortical secretion contributes 40% to total production of DHA sulphate, the remainder of production coming from peripheral conversion of DHA. The menopause has no impact on this contribution.

METABOLISM

The metabolism of DHAS differs during and outside of pregnancy.

Outside of pregnancy, DHAS is mostly metabolised in the liver. It is inter-converted to DHA which can be converted into androstenedione (D4A). D4A is then either metabolised into anderosterone and etiocholanolone which are excreted in urine, as sulpho- and glucurono-conjugated molecules, or converted into testosterone (T) which is metabolised into dihydrotestosterone (DHT) and then into androstanediol.

Other metabolic pathways exist but are quantitatively less important: interconversion with androstenediol sulphate and 7 or 16 hydoxylation.

During pregnancy, DHAS is produced by the foetal adrenal cortex and is 16 α hydroxylated in the foetal liver. 16 α-OH-DHA sulphate then passes into the placenta, loses is sulphate group and is converted into estriol *(cf estriol)*. It should be noted that the maternal adrenal cortex also produces DHAS although 16 α-hydroxylation is very limited in the mother's body. As its 16 α-hydroxylated precursors are predominantly produced in the foetus, estriol is considered to be a good reflection of foeto-placental metabolic activity.

INTRODUCTION

In terms of their biological activity, steroid sulphates can be converted into biologically active unconjugated steroids. DHAS can therefore be converted into androgens and into oestrogens. Administration of high doses of DHA in animals has revealed several effects, particularly anti-tumour, anti-diabetic, anti-lipaemic, anti-atherogenic, anti-obesity and anti-viral actions.

INDICATIONS FOR MEASUREMENT

As DHA sulphate mostly comes from the adrenal gland its measurement is recommended, particularly in the investigation of adrenocortical androgen production. It therefore forms part of the first line measurements for the investigation of ***hirsutism*** and ***virilism*** in women and girls. DHA and particularly DHAS measurements are also recommended in children to identify premature adrenocortical maturation.

Similarly, measurement of DHAS is used in the elderly as DHAS falls gradually with age, both in men and in women.

INFORMATION

■ SAMPLE

DHA and DHAS can be measured in EDTA or heparinised plasma or in serum, although serum is preferable when an automated analyser is used for measurement. In view of the lack of variations in DHAS during the circadian cycle or during the menstrual cycle in women, there is no essential time or day to take the blood sample. As the measurement forms part of the assessment of a person's androgen production however, the sample should be taken in the morning at the start of the follicular phase in women.

On the other hand, for DHA measurements the sample must be taken before 1000 hours in the morning in view of the circadian variation in peripheral concentrations. Stress should also be avoided.

■ NECESSARY INFORMATION

Any corticosteroid treatment, regardless of administration route (oral, local, intra-muscular or intra-articular) must be reported. In addition, for DHA, it is essential to report whether the measurement is part of a Synacthen® stimulation test.

Clearly the request should state whether the patient is receiving DHA.

■ SAMPLE STORAGE AND TRANSPORT

Plasma or serum should be stored at + 4°C and transported at this temperature to the laboratory for DHAS measurement.

ASSAY METHODS

DHA and DHAS are currently measured by an immunological method directly on the plasma or serum aliquot using an isotopic or non-isotopic label. DHAS can now be measured immunologically on several automated analysers.

NORMAL EXPECTED VALUES

(See table)

DHA results are expressed in ng/ml or nmol/l. Ng/ml is converted to nmol/l by multiplying ng/ml by 3.467.

DHAS results are expressed in ng/ml or µmol/l. Ng/ml is converted to µmol/l by multiplying ng/ml by 2.721.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

DHA and DHAS concentrations are very high at birth both in girls and in boys, and fall very rapidly during the first month and then more or less regularly during the first year of life, reflecting the large inter-individual variably due to regression of the foetal adrenocortical zone and sulphatase and sulphokinase activities.

From the first to sixth year of life concentrations remain low and similar in both sexes. They then increase abruptly in the seventh year of life both in girls and in boys. DHAS continues to increase and no age-related differences are seen until the age of 15 years old. From this age onwards, concentrations are higher in boys than in girls. Adult concentrations are achieved at P5 in girls although increase further after P5 in boys.

Variations in plasma or serum DHA and DHA sulphate with age and pubertal stage <u>in girls</u>

Age	2 – 11 d.	12 – 120d.	4 – 11m.	12 – 23 m.	< 9 yrs	9 – 12	9 – 13	10 – 15
Pubertal stage					1 a	1 b	2	3 – 4
DHA (ng/ml)	0.3 –4.3	0.3 – 4.8	0.1 – 0.5	<0.05 – 0.2	<0.05 – 2.0	0.4– 2.5	0.5 – 3.9	0.5 – 6.0
DHA (nmol/l)	1.04 – 14.91	1.04 – 16.64	0.35 – 1.73	<0.17 – 0.69	<0.17 – 6.93	1.39 – 8.67	1.73 – 13.52	1.73 – 20.80
DHAS (ng/ml)	380 - 1300	<100 – 1300	<100 – 400	<100 – 400	<100 – 340	200– 1030	220 – 1350	675 – 3000
DHAS (µmol)	1.03 – 3.54	<0.27 3.54	<0.27 – 1.09	<0.27 – 1.09	<0.27 – 0.93	0.54 – 2.80	0.60 – 3.67	1.84 – 8.16

Variations in plasma or serum DHA and DHA sulphate with age and pubertal stage <u>in boys</u>

Age	2 – 11 d.	12 – 120 d.	4 – 5m.	6 – 23 m.	< 11 yrs	11 – 13	11 – 15	15 – 18
Pubertal stage					1 a	1 b	2	3 – 4
DHA (ng/ml)	0.6 – 5.7	0.6 – 5.7	<0.05 – 2.5	<0.05 – 1.5	<0.05 – 1.9	0.3 – 3.6	0.6 – 6.6	0.8 – 7.1
DHA (nmol/l)	2.08 – 19.76	2.08 – 19.76	<0.17 – 8.67	<0.17 – 5.20	<0.17 – 6.59	1.04 – 12.48	2.08 – 22.88	2.77 – 24.62
DHAS (ng/ml)	360 - 2000	360 - 2000	<100 - 430	<100- 300	<100-675	120 - 1200	320 - 1900	390 - 3500
DHAS (µmol/l)	0.98 – 5.44	0.98 5.44	<0.27 – 1.17	<0.27 – 0.82	<0.27 – 1.84	0.33 – 1.20	0.87 – 5.17	1.06 – 9.52

Circulating DHA concentrations do not differ significantly between the sexes and generally range from 1 to 10 ng/ml in the morning before 1000 hours. Variations during the circadian cycle parallel those seen with cortisol.

In adults, circulating concentrations of DHAS are higher in men than in women *(cf. table)*. It is quantitatively the most important circulating plasma steroid and unlike DHA no changes in circulating DHAS concentrations are seen during the circadian cycle.

In pregnancy, DHAS concentrations fall markedly until approximately the 20th week of pregnancy. They then remain stable or continue to fall very gradually for the next twenty weeks.

In adults, after the age of 25-30 years old, DHAS begins to fall regularly, reaching very low values of approximately 10% of those seen in the 3rd decade in the 8th – 9th decade *(cf. table)*. On the other hand, DHA falls far less markedly with age.

Variations in serum DHA sulphate concentration with age and sex

AGE	WOMEN		MEN	
(yrs)	ng/ml	µmol/l	ng/ml	µmol/l
15 - 20	500 - 4000	1.36 - 10.88	750 - 5300	2.04 - 14.42
21 - 30	700 - 4250	1.90 - 11.56	1240 - 5500	3.37 - 14.97
31 - 40	650 - 4200	1.77 - 11.43	1000 - 5100	2.72 - 13.88
41 - 50	500 - 3750	1.36 - 10.20	750 - 4750	2.04 - 12.92
51 - 60	350 - 3000	0.95 - 8.16	700 - 3850	1.90 - 10.48
61 - 70	200 - 2150	0.54 - 5.85	500 - 2800	1.36 - 7.62
71 - 80	125 - 1500	0.34 - 4.08	400 - 1900	1.09 - 5.17

This does not reflect a change in DHA or DHAS metabolism but a fall in adrenocortical secretion. Cortisol secretion, however, is not affected by age. The fall in DHAS leads to an increase in the cortisol/DHAS ratio (partly responsible for the catabolic state which develops with ageing).

Although very large inter-individual variability is seen, concentrations remain remarkably stable in the same person and DHAS can therefore be used as a specific marker for a person.

■ FACTORS INFLUENCING PERIPHERAL CONCENTRATIONS

Because it is highly bound to albumin, DHAS has a long half-life (7-10 h) and low metabolic clearance (5 - 20 l/d). Circulating concentrations also do not vary during the 24 hour cycle. In addition, and unlike DHA and cortisol, DHAS concentrations are not affected by stress.

On the other hand, changes in serum albumin also result in changes in DHAS. DHAS concentrations fall in hypoalbuminaemia as in rheumatoid arthritis, cirrhosis and pregnancy.

Although ACTH stimulates adrenocortical DHAS synthesis, the rise in circulating DHAS concentrations is only seen after ACTH treatment for one to two days. No significant change in DHAS is seen immediately during stimulation by Synacthen®, whereas DHA rises in parallel with cortisol markedly after Synacthen®.

A relationship has also been found between serum insulin and DHAS. In the fasting state, serum DHAS concentrations correlate negatively with insulin concentrations and with the integrated insulin response to provoke hyperglycaemia. Insulin causes a fall in DHA and DHAS firstly through its inhibitory effect on the enzyme involved in the conversion of 17-hydroxypregnenolone into DHA and secondly by the increase in metabolic clearance of DHA.

PATHOLOGICAL VARIATIONS

Increased DHAS concentrations

Hypercorticism

In metabolic hypercorticism responsible for Cushing's syndrome and Cushing's disease, increased secretion not only of glucocorticoids but also of adrenocortical androgens is seen, together with a rise in circulating DHA and DHAS concentrations (particularly in an adrenocorticaloma).

Hyperandrogenism and hirsutism in women

A large rise in DHAS in hyperandrogenism is evidence in favour of the adrenal gland being the source of excess androgen production. DHA and DHAS concentrations are particularly raised in virilising adrenocortical tumours and also in congenital adrenal hyperplasia, due to 3β-hydroxysteroid dehydrogenase deficiency. This enzyme deficiency can be diagnosed from a rise in DHA and DHAS. Concentrations also rise but to a lesser extent in the other enzyme deficiencies (21-hydroxylase and 11β-hydroxylase). A diagnosis in these cases is made from the cortisol precursor's 17-hydroxyprogesterone (21-hydroxylase deficiency) and 11-Deoxycortisol (11β-hydroxylase deficiency).

Premature adrenarche

Premature maturation of adrenocortical function occurs very commonly in girls. Its laboratory diagnosis is straightforward as only the adrenocortical androgens (DHA and DHAS) are raised, whereas in true precocious puberty the gonadotropins (FSH and LH) and oestrogens are raised.

Hyperprolactinaemia

The hyperandrogenism which accompanies hyperprolactinaemia is characterised by raised DHA and DHAS.

Reduced DHA and DHAS concentrations

Hypocorticism

Adrenocortical androgen production falls markedly in hypocorticism with a profound fall in DHA and DHAS concentrations.

Chronic diseases

Low circulating DHA and DHAS concentrations are seen in people in poor physical health because of stress (accident and surgery) or immune system dysregulation (systemic lupus erythematosus, rheumatoid arthritis and AIDS) and in many chronic diseases (cancer, cardiovascular disease, cirrhosis, etc.).

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DIGOXIN

DEFINITION

Digoxin is a plant cardiotonic glucoside which is rapidly eliminated and used for its positive inotropic and negative chronotropic and dromotropic properties and its natriuretic effect, particularly in the treatment of congestive heart failure and supraventricular dysrhythmias (atrial fibrillation). Deslanoside is a cardiac glycoside used parenterally which is related to digoxin. It is measured using the same assay methods as digoxin (100% cross reaction with some methods).

METABOLISM

Following oral administration, digoxin is 70% absorbed and its concentration peak is reached between 1 and 5 hours post-dose. Digoxin is 20 – 25% bound to albumin in the circulation. Approximately 10% is metabolised in the liver although the proportion of metabolised forms may be higher explaining the resistance of some patients to treatment. Mono- and bis-digitoxoside derivatives of digoxigenin (active metabolites) are usually measured along with digoxin but the inactive metabolites (digoxigenin and dihydrodigoxin), are not theoretically measured by recent methods. The average half-life of digoxin is 36 hours. 90% is removed by the kidney.

INDICATIONS FOR MEASUREMENT

Digoxin has a narrow therapeutic margin and its measurement in the blood is therefore indicated in the following situations:

– Dosage adjustment in severe disease or when treatment is difficult, particularly in elderly or dysthyroid patients.

– When overdose is suspected (particularly with renal insufficiency). Symptoms of overdose are gastro-intestinal disorders (nausea, vomiting and anorexia), headaches, dizziness and particularly cardiac conduction disorders (change in the ECG).

– Inadequate efficacy at usual doses: Poor adherence, "resistance to treatment", poor gastro-intestinal absorption which may be due to interference from some drugs (antacids, activated charcoal, cholestyramine, aluminium hydroxides, salazopyrin, etc.).

– As an aid to the aetiological diagnosis of a dysrhythmia: whether related to overdose or the underlying heart disease.

– Acute poisoning (accidental overdose, attempt of suicide, ingestion of plants containing cardiotonic heterosides, etc.), plasma digoxin measurements can be used in diagnosis and follow-up (monitoring antidote treatment).

INFORMATION

SAMPLE

Serum or heparinised plasma. Avoid tubes containing gel (risk of adsorption).

For therapeutic monitoring the sample must always be taken at the same time, between 8 and 24 hours post-dose, generally in the morning, immediately before the next dose (trough concentration). Wait until the pharmaceutical steady state which is reached after 5 half-lives or 6 to 8 days after starting treatment (if renal function is normal) before measuring. For patients being treated intravenously the sample must be taken 6 to 8 hours after the end of the injection (contralateral arm to the infusion). If an overdose is suspected, then the sample can be taken whenever symptoms develop.

ESSENTIAL INFORMATION

In accordance with the French Nomenclature of Medical Laboratory Procedures, any request for drug measurements must include the reasons for the request (testing for efficacy or toxicity), the sampling time, the treatment start date and/or any change in dosage, dosage information (amount administered, frequency, route of administration) and the age, height and weight of the person whenever possible.

Current treatment? Ask about drugs which may interfere with gastro-intestinal absorption of digoxin or its metabolism;

– Increased blood digoxin as a result of reduced digoxin clearance which can be caused by amiodarone, hydroquinidine, quinidine and itraconazole;

– Increased blood digoxin due to increased absorption can be caused by clarithromycin and erythromycin;

– Reduced blood digoxin because of a reduction in gastro-intestinal absorption can be caused by antacids, local gastrointestinal agents (aluminium hydroxides, activated charcoal), cholestyramine, sucralfate where blood digoxin concentration is reduced by 50% if sucralfate is taken less than two hours before the digoxin; sulfasalazine can cause a reduction in blood digoxin concentration of up to 50%.

– Reduced blood digoxin during carbamazepine treatment.

SAMPLE STORAGE AND TRANSPORT

It can be stored for 1 week at + 4°C and 6 months at - 20°C.

Transport at + 4°C or frozen if the sample is already in its frozen state.

ASSAY METHODS

Immunological methods: FPIA, EMIT, radio-immunological assay etc.

NORMAL EXPECTED VALUES

The therapeutic range is between 0.9 and 2 ng/ml.

Below 0.45 ng/ml, the patient is underdosed.

Above 2.1 ng/ml signs of overdose may develop.

Toxic range > 3.1 ng/ml.

Conversion factor: 1 nmol/l x 0. 781 = ng/ml.

In acute poisoning, treatment is the administration of anti-digoxin Fab fragments. Patients are monitored by measuring free active digoxin unbound by antibodies. Many digoxin assay methods, particularly competitive methods, suffer interference from these antibodies (the interference varies depending on the method). The plasma of patients who have been treated with these antibodies within 3 days before measurement should be pretreated (1 hour in a water bath at + 55°C).

FOR FURTHER INFORMATION

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DIHYDROTESTOSTERONE

DEFINITION – SYNONYMS

Dihydrotestosterone (DHT) is the 5α-reduced derivative of testosterone. As it is the predominant androgen in many target cells and its activity is approximately twice as great as that of testosterone in most biological tests, DHT is recognised to be the mediator of androgen action on some target cells.

DHT is formed from testosterone in response to the action of 5α-reductase, which exists as two isoenzymes: 5α-reductase 1 expressed by sebaceous glands and the liver and 5α-reductase 2, expressed in the male urogenital tract, genital skin and liver.

Both the distribution and ontogenics of the two isoenzymes are different. Whereas isoenzyme 2 is expressed very early in the male urogenital tract during embryogenesis and isoenzyme 1 is only expressed in the skin after puberty.

During sexual differentiation of the male foetus, masculinisation of the internal genital tract (epididymis, vas deferens, seminal vesicles) from the Wolffian duct which has no 5α-reductase depends on testosterone whereas the development of the external genital organs (scrotum, penis), prostate and urethra depends on DHT.

In order to act, DHT is formed *in-situ* in the target cells and binds to a soluble receptor. The DHT-activated receptor complex migrates to the nucleus and binds to a molecule called *"heat shock protein"* 90. The complex formed binds to the nuclear site of action.

It is important to be aware that DHT and testosterone share the same cytosol receptor and that it is the tissue availability of 5α-reductase which determines the preferential binding of DHT. Muscle tissue, for example, is devoid of 5α-reductase activity and testosterone alone is the promoter of muscle development at puberty.

DHT is also a potent regulator of gonadotrophin secretion, particularly LH, and men treated with DHT have very low LH and testosterone concentrations.

DHT production depends on the amount of available testosterone to act as the substrate for 5α-reductase. Approximately 60% of testosterone in women comes from androstanedione which is therefore the most important precursor of DHT. Conversely, testicular testosterone is the main precursor in men. In both cases, circulating DHT does not reflect the action of androgens on target tissues as they are also formed in the liver.

Circulating DHT binds mostly to SHBG for which it has three times greater affinity than testosterone.

DHT is metabolised particularly via the 3α pathway resulting in the formation of 3α 17β-androstanediol. This occurs both in the liver and in target cells. Androstenediol is then glucurono-conjugated and excreted in urine.

INDICATIONS FOR MEASUREMENT

DHT measurement generally forms part of the investigation of hirsutism and virilisation in women and girls and in the investigation of male pseudohermaphroditism. Plasma DHT concentrations however do not reflect skin 5α-reductase activity and measurement of androstanediol glucuronide in so-called idiopathic hirsutism is preferred.

INFORMATION

SAMPLE

Measurements are performed in serum or EDTA or heparinised plasma as the anticoagulant does not interfere. Similarly, lipaemic or haemolysed samples do not raise difficulties as the assay method used includes extraction followed by chromatography.

INFORMATION REQUIRED

DHT administration is part of the treatment of hypogonadism in males and the treatment being taken must be known. Information should also include whether the measurement is for an hCG stimulation test.

SAMPLE STORAGE AND TRANSPORT

Separated plasma or serum should be stored at + 4°C and transported at this temperature to the laboratory.

They may be stored for 6 months to 1 year frozen at -30°C.

ASSAY METHOD

Measurements are performed using radio-immunological assay with a prior purification stage involving extraction followed by chromatography. DHT differs from testosterone only by the absence of the double bond between C4 and C5 and testosterone produces a considerable cross-reaction with anti-DHT antiserum. Only the chromatography method can separate the two steroids producing reliable DHT results with good specificity.

USUAL VALUES

Plasma or serum concentrations of DHT vary:

In adult men between 0.33 and 1.20 ng/ml.

In adult women between 0.06 and 0.30 ng/ml.

Results are expressed either in ng/ml or nmol/l. To convert ng/ml to nmol/l multiply the ng/ml by 3.443.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

DHT concentrations in boys are higher at birth than in cord blood and then fall, subsequently rising again in parallel to testosterone concentrations and reach a peak at around the third month after birth. DHT then falls and remains at very low concentrations until puberty when a rise begins in parallel to testosterone. Concentrations in girls are extremely low from birth to puberty.

Serum DHT concentrations are lower in post-menopausal women than in women whose ovaries are still active although like testosterone it falls proportionately less than oestrogens. Conversely, DHT remains unchanged or increases with age in men.

PATHOLOGICAL VARIATIONS

5α-reductase deficiency:

This involves deficiency of 5α-reductase characterised by male pseudohermaphroditism. The syndrome is familial and transmitted autosomal recessively. Pronounced ambiguous genitalia is present at birth and the newborn babies are declared to be female. At puberty these males with a female phenotype undergo variable degrees of somatic and behavioural virilisation, where muscle mass develops, the voice becomes deep and the external genital organs increase in size. Gynecomastia never occurs. Overall, post-pubertal morphology is increasingly male apart from pubic hair growth which is more female in distribution. The laboratory diagnosis is relatively easy in the post-pubertal period and uses the testosterone/DHT ratio which is very high because of the normal testosterone concentration and negligible or very low DHT. The ratio can vary from 30 to 50 compared to between 7 and 10 in normal people. In pre-pubertal or pubertal children the hCG stimulation test shows a good testosterone response without a concomitant rise in DHT.

Idiopathic hirsutism:

Increased cutaneous 5α-reductase activity is rarely accompanied by an increase in plasma DHT. Measurement of androstanediol glucuronide, the DHT metabolite, is preferred in this case.

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DIPHTHERIA

DEFINITION

Diphtheria is a highly contagious toxic bacterial infection caused by *Corynebacterium diphteriae*. Although practically eradicated in developing countries as a result of vaccination, it remains topical as it has increased again numbers in Eastern Europe in the last fifteen years and in some countries such as Algeria and Vietnam.

C. diphtheria is a non spore-forming, irregularly shaped gram positive bacillus with one or two club-shaped swollen extremities. On direct examination the bacteria are grouped in clusters and produce the characteristic appearances of a fence or alphabetical letters.

Synonym: *Corynebacterium diphteriae*, diphtheria bacillus and Klebs-Löeffler bacillus.

INTRODUCTION

EPIDEMIOLOGY

Diphtheria is a strictly human disease, notification of which is compulsory. It mostly affects adults who no longer have vaccine cover and unvaccinated children. People exposed to the disease are those in direct contact with patients or living in poor hygiene conditions. It should be suspected in any case of sore throat with a pseudomembrane occurring in a person coming from an endemic area, or in a person with poor living conditions, particularly if they are not up to date with their diphtheria vaccination (the diagnosis cannot be excluded if a past history of vaccination is given). Diphtheria is still endemic in tropical and subtropical regions in all continents. Human to human transmission occurs through droplet spread, from infected saliva or by direct skin contact.

SYMPTOMS

C. diphtheria has tropism for the upper respiratory tract and more rarely for the skin and conjunctivae. The disease is variably severe ranging from asymptomatic carriers to complicated forms of the disease.

Diphtheria sore throat:

This is the main clinical feature of diphtheria. It begins after an incubation period of 2 to 5 days and is characterised by a sore throat with swollen red tonsils covered with a white coating (pseudomembrane) associated with dysphagia, cervical lymphadenopathy, headaches, general malaise and a moderate fever. The characteristic pseudomembranes are extremely adherent and can invade the nasopharynx and larynx, causing "croup" or respiratory tract obstruction.

Malignant diphtheria sore throat:

This is a specific form of sore throat which develops suddenly with deterioration in general health, high fever, bilateral painful growth lymphadenopathy (proconsular neck), foetid breath and confluent, haemorrhagic pseudomembranes. Sever disease is due to the presence of toxic signs such as a large fall in blood pressure, profuse haemorrhage and renal damage. It is often fatal.

Cutaneous diphtheria:

This occurs particularly in tropical countries in the form of a skin ulcer covered by pseudomembranes complicating a pre-existing skin lesion.

Diphtheria toxaemia:

This is caused by strains of *C. diphtheria* carrying the "*tox* gene" which codes for an exotoxin responsible for cell damage. It causes systemic disease such as myocarditis, neurological disease with cranial and peripheral nerve paralysis, haemorrhage and renal failure, etc.

SEARCH INDICATIONS

– Diagnosis of diphtheria in an unvaccinated child or in an adult who is no longer protected.

– Testing for *C. diphtheria* carrier status in the patients close contacts.

– Testing for diphtheria in a person returning from an endemic area.

– Determining protective immune status after vaccination.

INFORMATION

SAMPLE

Samples from the inflamed areas of the nasopharynx or pseudomembranes using forceps or swabs. Several samples are recommended.

Samples from the edge of the pseudomembranes covering the ulceration for suspected cutaneous diphtheria.

Sera to test for protective immune status.

QUESTIONS FOR THE PATIENT

Vaccine status?

Clinical symptoms?

History of travel to an endemic area?

Current antibiotic treatment?

SAMPLE STORAGE AND TRANSPORT

Swabs must be transported immediately to the laboratory. If this is not possible then a semi-agar medium or one containing silica gel must be used. If the time to reach the laboratory exceeds 24 hours, tellurite enrichment is required. Regardless of the medium used, it must be kept at ambient temperature or at + 4°C.

ASSAY METHODS

BACTERIOLOGICAL DIAGNOSIS

Direct microscopic examination:

This involves examining for the presence of a flora predominantly containing irregular gram positive bacilli and their characteristic grouping on a smear. If a large number of Corynebacteria are found, serotherapy is occasionally required if clinical features support diphtheria.

Isolation and culture conditions:

The sample is inoculated in parallel onto a specific medium (Tinsdale medium or Cystine-tellurite agar) and onto a nutrient agar spiked with blood. Other enriched media such as Loeffler

or Mueller-Hinton media may be used. Colonies of *C. diphtheria* grown on Tinsdale medium are black and surrounded by a black halo. The colonies grown on blood agar are grey, convex and half of *C. diphtheria* strains are β-haemolytic. The suspicious colonies are observed microscopically and sub-cultured onto blood agar and ordinary agar.

- **Identification methods:**

Biochemical tests are performed using commercially available identification kits (such as API Coryne® from BioMérieux). *C. diphtheria* is identified from sugar fermentation tests and from their pyrazinamidase negative and α–glucosidase positive activities.

Testing for diphtheria toxin production via the isolated strain:

This can be performed *in-vivo* by revealing its pathogenic potential after inoculating a suspension of bacterial culture into guinea pig, or *in- vitro* using the Elek test (or agar medium immunoprecipitation test). In practice, the toxin is tested for by amplification of the *tox* gene.

INDIRECT DIAGNOSIS

This can be used to monitor protective antibody titres in a person after vaccination or to make a retrospective diagnosis in some situations. The techniques available are seroneutralisation of the cytopathogenic effect of the toxin on Vero cell cultures, ELISA and passive haemagglutination.

According to the World Health Organisation (WHO) criteria, an antibody titre of 0.1 IU/ml or more is deemed to be protective.

TREATMENT

CURATIVE

Diphtheria is a diagnostic and therapeutic emergency. If suspected, immediate treatment must be started using a combination of serotherapy and antibiotic therapy.

- **Serotherapy:**

This follows a specific therapeutic regimen recommended by the WHO and must be administered as soon as possible in order to inhibit the systemic action of the toxin.

- **Antibiotic therapy:**

The usual treatment is penicillin G or amoxicillin. Erythromycin may be used in patients allergic to β-lactams.

PREVENTATIVE

This involves vaccination with the diphtheria anatoxin, which has a mandatory vaccine regimen in France of 3 injections at one month intervals from the age of 2 months onwards followed by a booster at 10-18 months, 6 years, 11-13 years and 16-18 years old. Continued vaccination is not given to adults except for some categories of people, such as travellers to endemic areas, military personnel, medical care staff and nursery staff etc.

NB: Patients with presumed disease must be kept in isolation pending the results of samples to isolate *C. diphtheria* and then identify the toxin gene. This should be continued until 2 samples taken at least at 24 hour interval during antibiotic therapy (or less than 24 hours after stopping antibiotic therapy) are negative. All objects in contact with the patient must also be disinfected with an appropriate disinfecting agent.

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DIRECT ERYTHROCYTE COOMBS TEST

DEFINITION

The direct erythrocyte Coombs test and the direct Coombs test are former names for what should now be called the "direct antiglobulin test" (DAT). This test demonstrates the in-vivo binding of IgG antibodies and complement fractions to human red blood cells.

The principle of the direct antiglobulin test (DAT) is to demonstrate in-vivo sensitisation of red blood cells by human antiglobulins, the Fab portion of which recognises immunoglobulin (Ig) isotype markers or complement fractions specifically bound to the red blood cell. It can identify the class or classes of Ig or the complement fractions involved in autoimmune haemolytic anaemias (AIHA).

The method involves the mandatory ***simultaneous and independent*** use of an anti IgG antiglobulin and anti C3d, together with appropriate control reagents. Other reagents are only used in rare situations (antiglobulin binding to IgA, rarely responsible for autoimmune haemolysis).

INTRODUCTION/INDICATIONS

The autoimmune haemolytic anaemias (AIHA) are defined as haemolytic anaemias associated with the presence of an antibody against one or more erythrocyte antigen(s) on red blood cells and/or in plasma. The autoantibody is an IgG, IgM or more rarely IgA immunoglobulin. The red blood cells coated with antibodies are said to be "sensitised" and their life-span is shortened.

There are several classifications of the AIHA:

– Clinical classification: Distinguishing the acute AIHA from the chronic AIHA.

– Aetiological classification: Idiopathic or associated with other disease.

– Laboratory classification based on the optimum temperature of antibody-erythrocyte antigen binding. This classification has the advantage of being close to the clinical classification.

According to the classification based on optimal antibody temperature, the following can be distinguished:

"Warm" autoantibodies: Generally IgG and binding optimally at 37°C. Their ability to bind complement varies depending on the IgG subclass and the amount of autoantibodies present on the red blood cell surface. They recognise either HR system antigens (anti-public or anti-e, c, and E specificity) or other systems (LW, U, etc).

"Cold" autoantibodies (see cold agglutinins chapter) which are generally IgM with low optimal temperature (+4°C) and which bind complement. Cold agglutinins are usually of anti I, i, MNS specificity, etc.

Autoantibodies or biphasic haemolysins: These are IgG antibodies which bind complement at 37°C and detach in the cold, activating complement. They are of anti-P specificity.

The following are therefore distinguished:

– Warm autoantibodies AIHA which generally occur in a context of infection or autoimmunity. The DAT is IgG ± C3d.

– The cold autoantibodies AIHA which usually occur in a context of infection (generally in the young) or chronic lymphoproliferative disease (generally in the elderly). The DAT is C3d.

– Mixed warm and cold autoantibodies AIHA in which the DAT is IgG+C3d.

– Biphasic AIHA which are very rare and classically occur in a context of childhood infection. The DAT is C3d (the IgG binds warm although complement is activated when it detaches from the red blood cell).

The most common AIHA are IgG or mixed types.

The direct antiglobulin test is therefore used to identify a red blood cell surface auto-antibody in the diagnosis of AIHA although it is also used to:

– Demonstrate neonatal RBC sensitisation in neonatal haemolytic disease (NNHD). To identify maternal autoantibodies bound to the neonatal red blood cells.

– To demonstrate donor RBC binding of specific autoantibodies present in the recipient patient's serum (transfusion, haemolytic accident) to identify recipient autoantibodies on the donor's red blood cells.

– To investigate for RBC sensitisation in drug-induced haemolytic anaemia.

INFORMATION

SAMPLE

The DAT must be performed on a sample of blood taken into an anticoagulant, either citrate or EDTA, with a sufficient amount to perform controls and/or further investigations.

Haemolysed samples should be avoided as lysis weakens the agglutination reactions and makes them difficult to interpret. The antibodies can also become detached, with haemolysis and produce a false negative DAT.

QUESTIONS FOR THE PATIENT

Age?

Clinical context, chronic disease?

History of transfusion and date?

Medical drug history?

SAMPLE STORAGE AND T TRANSPORT

The DAT is performed on a sample under 2 days old stored under correct conditions at +4°C.

METHODOLOGY

Test tube agglutination techniques are available (washed red blood cells) although solid phase filtration methods (particularly gel filtration) are tending to replace the test tube methods. These have the advantage of being automatable and avoid the initial wash stage (which is known to elute some bound antibodies and be responsible for false negative results).

It also increases the sensitivity of the test as low ionic forces are used. This increase in sensitivity is accompanied by a reduction in specificity and raises a problem of establishing physiological and pathological thresholds.

More sensitive tests, particularly flow cytometry, are also available.

INTERPRETATION

In haemolytic anaemia the DAT may be positive in the following situations:

- AIHA
- NNHD
- Transfusion accident
- Drug-induced AH

Outside of the context of haemolytic anaemia the DAT may also be positive in the following situations:

- Polyclonal and particularly monoclonal dysglobulinaemias
- Cryoglubulins
- Administration of high dose, intravenous, polyvalent IgG (note also that the DAT may be positive in the new-born baby following injection of Rophylac in the mother, where passive anti-D is bound to neonatal red blood cells).
- Damage to the erythrocyte membrane
- Polyagglutinable red blood cells

Physiologically positive DAT may also be seen (minimal physiological binding of complement or IgG to red blood cells). According to some studies, 1% of the general population and even 10% of hospitalised patients have a positive DAT without any specific reason.

Conversely, the DAT may be "falsely" negative when:

-The number of antibodies bound is below the limit of detection

-Antibody affinity for red blood cells is weak, where free autoantibodies in serum are not bound to red blood cells

- Zone effect

-IgA autoantibodies AIHA

2 to 4% of AIHA have a negative DAT.

The diagnosis of AIHA must not therefore be based on the DAT alone. Whether positive or negative, in a suggestive clinical situation, the DAT must be followed by other tests, such as elution (more sensitive than DAT and defines antibody specificity) and studying serum at 37°C and 4°C (testing for free serum antibodies of identical or different specificity to the bound antibody).

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ECARIN TIME

DEFINITION AND SYNONYMS

The ecarin time was developed in 1993 by Nowak and Busha and is a coagulation test performed on citrated plasma or in some cases on whole blood as for example extra-corporial circulation (EEC) using the reagent ecarin, a purified protein extracted from the venom of the viper *Echis carinatus*. Ecarin converts prothrombin into meizothrombin and intermediary products. Meizothrombin then autocatalyses itself into alpha-thrombin and prothrombin 1+2 fragments. These reactions all occur in the absence of phospholipids and calcium ions. Direct inhibitors of thrombin (anti-IIa), such as hirudin, dabigatran or argatroban, inhibit meizothrombin, whereas anti-thrombin and heparin have almost no effect. A good correlation is seen between the ecarin time and concentrations of various direct thrombin inhibitors both in experimental animal models of venous thrombosis and in *in-vitro* studies. Compared to the activated cephalin time (ACT) or activated partial thromboplastin time, the ecarin time has the advantage of producing a linear response even for high concentrations of direct thrombin inhibitors, whereas the ACT reaches a plateau at highest concentrations of these.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

Measurement is indicated in monitoring treatment with direct thrombin inhibitors, hirudin and derivatives (lepirudin and desirudin), argatroban and dabigatran. Measurements may be performed on plasma (samples sent to another laboratory for analysis) or whole blood (samples analysed in real time, during, for example ECC).

INFORMATION

SAMPLE

The sample is taken into sodium citrate (1 volume of anticoagulant per 9 volumes of whole blood), at a concentration of 0.109 M (sodium citrate concentration 0.129 M is acceptable). The volume of anticoagulant may be adjusted in cases when the hematocrit is far from normal values (< 0.35 or > 0.55). Vacuum glass tubes are recommended (plastic tubes may be used if they have been validated).

A sample is taken from the antecubital fossa by direct venepuncture. The tube is mixed by turning over in succession (8 to 10 times) and then centrifuged at 2000 g for 15 minutes. A fasting sample is not required.

QUESTIONS FOR THE PATIENT

What medicinal products is the patient taking?

What dosage, route(s) of administration and administration time(s)?

For how long has the patient being taking these medicinal products?

SAMPLE STORAGE AND TRANSPORT

The samples can be stored for 4 hours at room temperature. Beyond this time the plasma needs to be separated and frozen in a suitably labelled polypropylene tube and frozen at -20°C (for less than a week) or at – 80°C for up to several months. Plasma samples must be transported frozen at -20°C if the laboratory which received the sample is not performing the test.

ASSAY METHOD

The assay method available is a chonometric method involving measuring the clot formation time, after mixing an aliquot of plasma and ecarin used to trigger coagulation of the plasma. The method is usually automated on automated analysers with electro-magnetic or optical detection.

A new method, the *ecarin chromogenic assay* (ECA) method has been developed and can be used on automated coagulometers.

NORMAL EXPECTED VALUES

The method is not well standardised between the laboratories and each laboratory must establish its own reference ranges depending on the conditions under which the test is performed (whole blood, plasma, relative proportions of test sample and reagent, instrument, concentration of ecarin used) and the origin of the ecarin used. Significant between-batch variability has also been described for batches of ecarin from the same manufacturer. It is therefore essential to conduct a between-batch validation before performing tests, when batches are changed. For reference, two main reference ranges are found in the literature, one around 15 seconds and the other around 30 seconds, depending on operating conditions.

PATHOPHYSIOLOGICAL VARIATIONS

The ecarin time is prolonged during treatment with thrombin inhibitors: lepirudin, desirudin, argatroban and dabigatran.

In clinical practice, to monitor desirudin prophylaxis in orthopaedic practice, samples should be taken before subcutaneous administration and 2 hours afterwards. Values at 2 hours (after administering 2 x 15 mg or 2 x 20 mg) are between 0.2 and 0.3 µg/ml in patients with normal renal function and then decline to values close to the limit of detection immediately before the next dose. Two-hour desirudin levels in cardiac surgery may be as high as 2.5 to 4 µg/ml.

The major limitation of the ecarin time is situations in which prothrombin or fibrinogen are greatly raised: the ecarin time can then no longer be used, as the levels produced are incorrect.

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ECP

(Eosinophil Cationic Protein)

DEFINITION

ECP is one of the 4 specific eosinophil cationic proteins. Increase in this protein due to low but frequent allergenic stimulation without symptoms is a marker of exposure to allergens. It is released in the late phase of asthma and reflects the severity of obstructive bronchial disease. It is a marker of bronchial inflammation.

INTRODUCTION

ECP is released from the intracytoplasmic granules in every situation in which eosinophils are activated, such as during some infections, parasitic infection, autoimmune diseases and atopic dermatitis. Its release in the bronchial mucosa in the late phase of asthma causes tissue damage and progressive destruction of the bronchial mucosa. Concentrations in serum reflect the inflammatory state of the bronchi in an asthmatic child or adult following exposure to allergens even before clinical signs develop.

INDICATIONS FOR MEASUREMENT

ECP is proposed in addition to functional testing:

– As an aid to the early diagnosis of asthma.

– As an assessment of the severity of asthma.

– For monitoring and modifying treatment for asthma in combination with respiratory function tests.

– To monitor patients with unstable asthma whose adherence with treatment is variable.

INFORMATION

■ SAMPLE

Venous blood taken into a tube preferably containing separator gel and not containing anticoagulant. Bronchio-alveolar lavage and nasal fluid may be used.

Activated eosinophils in blood release ECP during the first hour after sampling, during the coagulation phase. Beyond this the inactive eosinophils degranulate as a result of apoptosis. For these reasons the sampling protocol must be followed carefully.

Mix the venous blood obtained by turning the tube upside down gently five times. Leave to coagulate for 1 to 2 hours at ambient temperature (+ 20-24°C). Centrifuge for 10 minutes at 1350 g, at + 20-24°C and transfer the serum into a new tube and freeze.

Haemolysed serum and plasma cannot be used.

■ QUESTION FOR THE PATIENT

Current treatment?

■ SAMPLE STORAGE AND TRANSPORT

Store at - 20°C or preferably at - 70°C for at least 2 years.

If the sample is to be transported, the serum must be frozen within 4 hours after sampling and transported at - 20°C.

ASSAY METHODS

Immunofluorimetric method.

NORMAL EXPECTED VALUES

< 10 µg/l: No bronchial inflammation.

PATHOLOGICAL VALUES

> 15 µg/l: Inflammatory state present. However, when monitoring treatment, the patient acts as his/her own control and results should be interpreted against previous values.

POUR EN SAVOIR PLUS

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EPSTEIN-BARR (VIRUS)

DEFINITION

The Epstein-Barr virus was discovered in 1964 on electron microscopy by A. Epstein in cells derived from African Burkitt's lymphoma. It belongs to the Herpesviridae family, Gammaherpesviridae subfamily, Lymphocryptovirus ("virus hidden in lymphocytes"). It is identical in morphology to the other herpes viruses. The virions measure approximately 150 nm and are enveloped. The genome is a large linear double-stranded DNA. EBV can only multiply in-vitro within B lymphocytes, in which it induces lymphoblastic transformation and immortalisation. This transforming property has led EBV to be suspected of involvement in some human tumours. After primary infection, EBV becomes established in a latent state in B lymphocytes where the viral genome persists in an episomic form although it can also incorporate into the host cell chromosomal DNA. Reactivation is usually clinically silent and transient, except in cellular immunodeficiency. Two types of EBV (EBV1-EBV2) can be distinguished depending on a polymorphism in the EBNA nuclear antigens. The distinction between these requires the use of monoclonal antibodies or specific genomic markers and is of no practical use because of extensive serological cross-reactions between the two types. EBV type 1 is by far the predominant type in infected populations.

Synonyms: EBV, HHV4.

INTRODUCTION

EPIDEMIOLOGY

The reservoir for the virus is strictly human. More than 90% of adults have antibodies against EBV and therefore host the virus in its latent state. EBV is found in lymphocytes, and can be excreted asymptomatically for long periods of time in saliva and possibly in genital secretions. Transmission occurs through close contact mostly from saliva ("kissing disease") and sexual activity.

SYMPTOMS

The virus enters the oropharyngeal epithelium where it begins to multiply and then reaches the epithelial B lymphocytes which are then activated. Lymphocyte proliferation is usually controlled by the immune system.

In immunocompetent people

Primary infection

Primary EBV infection usually occurs in childhood. It is often asymptomatic or may present atypically (flu-like syndrome, asthenia and lymphadenopathy).

Infectious mononucleosis tends more to affect adolescents and young adults. It presents with pseudomembranous febrile sore throat with multiple lymphadenopathy and splenomegaly. Pronounced lymphocytosis is always present with atypical lymphocytes (activated CD8 lymphocytes). Asthenia lasts for long periods of time (it may last for several months), although generally has a good clinical outcome. Although recovery may be slow, complications (haemolytic anaemia, hepatitis and ruptured spleen) are rare. Ampicillin (used for suspected bacterial sore throat) causes a skin rash in 90% of affected cases. Biological impaired liver function and numerous transient immunological abnormalities (heterophil antibodies, rheumatoid factor, anti-organ antibodies and IgM antibodies against different viruses, such as cytomegalovirus or bacteria are found. These abnormalities are a result of polyclonal stimulation of B lymphocytes.

Primary infection can occasionally present purely as neurological disease, such as encephalitis and polyradiculoneuritis.

Malignant disease associated with EBV

Burkitt's lymphoma is found throughout the world, although is endemic particularly in East Africa where it is the most common cancer in children between 6 and 10 years old. In this area, which is also an area where malaria is highly endemic, EBV is associated with the lymphoma in more than 90% of cases compared to less than 15% of cases in non-endemic areas. Clinically, it presents as an indolent tumour from the maxillae to the orbits with exophthalmos. Approximately 30% of cases are associated with abdominal organ involvement (lymph nodes, ovaries, liver, kidney and adrenal glands) and neuromeningeal disease. It is a fatal disease.

Undifferentiated nasopharyngeal carcinoma affects adults between 20 and 50 years old. It has a particularly high incidence in Southern China and Northern Africa, although the reason for this is unknown. EBV is associated with the tumour in 100% of cases.

Hodgkin's disease occurs in the Western world where it is the commonest lymphoma. It affects people between 25 and 30 years and over 45 years old, particularly in higher socio-economic groups. It is suspected to be associated with EBV infection from epidemiological findings (increased risk of developing Hodgkin's lymphoma within 3 years of developing primary EBV infection), serological evidence (high titres of anti-VCA antibodies) and the finding of the EBV genome in malignant Reed-Sternberg cells. These findings vary, however, depending on the histological type of lymphoma and by geographical origin.

Centro-facial T lymphoma is mostly but not only seen in South-East Asia. It causes progressive erosion of the bony tissue of the nose and face. It is variably associated with EBV depending on geographical origin and whether or not the lymphoma is located in lymph nodes.

In immunosuppressed patients

Primary infection

Oral hairy leukoplakia is a specific form of primary EBV infection seen particularly in HIV positive people and characterised by vertical striation affecting the edges of the ventral surface of the tongue. The lesions are due to chronic production of EBV by tongue epithelial cells.

Purtilo syndrome (Duncan's disease – X chromosome linked lymphoproliferative syndrome) is an extremely severe form of primary EBV infections affecting boys suffering from an X-chromosome linked immunodeficiency which is usually fatal. The minority of children who survive are at high risk of subsequent Burkitt's lymphoma.

Viral reactivation and risk of lymphoma associated with EBV

Cases of lymphoproliferation, a large majority associated with EBV, are seen in transplant patients at a frequency of 20 to 50 times higher than in general population. Lymphomas occur in approximately 10% of HIV positive people and are immunoblastic in the late phase of AIDS and always associated with EBV. On the other hand, early lymphomas are usually Burkitt's type when an association with EBV is only seen in 30% of cases.

INDICATIONS FOR MEASUREMENT

Aetiological diagnosis of a mononucleosis-like syndrome.

Aetiological investigation of tumour.

Prediction of the development of lymphoma in an immunosuppressed patient.

Monitoring change in viral load.

Definition of serological status in organ donation in both donor and recipient.

INFORMATION

SAMPLE

To test for virus or viral genome: whole blood collected into EDTA or citrate, plasma or serum, nasopharyngeal aspirates salivary or CSF sample, biopsies taken into a sterile bottle without histological fixing agent.

To test for antibodies: unhaemolysed serum (possibly CSF).

QUESTIONS FOR THE PATIENT

Immune status where applicable, HIV seropositivity, geographical and ethnic origin, clinical signs, nature and onset of symptoms?

SAMPLE STORAGE AND TRANSPORT

It is recommended that samples be transported promptly to the laboratory (a few hours at ambient temperature if possible, and less than 3 days if refrigerated). CSF should be frozen and serum kept at + 4°C, if the laboratory is distant to the sampling site.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

Haematological examination

Hyperleukocytosis of between 10,000 and 20,000/mm^3 with between 30% and 90% *lymphocytes*. Cells are characteristic by their pleiomorphism. In particular, large hyperbasophilic mononucleated cells with abundant cytoplasm and an eccentric nucleus.

Identification of EBV molecular markers or antigens

In-situ hybridisation can reveal small RNA coded by EBV (EBER's for *Epstein Barr encoded small RNAs*) in cells chronically infected with the virus. Labelled monoclonal antibodies (fluorescence or peroxidase) are used to locate some proteins produced by EBV. These methods are mostly used to study tumour tissue.

Detection of the viral genome

The most widely used technique to identify the EBV genome is currently PCR, particularly to quantify viral load (real time PCR) in circulating blood, leukocytes, plasma or serum, and possibly CSF or tissue. This is an alternative to isolation of the virus.

Isolation of the virus

EBV can only be isolated from B lymphocytes obtained from cord blood, although the culture techniques are long and time-consuming and cannot be used for routine diagnosis. *In-vitro* cell lines can also be prepared, derived from tumour tissue chronically infected with the virus. These methods are reserved for specialist virology laboratories.

INDIRECT DIAGNOSIS

Detection of heterophile antibodies

EBV infection of B lymphocytes stimulates the production of non-specific IgM antibodies (Ab) called heterophile Ab. In infectious mononucleosis these Ab can agglutinate red blood cells from some species of animals (sheep, cow and horse).

The IM test (Monospot) is a rapid agglutination method using stabilised horse red blood cells.

The Paul-Bunnell-Davidsohn reaction (PBD) distinguishes IM heterophile antibodies from natural Forsmann antibodies and quantifies the heterophile antibodies by differential absorption of sheep anti-red blood cell agglutinins onto beef red blood cells and guinea pig kidney.

Detection of specific anti-EBV Ab

By indirect immunofluorescence (reference method). This method requires chronically EBV infected cell lines expressing the capsid antigen (VCA), the early antigens (EA) and the nuclear antigen (EBNA). Antibodies titration against this set of antigens provides information about the person's EBV serological status.

ELISA method. Many kits are commercially available, often producing different values (there is as yet, no consensus on the antigens used). The best methods distinguish IgM and/or IgG antibodies against different EBV antigens and results are similar to interpret to immunofluorescence. Tests are also available which identify some antibodies by rapid ELISA (10 minutes) on a test strip or rod. IgG and IgM antibodies against a synthetic peptide derived from the EBNA-1 antigen can for example be detected.

Immunoblot identifies IgM and IgG antibodies against 5 recombinant proteins derived from the VCA, EA and EBNA antigens. This is a qualitative test and useful for small analytical batches.

INTERPRETATION

DIAGNOSIS OF PRIMARY EBV INFECTION

Infectious mononucleosis (IM)

The IM test is usually positive from the second week of clinical signs and lasts for approximately 6 to 8 weeks. False negative results can be found in 10 to 15% of cases (particularly in young children). False positive reactions also occur (blood disorders, autoimmune diseases, etc.).

The Paul-Bunnell-Davidsohn reaction becomes positive after 12 to 15 days and antibodies generally persist for 2 to 3 months. It is more specific than slide-based tests but can produce false negative results in young children and in "blood group A" people.

Specific EBV serology is always the method of choice. Anti-VCA IgM antibodies develop within a week after the onset of clinical signs, followed by IgG a few days later. IgG Anti-EA antibodies develop transiently but variably during the acute phase (only in 70 to 80% of cases) and disappear during convalescence. Anti-EBNA antibodies are always negative at this stage and only become positive after a few months. Note, however, that antibodies may be detected late or may not develop in severe immunosuppression (Purtilo syndrome).

- **Primary EBV infection without true infectious mononucleosis**

A diagnostic problem may arise in young children (asthenia and lymphadenopathy) or in those with neurological disease (encephalitis and polyradiculoneuritis). Testing for heterophil antibodies is of limited value. Specific EBV serology can be used or the viral genome can be identified by PCR.

DIAGNOSIS OF ACTIVE SECONDARY INFECTION

Serology often contributes little information, particularly in immunosuppressed patients. Detection of significant titres of IgG anti-EA antibodies is a useful indicator but these are variably present. On the other hand, detection of significant titres of IgA anti-VCA and anti-EA antibodies is an important marker for the diagnosis of EBV nasopharyngeal carcinoma.

PCR testing for markers of viral replication is needed in most cases. The finding of the viral genome in an anatomical site or biological fluid must also be interpreted with caution, in light of the clinical context and patient's immune status.

Measurement of viral load in blood, CSF (cerebral lymphoma) or tissues is believed to predict the development of an EBV lymphoproliferative syndrome. This is useful to monitor changes in the infection over time, depending on the treatment used.

DETERMINATION OF EBV SEROLOGICAL STATUS

In a clinical syndrome of mononucleosis, an old EBV infection profile excludes the disease and suggests other causes (cytomegalovirus, toxoplasmosis and HIV).

Positive serology can be used to identify transplant recipients or those who are to be given long-term immunosuppressant treatment who are EBV-seronegative and who are therefore at risk of severe primary infection. EBV serological status should also be determined whenever possible in organ or tissue donations.

The different possible serological situations which may be found on indirect immunofluorescence (antibody titres) are summarised in the table below.

	anti-VAC IgG	anti-VCA IgM	anti-VCA IgA	anti-EA IgG	anti-EA IgA	anti-EBNA IgG
Seronegative (receptive person)	< 5	< 5	< 5	< 5	< 5	< 5
Primary infection	40 - 1280	20 - 640	< 5 - 40	< 5 - 80	< 5	< 5
Past Infection (latent)	40 - 640	< 5-10	< 5	<5-10	< 5	20 - 320
Possible reactivation	> 640	< 5 - 80	<5- 40	< 5-320	<5-40	20-320
EBV associated Burkitt's lymphoma	> 640	< 5	< 5	80-640(*)	< 5	< 5-160
Nasopharyngeal carcinoma	> 640	< 5	80-1280	80-1280(**)	40-160	80-1280

(*) "Reduced" fluorescence appearance (R)

(**)"Diffuse" fluorescence appearance (D)

TREATMENT

CURATIVE

There are no antiviral agents effective against EBV.

Primary infection occurring in an immunocompetent person only requires symptomatic treatment. Short-term corticosteroids may be used for severe symptoms.

In severe EBV infections in immunosuppressed patients, antiviral agents such as aciclovir, ganciclovir, foscarnet and cidofovir have been tried with variable results. In lymphoproliferative syndromes, apart from radiotherapy and chemotherapy, trials with stimulation or restoration of cytotoxic response are ongoing.

PREVENTATIVE

Currently, there is no vaccine against EBV. A vaccine (under investigation) against the GP350 envelope protein induces a humoral response with neutralising antibodies and a cytotoxic cellular response. This type of vaccine may be useful, firstly to prevent infection and secondly to reduce viral spread of reactivation in an infected person. It would therefore be useful in regions where nasopharyngeal carcinoma and Hodgkin's disease are highly endemic. In addition to leukocyte-depleted blood, EBV-seronegative blood donors can be selected for transfusions to high risk people.

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ERYTHROPOIETIN

DEFINITION

Erythropoietin (EPO) is a glycoprotein hormone which stimulates erythropoiesis and is the specific growth factor for the erythroblast cell line. It is produced mostly by renal peritubular cells (90%) and in small amounts by the liver (10%). Endogenous EPO production in the body is low, approximately 2 IU/kg/24 hours. EPO synthesis is regulated by tissue oxygenation and is increased in hypoxia, particularly after spending time at altitude and falls in hyper oxygenation or polycythaemia. There is no erythropoietin reserve in the kidney and its release requires de *novo* synthesis. Erythropoietin is found in the blood and urine. It is removed by the liver and in urine with a half-life of approximately 4 hours. The natural hormone was isolated from urine in 1977 and produced by genetic engineering in 1985. Glycosylation of the recombinant hormone which is essential for its *in-vivo* biological activity was achieved through the production of mammal cells. Recombinant human erythropoietin (rh-EPO) is the treatment of choice for normochromic, normocytic anaemia in chronic renal failure due to defective EPO synthesis. It is also very effective in other forms of anaemia, particularly those associated with cancer, chemotherapy, AIDS, thalassemias and anaemia of the premature infant. Finally, it is an alternative to autologous blood transfusions in some cases of programmed surgery. Although its non-therapeutic use is prohibited, some athletes use it to artificially improve their performance.

INTRODUCTION

EPO acts on bone marrow, erythroid progenitor cells to regulate the production of erythrocytes in mammals. Its target cells are the mature *Burst-forming unit-erythroid* (BFU-E) and the *Colony-forming unit-erythroid* (CFU-E) cells, which are the erythroblast progenitors. In order to exert its action, EPO binds to its specific *"oxygen sensor"* receptor which is present on the cell surface in two forms of different molecular weight and affinity. It has no further effect once the cell has reached the erythroblast stage, as its receptor is no longer expressed. After EPO binds to its receptor, CFU-E and BFU-E stimulate the formation of erythroblast colonies in 7 and 15 days respectively. EPO probably acts on these target cells by stimulating mitosis and terminal differentiation and preventing (or delaying) programmed cell death. In anaemia or tissue hypoxia, increased EPO synthesis "saves" many erythroid progenitors from programmed death, thereby maintaining a sufficient number of circulating erythrocytes. Conversely, in some polycythaemic conditions (repeated transfusions, etc.) reduced circulating EPO concentrations cause an increase in erythroid progenitor cell apoptosis and a reduction in the circulating erythrocyte pool.

When used for doping purposes, raised circulating EPO increases haematocrit and blood oxygen transport capacity which improves endurance sport performance by increasing oxygen supply to muscle tissue. The effects which are obtained are long lasting, for more than 3 months. In addition, doping tests are difficult as blood immunological assays cannot differentiate endogenous EP from rh-EPO. EPO however is not without risk, as increased red blood cells and haematocrit raises the blood viscosity and secondarily increases systemic arterial resistance. The main side effects are an increased incidence of cardiac and thrombo-embolic events, hypertension and epilepsy.

INDICATIONS FOR MEASUREMENT

Aetiological diagnosis of some forms of non-regenerative anaemia, which are liable to be treatable with rh-EPO, or complex polycythaemia.

Screening for EPO doping.

INFORMATION

SAMPLE

Serum sample or heparinised plasma. Always take the sample at the same time, preferably in the morning (circadian cycle). Do not use EDTA anticoagulant and avoid haemolysed or lipaemic samples.

QUESTIONS FOR THE PATIENT

Are you taking any medicines? Treatment with recombinant human EPO increases plasma EPO concentrations. Various hormones can also increase EP synthesis, such as thyroxin, androgens, insulin and prolactin.

Have you spent time at high altitude? Spending time at high altitude increases plasma EPO concentrations.

SAMPLE STORAGE AND TRANSPORT

Sample can be stored and transported for a few days at + 4°C.

ASSAY METHODS

"Usual methods": Immunological (immuno-radiometric, ELISA and chemoluminescence).

Specialist methods to distinguish endogenous EPO from rh-EPO, such as capillary electrophoresis, possibly linked to mass spectrometry, and, *isoelectric focusing*.

NORMAL EXPECTED VALUES

As an indication: 5 to 25 mIU/ml by chemoluminescence.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Circadian cycle is a minimum plasma concentration at 08:00 hours and a maximum at 20:00 hours.

EPO rises slightly during pregnancy from the eighth week to term.

PATHOLOGICAL VARIATIONS

Increased plasma EPO concentrations:

– EPO doping (if the blood measurement is performed within 48 hours after a dose). Blood measurements, however,

cannot distinguish endogenous EPO from rh-EPO. Endogenous EPO is subject to wide inter-individual variation and the half-life of endogenous and exogenous EPO is extremely short. For this reason, testing for rh-EPO use in sportsmen/women currently relies on changes in other blood indices, such as haematocrit or the soluble transferrin receptor and a urine test (immunoblot with *isoelectric focusing* or alternatively using capillary electrophoresis).

– rh-EPO treatment: After IV injection, plasma EPO concentrations rise quickly and very transiently (1/2 life approximately 4 hours). Following subcutaneous injection, plasma EPO concentrations are well below those obtained after IV injection (ratio 1/20). Values increase slightly reaching a peak after 12 to 18 hours. EPO measurement is not used for therapeutic monitoring (rh-EPO has a short half-life, and rapid changes in haemoglobin provide an easier way of monitoring).

– Anaemia with normal renal function: Anaemia with raised plasma EPO excludes renal insufficiency. In this case iron deficient or megaloblastic anaemia, haemolysis (thalassemia, etc.), haemorrhage or ineffective erythropoiesis (bone marrow aplasia, in AIDS, etc.) should be considered.

– Polycythaemia secondary to some malignant tumours, such as renal, liver, ovarian and lung cancers. Raised EPO is not always seen. Very high plasma concentrations, however, are a pointer towards secondary polycythaemia, particularly in renal cancer (over-excretion of EPO). In this context, EPO appears to correlate with the histological grade and may be of prognostic value.

– Polycythaemia secondary to tissue hypoxia, seen with respiratory insufficiency, cardiac disease and chronic smoking.

- **Reduced plasma EPO concentrations:**

– Anaemia with low plasma EPO: Anaemia associated with chronic renal insufficiency or acute tubular necrosis (reduced EPO secondary to loss of EPO-producing cells), pituitary failure, thyroid failure or cirrhosis.

– Head and neck cancers and Hodgkin's disease.

– EPO is reduced or normal in primary polycythaemia. It is inadequately sensitive or specific however to diagnose this disease.

FOR FURTHER INFORMATION

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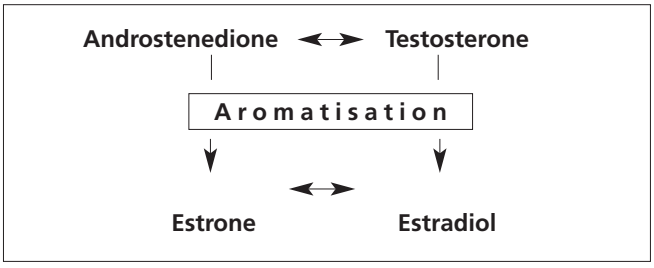
ESTRADIOL

DEFINITION

Estradiol (E2) is the major circulating oestrogen. It is synthesised in the gonads (ovaries and testes) and placenta from testosterone, which is aromatised. E2 is in equilibrium with estrone (E1) which is produced from aromatisation of androstenedione.



In women of childbearing age, E2 comes from a single origin, the ovaries. After the menopause, ovarian secretion falls dramatically and both E2 and E1 are obtained from the peripheral conversion of androgens. This aromatisation takes place not only in the liver but also in adipose tissue, the central nervous system and muscle.

In men, a small proportion of E2 comes from testicular secretion and a large proportion from the peripheral conversion of androstenedione.

Regardless of origin, E2 circulates in plasma mostly bound to carrier proteins: TeBG *(Testosterone – estradiol Binding Globulin)* or SHBG *(Sex Hormone Binding Globulin)* and serum albumin.

Estradiol is either conjugated in the liver into sulphate and glucuronide or converted into estriol (E3) (cf. Estriol). E2 can be inter-converted to estrone (E1) (cf. Estrone) in peripheral tissues, particularly fatty tissue. Estrone can be conjugated with glucuronide or sulphate.

The conjugates of the three oestrogens (E1, E2 and E3) either pass into the systemic circulation and are excreted by the kidney or are excreted in bile and undergo entero-hepatic cycling.

Synonyms: Estradiol = E2 = 17β-estradiol.

INTRODUCTION

Estradiol has four main types of effects:

Effects on the female genital tract: E2 stimulates proliferation and endometrial vascularisation in the uterus. In the cervix, it promotes secretion of clear thready mucus and in the vagina E2 stimulates proliferation of the mucosa from the basal layers and sloughing of the superficial layers.

Effects on development and maintenance of secondary sexual features: Oestrogens are responsible for the development of secondary sexual characteristics at puberty, such as changes in the external genital organs, feminisation of the figure, female appearances of the skin and integument and development of the mammary gland.

Metabolic effects: E2 has indirect effects on bone metabolism through calcitonin and parathyroid hormone and direct effects by acting on osteoblasts.

E2 inhibits total cholesterol and triglycerides and increased the HDL/LDL ratio. In addition to this anti-athrogenic effect, it has a beneficial direct effect on the endothelium, which may explain the relative protection women have against vascular diseases whilst their ovaries are active.

In the liver, E2 stimulates the synthesis of carrier proteins *(SHBG, TBG or Thyroxin Binding Globulin, CBG or Corticosteroid Binding Globulin)*, ceruloplasmin and prothrombin.

Effects on the hypothalamo-pituitary axis:

During the follicular phase, E2 exerts positive feedback control on both the hypothalamus and the pituitary. The ovulatory LH peak is induced by the pre-ovulatory E2 peak. In the luteal phase, E2 inhibits FSH secretion.

INDICATIONS FOR MEASUREMENT

In young girls, E1 and E2 measurement is recommended to identify hyperestrogenaemia, to diagnose precocious puberty and then monitor its treatment with GnRH agonists. On the other hand in adult women serum E2 concentrations are the only good indicator of follicular activity.

Measurement of E2 when monitoring for ovulation can be used to assess the follicular growth and maturation during a spontaneous induced or stimulated cycle. In the latter situations, repeated E2 measurements can be used to monitor the ovarian response in order to adjust treatment and avoid hyperstimulation.

Finally, in menopausal women, E1 and E2 are measured to assess replacement therapy provided that it is E2 and not another oestrogen which is being administered.

E1 and E2 measurements are performed in boys and men to investigate gynaecomastia to reveal oestrogen excess.

INFORMATION

SAMPLE

Estradiol may be assayed both in serum and in plasma (EDTA or heparinised). Haemolysed and lipaemic samples should be discarded.

ESSENTIAL INFORMATION

In addition to clinical information (precocious puberty, menopause, gynaecomastia, etc.), oestrogen treatments must be reported. Similarly, the information must include whether measurement is for an hCG stimulation test in men or boys.

SAMPLE STORAGE AND TRANSPORT

Separated plasma or serum is stable at +4°C for 1 week. They may be stored for 6 months to 1 year if frozen at –30°C.

ASSAY METHODS

E2 is assayed by immunological techniques. The most suitable to date have been methods using a radio-isotopic label. Some of the antisera used are sufficiently specific to assay it directly in the plasma or serum aliquot without prior

extraction. Immunoassays with non-isotopic labels are less sensitive than isotopic methods, although can be used to monitor kinetics and when levels are high, as may be seen in induction of ovulation.

UNITS

Results are expressed in pg/ml or pmol/l. To convert into pmol/l multiply the pg/ml result by 3.468.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Serum oestrogen concentrations (E1 and E2) at birth are high and then fall very rapidly during the first 5 days after birth, reaching very similar values in both sexes. Between 3 and 10 months old, E2 rises in girls and concentrations thereafter fall and remain stable until the start of puberty.

In women of childbearing age, circulating concentrations are extremely low at the start of the menstrual cycle and then rise progressively during the follicular phase. At the end of the follicular phase the rise becomes exponential reaching a pre-ovulatory peak and concentrations then fall markedly before ovulation. They then rise again during the luteal phase. Regardless of phase of the cycle, E2 secretion is pulsatile and serum concentrations are higher in the morning than in the afternoon.

During the follicular phase the E2/E1 ratio is less than 1, although rises to above 1 in the luteal phase.

	FP (1st week)	FP (2nd week)	Peak Pre-ovulation	LP
Estradiol (pg/ml)	20 - 94	57 – 260	100 - 420	60 – 230

FP = follicular phase; LP = luteal phase (D +4 to D +9 after the LH peak)

E2 concentrations rise from the start to the end of pregnancy following a sigmoid curve.

In the perimenopausal period characterised by a progressive increase in FSH, ovarian oestrogen secretion continues, although cycles become increasingly anovulatory. Relative oestrogen excess then develops because of the disappearance of progesterone secretion.

At the menopause, ovarian E2 concentration stops and circulating concentrations from the peripheral conversion of androstenedione into E1 are low and continue to fall with age *(cf. table)*..

	Age (years)		
	< 60	60 – 70	> 70
Estradiol (pg/ml)	< 3 – 50	< 3 – 30	< 3 - 30

In post-menopausal women, E1 and the E1 sulphate represent quantitatively, most important circulating oestrogens. Because of this the E1/E2 ratio is over 1.

In man, E2 concentrations are always lower than E1 (E2/E1 ratio < 1), between 10 and 40 pg/ml before 60 years old and then rise gradually with age.

PATHOLOGICAL VARIATIONS

Serum E2 concentrations are a good index of follicular activity but only E2 measurement is a useful parameter to assess oestrogen impregnation in the women. This is seen particularly during the pre- or peripubertal period, when the menopause begins and with amenorrhoea.

E2 concentrations are low in women with hypogonadism regardless of cause, primary ovarian or secondary due to hypothalamo-pituitary deficiency.

Conversely, E2 is raised in feminising ovarian tumours, such as granulosa tumours, dysgerminomas and gynadroblastomas, choriocarcinomas and Sertoli cell tumours.

Over excretion of oestrogens in young girls results in isosexual precocious puberty with development of secondary sexual characteristics and menstrual periods. Signs of oestrogen excess in women of child-bearing age are metrorragia, mastodynia and pre-menstrual syndromes.

Overproduction of oestrogens with concomitant increases in E1 and E2 in men and boys causes gynaecomastia.

This oestrogen excess may:

– Be due to tumour secretion, with the tumour lying in the testes or an adrenal cortex or be secondary to chorionic gonadotrophin (hCG) secreting choriocarcinoma which stimulates Leydig cells.

Oestrogen excess in men may also result from:

– Defective oestrogen metabolism as is seen in liver disease (particularly cirrhosis) and increased aromatase activity in peripheral tissues.

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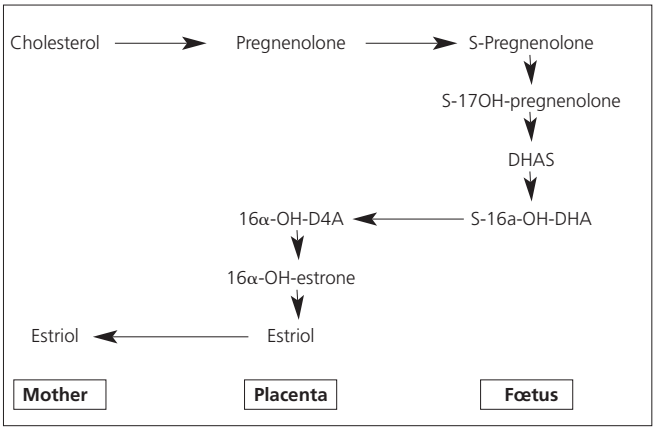
- Carr B.R., *Disorders of the ovaries and female reproductive tract*. In: Wilson J.D., Foster D.W., Kronenberg H.M., Larsen P.R., eds. Williams Textbook of Endocrinology, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, 1998:751-817.

ESTRIOL

DEFINITION

Outside of pregnancy, estriol (E3) is formed in the liver from estrone and estradiol via 16α-hydroxyestrone. In pregnancy, E3 is synthesised in the foeto-placental unit as shown in the diagram below. Starting from cholesterol, the placenta synthesises pregnenolone, which passes to the foetus where it is converted in the adrenal gland into 17-hydroxypregnenolone sulphate and then dehydroepiandrosterone (DHAS).



DHAS is hydroxylated in the foetal liver into 16α and then 16α-hydroxy-DHA (16α-OH-ΔHA) sulphate, and then it passes into the placenta, where it loses its sulphate group following the action of sulfatase and is converted into estriol via 16α-hydroxyandrostenedione (16α-OH-Δ4A) and 16α hydroxyestrone.

It should be noted that the maternal adrenal gland is also able to synthesise DHAS, although 16α-hydroxylation is extremely limited in the mother's body, as the production of S-16α-OH-DHA is extremely low. Given the predominantly foetal origin of its precursors, maternal serum estriol is considered to be a good reflection of the metabolism of the foeto-placental compartment.

Estriol is glucurono- and sulpho-conjugated in the mother's body before being excreted in urine.

Synonyms: Estriol = E3 = 1,3,5 (10) -Estratriene-3β, 16α, 17β-triol.

INDICATIONS FOR MEASUREMENT

Serum or plasma UCE3 (unconjugated Estriol) or TE3 (total estriol) and UE3 (urinary estriol) measurements have no use in the menstrual cycle. They should only be used in pregnancy to assess foetal well-being. Measurements are indicated in three main pathological conditions:

– Pre-eclampsia with hypertension,

– Intra-uterine growth retardation,

– Diabetes.

E3 measurement has nowadays lost much of its use with the development of modern monitoring methods, particularly ultrasound. It is therefore very rarely requested outside of the context of antenatal laboratory screening for foetal trisomy 21 in the second trimester of pregnancy. Maternal UCE3 is one of the markers used to calculate the risk.

INFORMATION

SAMPLE

Unconjugated E3 is measured in serum or plasma. Urinary estriol is measured in an aliquot from a 24 hour urine collection.

ESSENTIAL INFORMATION

As E3 is measured during pregnancy it is essential that the term of the pregnancy is stated. In addition, if the test is to screen for increased risk of trisomy 21, other information is also required, including date of birth and weight of the patient, date of pregnancy (determined by ultrasound), whether one or two foetuses are present and if the mother is a smoker.

SAMPLE STORAGE AND TRANSPORT

Separated serum is stable for a few days at +4°C and can be transported at this temperature to the laboratory. Serum samples can be frozen for 1 year at –20°C. There is also a legal requirement (in France) to store these for 1 year at –20°C if the measurement is requested in the context of screening for increased risk of trisomy 21. Urine samples can be stored at +4°C.

ASSAY METHODS

Unconjugated (UCE3) and total estriol (TE3) representing the sum of UCE3 and estriol conjugates (glucuronide and sulphate), can be measured in serum or plasma. On the other hand, only total estriol is measured in urine.

The methods use the same principle. These are generally immunoassays with an isotopic or non-isotopic label. For TE3 measurement, the conjugates are firstly hydrolysed and the estriol released is measured using the same methods as those for UCE3.

It should be noted that some immunoassays using non-isotopic tracers have been entirely automated. UE3 is always measured by physicochemical techniques which were developed many years ago.

RESULTS

Results during pregnancy are generally expressed in ng/ml. To convert ng/ml to nmol/l multiply by 3.468.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Plasma estriol:

Whereas unconjugated estriol (UCE3) is found in trace amounts during the menstrual cycle, it only becomes measurable in plasma by the 5th week of pregnancy. Subsequently, concentrations rise in a characteristic curve which can be broken down into two parts. The first longer part is up to 34 weeks, and the second shorter, more abrupt part continuing to term. The first represents the growth of the

placenta and foetal adrenal glands and the second, the extensive development of the foetal zone of the adrenal glands.

Plasma unconjugated estriol in pregnancy

Week of pregnancy	UCE3 (ng/ml) (2.5 – 97.5 percentiles)
15	0.5 - 1.7
16	0.8 - 2.0
17	1.1 - 2.8
18	1.4 - 3.7
19	1.4 - 4.3
20	1.5 - 4.5
21	1.6 - 4.8
30	1.8 - 8.0
31	1.9 - 9.0
32	2.0 - 10.0
33	2.1 - 11.0
34	2.3 - 12.0
35	2.4 - 13.0
36	2.6 - 15.0
37	2.9 - 16.0
38	3.2 - 18.0
39	3.6 - 21.0
40	3.9 - 24.0

Total estriol (TE3) follows a similar curve to UCE3 although the levels and ratios are different in pregnancy. At the start of pregnancy, TE3 is approximately 90 times higher than UCE3, this ratio falling to 20 at the end of the pregnancy.

Urinary estriol:

The urinary estriol curve is similar to the curves found for UCE3 and TE3 in plasma.

week of pregnancy	E3U (mg/24 hours)
5 - 8	30 - 140
9 - 12	125 - 770
13 - 15	240 - 3570
16 - 18	900 - 5200
19 - 22	1750 - 8200
23 - 25	2800 - 11000
26 - 28	3500 - 12500
29 - 31	4500 - 17000
32 - 34	6000 - 21000
35 - 36	8400 - 28000
37 - 40	9000 - 31000

PATHOLOGICAL VARIATIONS

Abnormalities of foetal well-being:

Both serum and urine estriol concentrations are reduced in pre-eclampsia with hypertension, intra-uterine growth retardation and diabetes.

In severe eclampsia with renal damage, however, UE3 may be reduced whereas serum TE3 or UCE3 are raised. Abnormalities of the foetal adrenocortical zone which may be seen in anencephalic foetuses are characterised by very low E3 concentrations.

Placental abnormalities:

Serum and urine E3 concentrations are very low in placental sulfatase deficiency, the enzyme which hydrolyses 16α-OH-DHA sulphate releasing 16α-OH-DHA, the precursor of E3. Plasma aromatase deficiency is also accompanied by very low estriol concentrations although androgen concentrations (DHA, androstenedione and testosterone) are very high and result in androgenisation of both the female foetus and the mother.

Similarly, UCE3 is extremely low in the Smith-Lemli-Opitz syndrome, an autosomal recessive disorder, the biochemical marker of which is the inability to convert 7-dehydrocholesterol into cholesterol.

Screening for increased risk of trisomy 21:

This test has found a new use since it was shown that serum UCE3 is low in the second trimester of pregnancy with a trisomy 21 foetus. Median UCE3 values are approximately 0.7 of those of normal age match to pregnancies. The detection rate for trisomies obtained by measurement of UCE3 combined with maternal age is only 40% compared to a detection rate of 70 to 75% when joint measurements of hCG or free beta-hCG and alphafoetoprotein are used. The improvement in successful screening rates obtained by adding UCE3 measurement is controversial. In addition, the most recent recommendations (2009) are to screen for trisomy 21 in the first trimester using ultrasound and first trimester markers (PAPP-A and βHCG).

POUR EN SAVOIR PLUS

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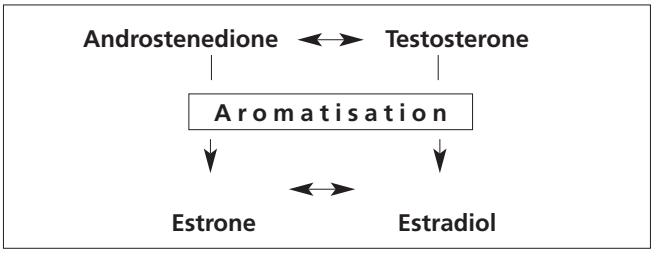
ESTRONE

DEFINITION

Estrone (E1) is the precursor of estradiol (E2) which is the major circulating oestrogen. It is synthesised in the gonads (ovaries and testes) from androstenedione which undergoes aromatisation. During pregnancy, both E1 and estradiol are synthesised in the placenta from dehydroepiandrosterone (DHA), which is formed from foetal and maternal DHA sulphate by the action of the sulphatase. E1 is in equilibrium with E2 which is obtained from aromatisation of testosterone.



E1 has many origins in women of child-bearing age, such as, ovarian and adrenal secretion and peripheral conversion. The ovarian contribution is no more than 70%. A large proportion of E1 comes from the conversion of androstenedione not only in the liver but also in adipose tissue, the central nervous system and muscle.

During pregnancy, both estrone and estradiol are synthesised in the placenta from dehydroepiandrosterone (DHA).

After the menopause, ovarian secretion falls markedly and E1 is obtained mostly from the peripheral conversion of androgens, becoming the major circulating oestrogen.

In men, a small proportion of E1 comes from testicular secretion and a large proportion from peripheral conversion of androstenedione.

Regardless of its origin, E1 circulates in plasma, bound mostly to serum albumin (approximately 80%) and to a small extent (7 – 16%) to TeBG *(Testosterone estradiol Binding Globulin)* or SHBG *(Sex Hormone Binding Globulin)* or SBP *(Sex Steroid Binding Globulin)* as the molecule does not contain a 17β hydroxyl group which is an essential condition for its binding to SBP.

Estrone is either conjugated in the liver into sulphate and glucuronide or converted into estriol (E3) (cf. Estriol). Estrone can be inter-converted to estradiol (E2) in peripheral, particularly adipose, tissue (cf. Estradiol). Estradiol can then be conjugated into glucuronide or sulphate.

The estrone sulphate formed is the quantitatively most important plasma oestrogen and forms a type of reservoir as it can be converted into estrone at any time by the action of sulphatases.

Conjugates of the three oestrogens (E1, E2 and E3) have two destinations. They can pass into the general systemic circulation and be excreted by the kidney or are excreted in bile and undergo entero-hepatic cycling.

Synonym: Estrone = E1.

INDICATIONS FOR MEASUREMENT

E1 and E2 measurements are recommended in young girls to identify hyperoestrogenaemia and diagnose precocious puberty and then monitor treatment with GnRH agonists. On the other hand, in adult women serum E2 and not E1 concentrations are a good indicator of follicular activity. E1 measurement therefore is of limited use in women of child-bearing age.

In post-menopausal women, E1 and E2 are measured to assess replacement therapy. E1 concentrations can be high after oral administration.

E1 and E2 measurements in boys and men are used to investigate gynaecomastia to identify hyperoestrogenaemia.

INFORMATION

SAMPLE

Estrone can be measured both in serum and in EDTA or heparinised plasma. Haemolysed or lipaemic samples do not pose any problem to analytical quality.

ESSENTIAL INFORMATION

In addition to clinical information (precocious puberty, menopause, gynaecomastia, etc.), oestrogen treatment must be reported. Similarly, information must be provided about whether measurement is part of an hCG stimulation test in men or boys.

SAMPLE STORAGE AND TRANSPORT

Separated plasma or serum is stable for one week when stored at +4°C. Please ensure that if the sample is being referred to another laboratory that the transit time does not exceed one week. Otherwise, the samples should be frozen at –20°C. Samples can be stored for 6 months to 1 year if frozen at –20°C.

ASSAY METHODS

In view of the low circulating concentrations, only immunoassay can be used and until now the most suitable methods use a radio-isotopic label. The antisera, however, are not sufficiently specific for direct assay on the plasma or serum aliquot without prior extraction. Extraction and chromatographic purification are still needed in order to obtain reliable results.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Serum oestrogen (E1 and E2) concentrations are high at birth and then fall very quickly during the first 5 days of life to very low levels, which are similar in both sexes. E2 rises in girls at between 3 to 10 months old, after which concentrations fall and remain low until the start of puberty.

Circulating concentrations are extremely low at the start of the cycle in women of child-bearing age and then rise gradually during the follicular phase. The rise becomes exponential at the end of the follicular phase reaching a pre-ovulatory peak following which concentrations fall markedly before ovulation. They then rise again during the luteal phase. The E1/E2 ratio is over 1 in the follicular phase but falls to less than 1 in the luteal phase.

	FP (1st week)	FP (2nd week)	Pre-ovulatory peak	LP
Estrone (pg/ml)	0 - 86	29 - 120	40 - 270	40 – 160

FP = follicular phase; LP = luteal phase (D+4 to D+9 after the LH peak)

E1 concentrations increase from the start to the end of pregnancy following a similar curve to that of E2.

Concentrations fall markedly at the menopause although E1 concentrations remain higher than E2 such that the E1/E2 is greater than 1.

	Age (years)		
	< 60	60 – 70	> 70
Estrone (pg/ml)	10 – 57	10 – 60	10 - 55

■ PATHOLOGICAL VARIATIONS

E1 and even E2 concentrations rise in young girls in premature puberty providing the diagnosis.

Estrone is raised in women with the polycystic ovarian syndrome and in obesity due to an increase in peripheral androgen aromatisation. Similarly, it is increased in women with ovarian or adrenal tumours which can secrete either oestrogens or androgens which are then aromatised in peripheral tissue.

Excess oestrogen production in boys and men with concomitant rises in E1 and E2 results in gynaecomastia. The hyperoestrogenaemia may be:

– Due to tumour secretion, if the tumour is located in the testes or adrenal cortex.

– Be secondary to choriocarcinoma secreting chorionic gonadotrophin (hCG), which stimulates the Leydig cells.

Hyperoestrogenaemia may result in:

– Disordered oestrogen metabolism, as seen in liver disease (particularly cirrhosis).

– Increased aromatase activity in peripheral tissues.

POUR EN SAVOIR PLUS

■ Nahoul K., Roger M., *Dosages hormonaux*. Dans: Papiernik E, Rozenbaum H, Bellaisch-Allart J. Paris, Medecine-Sciences Flammarion, 1990: 201-21.

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ETHOSUXIMIDE

DEFINITION

Ethosuximide is a first generation anticonvulsant. It is marketed in the form of 250 mg capsules and syrup (250 mg/5 ml) and is indicated for use in the treatment of generalised epilepsy: absences, myoclonic and atonic seizures, in children and adults as monotherapy or in association with another anticonvulsant.

Ethosuximide is a specific anti-epileptic agent for absences. It acts by abolishing the paroxysmal 3 cycle per second spike wave activity associated with loss of consciousness, which is characteristic of absences seizures.

The effective dosage in adults is around 20 mg/kg/d or 6 capsules per day (1.5 g). The maximum dose is 2 g/day.

PHARMACOKINETICS

Oral absorption	Rapid and almost complete
Plasma peak (Tmax)	3 to 7 hours
Steady state	7 days
Metabolism	Extensive, mostly by oxidation into at least 3 inactive metabolites, the main one of which is 2-(1-hydroxyethyl)-2-methyl suximide.
1/2 life of elimination	Approximately 60 hours in adults; 30 hours in children.
Elimination	Mostly renal in the unchanged form (20%) and as free and conjugated metabolites.

INDICATIONS FOR MEASUREMENT

Ethosuximide measurements are justified, as a relationship exists between plasma anticonvulsant concentrations and reduced incidence of epilepsy attacks. Measurements are indicated if treatment is ineffective (poorly controlled seizures) or if signs of toxicity develop. They can also be used for monitoring purposes every 6 months.

INFORMATION

■ SAMPLE

Serum or plasma sample taken into EDTA or heparin. Avoid tubes with separator gel. Take the sample immediately before the next dose (trough concentration) at steady state (after treatment for 1 week).

■ QUESTIONS FOR THE PATIENT

Are you taking carbamazepine? Concomitant use of carbamazepine causes a fall in serum/plasma ethosuximide concentrations.

■ SAMPLE STORAGE AND TRANSPORT

Store plasma/serum at +4°C.

ASSAY METHODS

Immunochemical methods: EMIT, FPIA (the most widely used methods); liquid or gas phase chromatography.

NORMAL EXPECTED VALUES

Steady state therapeutic range: 40 to 100 µg/ml (285 to 710 µmol/l). Measurement is more useful to adjust doses in order to achieve effective concentrations than to prevent toxicity (which is mild with this compound). Signs of overdose are nausea, vomiting, diarrhoea, anorexia, drowsiness, dizziness, poor coordination, irritability, headaches, confusion and psychological or psychiatric disorders. There is no specific antidote and treatment is gastric lavage combined with symptomatic adjuvant treatment taking account of the long half-life of ethosuximide.

FOR FURTHER INFORMATION

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ETHYL ALCOHOL

DEFINITION

Ethyl alcohol is a psychoactive substance of which the effects may vary according to the individual involved, the dose ingested and the circumstances of consumption. It is one of the products most often involved in acute and chronic intoxication in France. Its effects can be sedative or psycho stimulating or cause anxiety, euphoria or depression. In certain cases (complicated acute intoxication or atypical inebriation), it can lead an individual to commit offences or, exceptionally, to attempt suicide. Alcohol is also used in human therapy as an excipient in numerous preparations, and as a disinfectant.

Synonyms: ethanol (ethanolaemia), alcohol (alcoholaemia).

INTRODUCTION

After being ingested, ethanol is absorbed in the stomach (20%) and small intestine (80%) in 2 to 6 hours in the best case if no food is consumed. It diffuses rapidly to all parts of the body, crossing in particular the haematomeningeal and placental barriers. It is almost completely metabolised in the liver (less than 10% being eliminated unchanged in urine or through the lungs) in a manner which is independent of concentration (0.3 g/l for ethanolaemias). In adults, the rate of methanol metabolism is 7 to 10 g per hour, which equates to an average drop in plasma concentrations of 0.15 to 0.20 g/hour, although this speed of metabolism can be doubled in chronic consumers. The half-life thus varies from 2 to 14 hours, depending on individual and their usual mode of consuming alcoholic drinks.

Alcohol consumption engenders three types of risk. In acute terms, toxicity manifests itself by a change in the equilibrium between excitatory and inhibitory systems in the central nervous system. Chronic intoxication has damaging effects on all organs, while the third risk is that of the development of dependency, seen in some 5% of alcohol consumers.

In clinical terms, acute intoxication is classically revealed by an initial phase of psychomotor excitation (inebriation), usually occurring at ethyl alcohol concentrations in the plasma between 0.5 and 2 g/l. The second phase is one of drunkenness, characterised by difficulties in motor coordination and in a subject who is not a chronic alcoholic, this phase is observed at plasma ethanol concentrations between 1.5 and 4 g/l. Beyond 3.50 g/l, coma may occur, which is usually calm and hypotonic with hypotension, hypothermia and respiratory depression. Death due to respiratory arrest is possible at ethanol levels of 5 g/l or higher (tolerance is however highly variable from one individual to another). There is no specific treatment for acute intoxication. Simple observation of the patient is usually sufficient, but symptomatic treatment may be indicated, including the correction of associated metabolic problems, including hypoglycaemia (particularly in children), and the prevention of hypothermia and circulatory disorders.

From the pathophysiological viewpoint, the acute toxicity of ethanol is believed to be due to changes in the neurotransmission systems. The most probable theory for subjects who are not chronically alcoholic is an inhibition of the operation of NMDA (N-methyl-D-aspartic acid) receptors and a stimulation of GABA (gamma-aminobutyric acid) receptors, which cause the anxiolytic and sedative effects observed in the majority of subjects. This imbalance is thought to be accentuated in chronic excessive drinkers, leading, if consumption is stopped suddenly, to excitotoxicity.

INDICATIONS FOR MEASUREMENT

Search and confirmation of acute alcoholic poisoning, either in a medical context (agitation or coma), or in connection with criminality such as drunkenness in public places, drunk driving, criminal offences, etc.

Monitoring of acute, alcoholic intoxication, whereby a series of measurements allows the alcohol metabolism to be assessed.

INFORMATION

SAMPLE

In clinical toxicology: Serum or plasma collected in EDTA or heparin; the sampling location must not be disinfected with alcohol or ether; use a pad impregnated with a quaternary ammonium compound.

Measurement of ethyl alcohol in the blood is a clinical emergency. The shorter the lapse of time between intoxication and sample collection, the better the evaluation of alcohol intoxication.

QUESTIONS FOR THE PATIENT

Circumstances of the analysis request: Criminology, drunk driving, etc.

Try to detail the circumstances of intoxication: Quantity of drinks consumed, time lapse since ingestion, concomitant consumption of food or medicines (alcohol resorption slows down during a meal; alcohol potentialises the action of many medicines, particularly in the case of psychoactive substances, of which the consumption should be determined).

SAMPLE STORAGE AND TRANSPORT

Storage at +4° C for as short a time as possible or several months at -20° C.

Transport: If the analysis is being referred then decant serum or plasma immediately (< 30 minutes) and freeze to -20° C.

In a context of criminology: Plasma collected on sodium fluoride can be conserved for a few hours between 0 and +4° C before analysis, but the longer the delay, the less the analysis is justified. In practice, this means that the measurement is urgent.

ASSAY METHODS

Enzymatic methods (with alcohol dehydrogenase): Routinely used in clinical toxicology.

Gas phase chromatography: The reference method.

NORMAL EXPECTED VALUES

Alcohol levels are nil in abstinent subjects. In France, the criminal threshold for drivers is 0.5 g/l in the blood and 0.25 mg/l in exhaled air.

INTERPRETATION

■ **Measurement of ethyl alcohol in the blood diagnoses or confirms acute intoxication.**

The symptoms are theoretically dependent on the concentration measured in plasma. It is important, however, to underline the extreme variability of the individual response to alcohol ingestion. In addition, enzymatic methods of measurement may produce false positives which are not possible to predict.

In broad outline (according to M. Deveaux & T. Danel):

– Alcoholaemia < 0.3 g/l: no obvious clinical symptoms

– 0.3 to 1 g/l: Exhilaration, slight euphoria

– 0.9 to 2 g/l: Inebriation, excitement; driving is dangerous

– 1.5 to 3.0 g/l: Clinical drunkenness, confusion

– 2.5 to 4 g/l: Severe to very severe, stupor

– 3.5 to 5 g/l: Coma, possible death of individuals who are old or in a poor general condition

– ≥ 5 g/l: Possible death (respiratory arrest) in the absence of treatment

With regard to the ability to drive a motor vehicle, it is accepted that the accident risk is doubled if alcoholaemia is > 0.5 g/l; it is multiplied by 5 at 0.7 g/l and by 10 beyond 0.8 g/l.

Reminder: The alcohol level in a drink indicates the volume of pure alcohol contained in 100 volumes of the drink. For example, one litre of wine at 12° contains 12% of alcohol i.e. 120 ml. Knowing the density of alcohol (0.8), the quantity of ethanol contained in 1 litre of wine is 120 x 0.8 = 96 g and, considering glasses of standard size, each glass carries approximately 12 g of ethanol.

In adults, the lethal dose of pure ethanol is 300 to 400 ml (i.e. 240 to 320 g) ingested in less than one hour.

■ **Monitoring acute intoxication:** Repetitive measurements of ethyl alcohol in the blood can avoid the onset of withdrawal symptoms, which can be lethal. The rate of decrease of alcoholaemia is restated above: it can vary significantly from one individual to another and according to the circumstances of consumption.

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ETHYLENE GLYCOL

DEFINITION

Ethylene glycol (1,2-ethanediol) is a diol derived from the saturated aliphatic hydrocarbon family, which is very widely used in industry (antifreeze, explosives, plastics, synthetic agent, etc.).

It takes the form of a colourless, odourless, syrupy liquid which is extremely hygroscopic, very soluble in water, insoluble in oils and stable up to 500/600°C.

Ethylene glycol is easily absorbed through the respiratory and gastro-intestinal tracts and by the skin. Initially it is almost non-toxic and is a mucosal membrane irritant. Its metabolites (glycolic and oxalic acids) are worrying particularly because of their effects on the CNS, kidney and myocardium.

– T1/2 ethylene glycol (blood) approximately 3-5 h in adults and 2.5 h in children.

– Renal clearance = 20 ml/min.

INTRODUCTION

After absorption (which is rapid and complete), approximately 25% of the ethylene glycol absorbed is excreted unchanged by the kidney. The remainder is oxidised into glycolaldehyde and then into glycolic acid (40%) by hepatic dehydrogenases. A small proportion of the glycolic acid is then metabolised in particular into glyoxylic and oxalic acids (3-4%). All of these detoxification products are removed mostly in urine as salts.

Acute poisoning is accompanied rapidly (after a few hours) by CNS depression (loss of consciousness, narcosis, confusion and coma) and more or less severe gastro-intestinal signs (nausea, vomiting and abdominal pain). The metabolic disorders are obvious and characteristic (metabolic acidosis, raised anion gap, hyperglycaemia, leukocytosis and occasionally hypocalcaemia). Myocardial damage is a constant finding after 24 hrs (hypotension, dyspnoea, heart failure, pulmonary oedema, etc.). Renal failure occurs after 1 to 3 days as a result of oxalic acid precipitation (causing proteinuria, haematuria, acute oliguric or anuric tubulopathy and necrosis, etc.).

Lethal oral doses: adults = 1.4 ml/kg
children = 1 ml/kg.

Chronic poisoning is limited because of the irritant properties of the substance and causes mostly central signs (headaches, dizziness and nausea). It may be accompanied by skin and haematological signs (hyperlymphocytosis).

INDICATIONS FOR MEASUREMENT

Monitoring exposed people.

Follow up of acute and chronic poisoning.

INFORMATION

SAMPLE

Measurements may be performed on serum, plasma or urine (and gastric lavage fluid).

They are generally measured on serum. For plasma, the blood is drawn by venepuncture into a heparinised tube with no other additive.

Urine: First morning sample or 24 hr urine without additive (20 ml).

In occupational medicine samples are taken depending on purpose of the measurement either at the start and/or end of the day or at the end of the week (after exposure).

QUESTIONS FOR THE PATIENT

Suspected diseases, clinical information (renal failure) and current treatment?

Environmental information?

Dietary habits (alcohol consumption)?

SAMPLE STORAGE AND TRANSPORT

Serum and plasma after centrifugation and separation, must be stored at +4°C until assay which should be performed without delay. For later analysis, freeze and store at -20°C.

Urine: First morning or 24 hr urine. Store as for serum after centrifugation.

IMPORTANT NOTES

Because of the specific properties of the substances tested:

– Assay samples without delay after sampling.

– Collect biological samples into hermetically sealed tubes or bottles.

– Fill the tubes full and close tightly to limit volatile losses.

ASSAY METHODS

Enzymatic methods: use glycerol dehydrogenase.

Non-specific assay, cross-reaction with other aliphatic alcohols (2-3 butanediol, propylene glycol and endogenous glycerol). Glycerol interference can be removed by sample pre-treatment with glycerokinase.

Most assay methods use prior serum or plasma deproteinisation to remove interference from lactic acid.

Chromatographic methods:

– High performance liquid chromatography with light diffusion detector.

– Gas phase chromatography with flame ionisation detector, whether or not coupled with mass spectrometry, is currently the method of choice.

NORMAL EXPECTED VALUES

Unexposed people:

Serum, plasma = trace.

Urine = less than 1 µg/l.

PATHOLOGICAL VALUES

Values of 0.25 mmol/l or more indicates poisoning. Haemodialysis is indicated above 8 mmol/l.

Comments

Blood ethylene glycol measurements are used particularly in acute poisoning. End of shift and/or end of week urine measurements reflect the person's overall exposure.

Ethanol (the classical antidote for poisoning, alongside 4-methylpyrazole) delays the oxidation of ethylene glycol (competitive substrate for alcohol dehydrogenase), prolonging its plasma half-life (T1/2 = 11 to 18 h) and promotes its excretion in the unchanged form.

Results are interpreted in the context of laboratory results (including blood gasses), treatment given and the time between sampling and patient management.

FOR FURTHER INFORMATION

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- www.inrs.fr.

EVEROLIMUS

DEFINITION

Everolimus is an immunosuppressant belonging to the mTOR protein *(mammalian target of rapamycin)* inhibitor pharmacotherapeutic class – the other member of which is sirolimus. Everolimus acts by binding to the intracellular FKBP-12 protein, forming a complex which inhibits mTOR activity. mTOR inhibition blocks several specific signal transduction pathways leading to inhibition of lymphocyte activation and causing immunosuppression. Everolimus obtained marketing authorisation (MA) in France in 2004 for the indication of, prevention of organ rejection in adult patients at mild to moderate immunological risk, who have received a renal or cardiac allotransplant. Everolimus must be used in conjunction with cyclosporin (as a microemulsion) and corticosteroids. The French National Authority for Health produced recommendations in February 2006 on the measurement of blood everolimus in this situation.

In addition, mTOR is a serine threonine kinase, the activity of which is known to be unregulated in many human cancers. Through this pathway, everolimus reduces vascular endothelial growth factor (VEGF) concentrations involved in the tumour angiogenesis process: it inhibits growth and proliferation of tumour cells, endothelial cells, fibroblasts and vascular smooth muscle cells and it reduces tumour cell glycolysis both *in vivo* and in vitro. It obtained MA in France in August, 2009 for this action in the treatment of advanced renal cancer in patients who had progressed on or after targeted anti-VEGF therapy. There are no recommendations for laboratory monitoring in this indication.

PHARMACOKINETICS

Peak concentration	1 to 2 h after administration of 5 to 10 mg, fasting (or with a light fat-free meal)
Half-life of elimination	28 h ± 7 h
Protein binding	Approximately 74% in healthy people
Metabolism	By CYP 3A4 and PgP. After oral administration, everolimus is the main compound circulating in the blood and therefore responsible for the majority of its overall pharmacological activity; 6 principal metabolites have also been identified in human blood.
Elimination	80% in faeces; approximately 5% in urine.

INDICATION

Measurement of blood everolimus is indicated in the therapeutic monitoring of cardiac and renal transplant patients treated with everolimus. The MA stipulates:

"Regular monitoring of whole blood everolimus concentrations is recommended. A relationship has been established between the mean trough everolimus concentration during the first 6 months after transplantation and the incidence of acute biopsy-confirmed rejection and thrombocytopaenia in renal and cardiac transplant patients. Analysis of the efficacy-exposure and tolerability-exposure relationships, has demonstrated, in renal and cardiac transplantation that the incidence of acute rejection confirmed on biopsy is lower in patients who achieve trough whole blood everolimus concentrations of 3 ng/ml or more, compared to patients whose trough concentrations are under 3 ng/ml. The upper limit of the recommended therapeutic range is 8 ng/ml. It is particularly important to monitor blood everolimus concentrations in patients with hepatic insufficiency, when potent inducers or inhibitors of CYP3A4 are administered concomitantly, when a pharmaceutical form is changed and with cyclosporin and/or when doses of cyclosporin are greatly reduced. Optimally, everolimus doses should be adjusted accordingly on trough concentrations, in samples taken more than 4-5 days after a previous change in dose".

ASSAY METHODS

Liquid phase chromatography coupled to tandem mass spectrometry (LC/MS-MS). This is the reference method.

INFORMATION

SAMPLE

– Whole EDTA blood (minimum 2 ml). Heparinised plasma must not be used.

– Sample at steady state after treatment for approximately 2 weeks, measuring the trough everolimus concentration (C0) immediately before the next dose. Wait for at least 4 to 5 days before sampling for measurement after changing dose.

ESSENTIAL INFORMATION

Any request for drug measurements must contain the reasons for the request (efficacy or toxicity), the sampling time, date when the treatment was started and/or any change in dosage, dosage information (amount administered, frequency and administration route), and the age, height and weight of the person whenever possible.

– State indication.

– Concomitant treatments at risk of therapeutic interactions with everolimus. The absorption and removal of everolimus can be influenced by drugs which act on CYP3A4 and PgP.

CYP3A4 and PgP inhibitors can increase blood everolimus concentrations by reducing the metabolism or efflux of everolimus from intestinal cells. In particular, these are potent inhibitors, such as ketoconazole, itraconazole, posaconazole, voriconazole, erythromycin, telithromycin and clarithromycin, nefazodone, ritonavir, atazanavir, nelfinavir, indinavir, saquinavir, amprenavir, lopinavir and grapefruit juice.

CYP3A4 and PgP inducers can reduce blood everolimus concentrations by increasing its metabolism or efflux from intestinal cells. In particular these are potent inducers, such as carbamazepine, phenobarbital, phenytoin, primidone, rifampicin, rifabutin, efavirenz, nevirapine and St. John's Wort.

NB: Cyclosporin and everolimus interact, and as a result everolimus concentrations may fall when cyclosporin exposure is greatly reduced (trough concentration < 50 ng/ml).

SAMPLE STORAGE AND TRANSPORT

Freeze within 4 hours of sampling. Transport frozen at – 20°C.

EXPECTED VALUES - INTERPRETATION

Results must be interpreted taking account of the clinical situation or therapeutic co-prescriptions which alter blood everolimus concentrations.

The trough everolimus concentration during maintenance treatment to prevent cardiac or renal transplant rejection must lie between 3 and 8 ng/ml, when it is used in association with low dose cyclosporin.

FOR FURTHER INFORMATION

- Dictionnaire Vidal®.
- http://www.has-sante.fr.

FACTOR II

DEFINITION

Factor II is a glycoprotein produced by the liver and is a serine protease zymogen. It is a vitamin K-dependant coagulation factor belonging to the factors making up the prothrombin complex: II, V, VII, X. It has a plasma half-life of 50 to 120 hours and a minimum level of 20 to 30% is sufficient to provide normal haemostasis.

Synonym: Prothrombin.

PHYSIOLOGICAL ROLE

During the coagulation cascade prothrombin is activated by the prothrombinase complex (FXa, FVa in the presence of phospholipids and calcium) into thrombin, which plays a key role in the coagulation process. It converts fibrinogen into fibrin, amplifies its own formation and activates the protein C, TAFi systems and platelets.

INDICATIONS FOR MEASUREMENT

It is a second line measurement for the diagnosis of constitutional or acquired factor II deficiency suggested by prolongation of the Quick time and/or activated partial thromboplastin time.

INFORMATION

SAMPLE

Samples should be collected into citrate, concentration 3.2% (0.109 M), 1/10 (0.5 ml per 4.5 ml of blood). 3.8% (0.129 M) are acceptable. CTAD tubes (citrate, theophylline, adenine, dipyridamole) are recommended in patients receiving heparin. No other anticoagulant may be used.

The sample should preferably be taken between 7:00 and 11:00 am with the patient resting, seated, for at least 5 minutes. A light low fat snack is permitted but caffeine, tobacco and physical activity must be avoided during the hour before the sample is taken. Samples must be centrifuged promptly after being taken and the test performed within 2 to 4 hours of sampling. Check that no micro-clots are present and discard haemolysed or lipaemic samples.

For more information refer to the "General pre-analytical conditions in haemostasis" section.

QUESTIONS FOR THE PATIENT

Clinical context?

Results of standard haemostasis tests?

Current treatment with anti-vitamin K agents?

SAMPLE STORAGE AND TRANSPORT

Can be stored for 2 to 4 h, at laboratory temperature (never store the sample at + 4°C); for 2 weeks at – 20°C and for 6 months at – 70°C.

It is recommended that samples be thawed promptly in a water bath at 37°C and mixed thoroughly before the test.

Transport at – 20°C, if the sample is to be transported, plasma must be frozen at – 20°C within 2 hours of sampling.

ASSAY METHODS

Routine method: functional chronometric method (measurement of activity): measurement of the Quick time in an equal volume of patient plasma diluted 1/10 and factor II-deficient reagent plasma. The time is converted into percentage activity with reference to a calibration line from control plasma with 100% activity.

Immunological antigen assay to distinguish quantitative from qualitative deficiency (specialist laboratory use).

Molecular biology gene study (chromosome 11): testing for the mutation responsible for constitutional factor II deficiency.

NORMAL EXPECTED VALUES

Values are usually expressed as a percentage of normal or in IU/ml, 1 IU/ml = 100%.

Normal values in children from 1 year old and in adults: 70 to 140% (0.70 to 1.40 IU/ml).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

– Activity is reduced in the newborn babies: normal values between 25 and 70%

– Activity is occasionally increased in pregnancy: the values found being between 70 and 200%.

PATHOLOGICAL VARIATIONS

Constitutional factor II deficiency:

Constitutional factor II deficiency, which is autosomal recessive in transmission, is extremely rare. This may be quantitative (antigen and activity reduced) or more often, qualitative (antigen level normal, activity reduced). Seven mutations have been found to be responsible for this defect in the factor II gene. The gene is located on chromosome 11.

Clinically, homozygous or composite heterozygous deficiency is characterised by moderate bleeding (post-traumatic, bruising, epistaxis). Heterozygotes are usually asymptomatic.

The diagnosis of constitutional deficiency is only made after checking the deficiency on a second sample taken later and after excluding causes of acquired deficiency, which is far more common. Family studies are required.

- **Acquired factor II deficiency (commonest) may be seen in:**
  - – Treatment with anti-vitamin K agents
  - – Deficient vitamin K intake
  - – Malabsorption (abnormal vitamin K absorption), sprue
  - – Hepatocellular insufficiency
  - – Fibrinolysis, disseminated intravascular coagulation (DIC)
  - – In the presence of anti-factor II antibodies or in the anti-phospholipid syndrome in adults (particularly with systemic lupus erythematosus) or associated with childhood viral infection.

  The aetiological diagnosis is based on further measurements of vitamin K dependent factors (VII, IX, X) and factor V.

  - – If all of these are normal, the factor II deficiency is isolated (congenital deficiency or anti-factor II auto-antibodies).
  - – If factors VII, IX and X are reduced and factor V is normal: treatment with anti-vitamin K, hypovitaminosis K (deficient intake or malabsorption) or moderate hepatocellular insufficiency.
  - – If factors VII, IX, X and V are all reduced: the cause may be severe hepatocellular insufficiency, defibrination syndrome or DIC.

FOR FURTHER INFORMATION

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FACTOR IX

DEFINITION

Factor IX is a glycoprotein produced by the liver and is a serine protease zymogen. It is a vitamin K-dependent co-factor with a plasma half-life of 20 to 24 hours. Minimum levels required for normal haemostasis are 30% to 40%. Hereditary factor IX deficiency causes haemophilia B.

Synonym: anti-haemophilia B factor.

INTRODUCTION

Factor IX can be activated into activated factor IX (IXa) by factor XIa or by factor VIIa in the presence of phospholipids and calcium. FIX activation occurs in two stages: the initial formation of inactive FIXα, then conversion into FIXa by the action of FVIIa – tissue factor complex.

In the presence of FVIIIa, phospholipids and calcium, FIXa is the intrinsic activator of FX or *intrinsic tenase*, which amplifies the activation of FX into FXa. This pathway is essential for the activation of factor X.

Factor IXa activity is inhibited by antithrombine.

INDICATION FOR MEASUREMENT

This is a specific measurement used to diagnose factor IX deficiency (constitutional deficiency: haemophilia B or acquired deficiency) suggested by isolated prolongation of the activated partial thromboplastin time and/or from bleeding. It is also used to monitor haemophiliac patients treated with factor IX replacement.

INFORMATION

SAMPLE

Samples should be collected into citrate, concentration 3.2% (0.109 M), 1/10 (0.5 ml per 4.5 ml of blood). 3.8% (0.129 M) are acceptable. CTAD tubes (citrate, theophylline, adenine, dipyridamole) are recommended in patients receiving heparin. No other anticoagulant may be used.

The sample should preferably be taken between 7:00 and 11:00 am with the patient resting, seated, for at least 5 minutes. A light low fat snack is permitted but caffeine, tobacco and physical activity must be avoided during the hour before the sample is taken. The samples must be centrifuged promptly after the sample is taken and the test performed within 2 to 4 hours of sampling (if not freeze the plasma at –20° C. Check that no micro-clots are present and discard haemolysed or lipaemic samples.

For more information refer to the "General pre-analytical conditions in haemostasis" section.

QUESTIONS FOR THE PATIENT

Clinical context, particularly personal or family history of bleeding? Bleeding after tooth extraction?

Results of standard haemostasis tests?

Are you taking anticoagulant therapy (antivitamin K agents, dabigatran, rivaroxaban)? These treatments reduce factor IX levels

SAMPLE STORAGE AND TRANSPORT

Can be stored for 2 to 4 h, at laboratory temperature (never store the sample at + 4°C); for 2 weeks at – 20°C and for 6 months at – 70°C.

It is recommended that samples be thawed promptly in a water bath at 37°C.

Transport at – 20°C, if the sample is to be transported, plasma must be frozen at – 20°C within 2 hours of sampling.

ASSAY METHODS

– Routine method: functional chronometric method (measurement of activity): measurement of the Quick time in an equal volume of patient plasma diluted 1/10 and factor-IX deficient reagent plasma. The time is converted into percentage activity with reference to a calibration line from "control plasma" with 100% activity.

NB: circulating anticoagulant can interfere with the assay. In this situation factor IX should be assayed using higher dilutions of the test plasma (1/20, 1/40 and 1/80); this test is used to distinguish circulating lupus anticoagulant from anti-factor IX antibody. If the deficiency is corrected at higher dilutions a lupus circulating anticoagulant is present, the inhibitory effect of which reduces with increasing plasma dilutions. In extremely rare situations a very potent lupus anticoagulant can inhibit the coagulant activity of factor IX contained in the reagent plasma and invalidate the results.

– Immunological protein assay to distinguish quantitative from qualitative deficiency (specialist laboratory use).

– Molecular biology gene study (X chromosome): testing for the mutation responsible for constitutional factor II deficiency) (specialist laboratory use).

NORMAL EXPECTED VALUES

Results are usually expressed as a percentage of normal or in kIU/l, 1 kIU/l = 100%.

Reference values in children from 1 year old and in adults: 0.70 to 1.20 kIU/l (70 to 120%).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Newborn babies: reference values are between 20 and 80%.

Pregnancy: unchanged activity or reduced by 10 to 30%.

PATHOLOGICAL VARIATIONS

Constitutional factor IX deficiency: Haemophilia B

Constitutional factor IX deficiency or haemophilia B is an inherited coagulation disorder, which is sex-linked recessive in transmission affecting 1/30000 new male births. It is characterised by mostly muscle and joint (haemarthroses), bleeding, bleeding from skin wounds, and more rarely with mucosal or intracranial haemorrhage.

A range of genetic disorders is involved: deletions, point mutations and an abnormality in the haemophilia B Leiden promoter region (haemophilia which corrects at puberty).

Laboratory deficiency varies, and defines several forms of haemophilia:

– Severe haemophilia B: factor IX level < 1%

– Moderate haemophilia B: 1% ≤factor IX < 5%

– Minor haemophilia B: 5% ≤factor IX < 25%. Between 25 and 50%, we refer to "borderline" levels. The risk of bleeding increases when factor IX levels are < 25%.

Some patients with haemophilia B treated with factor IX concentrates develop anti-factor IX Ab, which are titred in Bethesda units (*cf* factor VIII section).

The severity of bleeding is related to the circulating factor IX level.

– Severe haemophilia: first accidents when the child begins to walk: multiple bruising, frontal and buttock haematomas or haematomas after vaccination, bleeding after tongue bites; frequent, recurrent, spontaneous or provoked accidents.

– Moderate haemophilia: bleeding begins later, is less common and is often post-traumatic.

– Minor haemophilia: accidents are rare and only provoked.

The diagnosis of constitutional deficiency is only made after confirming the deficiency on a second sample taken some time after the first and after excluding causes of acquired deficiency. Family studies are required.

Note: in very occasional cases in which a factor IX gene propeptide mutation is present, profound factor IX deficiency occurs only when the patient takes an anti-vitamin K. This mutation does not change the INR but may cause severe bleeding due to the profound factor IX deficiency.

- **Acquired factor IX deficiencies**

  – Treatment with anti-vitamin K agents,

  – deficient vitamin K intake,

  – malabsorption (abnormal vitamin K absorption, some repeated or prolonged antibiotic treatments),

  – hepatocellular insufficiency,

  – circulating anti-factor IX autoantibodies. An anti-IX autoantibody develops in 5 to 10% of treated haemophiliacs making them refractory to factor IX concentrates. These must be tested for regularly (every 3 to 6 months and particularly after a new dose of a factor IX preparation).

  Isolated deficiency suggested haemophilia B or the presence of anti-factor IX autoantibodies (the differential diagnosis is made from assaying factor IX with higher plasma dilutions).

  Factor IX deficiency associated with factors II, VII and X deficiency suggests hypovitaminosis K (deficient intake or malabsorption) or moderate hepatocellular insufficiency; association with factor V deficiency suggests severe hepatocellular insufficiency.

- **Increased factor IX**

  Significantly increasing activated factor IX levels are seen in patients who have had a cerebrovascular accident, transient ischaemic attack (average: 156%) or a venous thrombo-embolic event (average: 165%). Increased factor IX levels therefore appear to be associated with both venous and arterial events.

- **Factor IX mutation**

  A new variety of familial thrombophilia was found in 2009 due to a mutation in the factor IX gene (factor IX Padua): substitution of an arginine by a leucine in position 138 leading to "functional gain", i.e. the factor IX is produced at normal concentrations in plasma but is 7 to 8 times more active than the normal protein (the first case published was a 23 year old young man with normal factor IX Ag but factor IX activity of 776%).

FOR FURTHER INFORMATION

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FACTOR V LEIDEN MUTATION
ACTIVATED PROTEIN C RESISTANCE

DEFINITION

90 to 95% of cases of activated protein C resistance (PCaR) reflect a single mutation in the gene coding for coagulation factor V: this is referred to as Factor V Leiden from the name of the town in which the abnormality was discovered. This mutation results in an arginine being replaced by a glutamine in position 506 (R506Q) which affects one of the factor V cleavage sites by activated protein C (PCa) which then inactivates its substrate less effectively. In practice, factor V Leiden ceases to act as a cofactor for the protein C system, i.e. a coagulation inhibitor system. Conversely it retains its pro-coagulant properties. Other cases of PCaR are acquired or due to one or more different mutation(s).

Synonyms: mutation 1691G > A; mutation R506Q; mutation Q506; Factor V Leiden, FVL.

INTRODUCTION

The FV Leiden mutation causes hyper-coagulability by two mechanisms. Replacement of an arginine with a glutamine in position 506 leads to loss of the activated protein C (PCa) cleavage site on FV and FVa. This results in defective inactivation of FVa by PCa and a loss of the PCa co-factor activity for FV.

In addition, FV Leiden can no longer act as a co-factor for PCa (in the presence of phospholipids, calcium and protein S) to inactivate factor Va and therefore increases coagulation.

INDICATIONS FOR MEASUREMENT

Testing from FV Leiden mutation by PCR (unequivocal diagnosis) may be indicated possibly after a phenotype screening test (resistance to the anticoagulated action of activated protein C, PCaR) rendered specific for FV by diluting the plasma in FV-deficient plasma, in the following situations:

– In a person (index case) under 60 years old with a first spontaneous venous thrombo-embolic event (proximal deep vein thrombosis and/or pulmonary embolism), or in a woman of childbearing age if the event is spontaneous or provoked. The thrombophilia screen is also indicated for a recurrent proximal DVT and/or PE whether or not provoked after an initial episode occurring before the age of 60 years old and in recurrence of unprovoked distal DVT when the first episode occurred before 60 years old.

– For family studies the test may be performed in first degree relatives if a homozygous FVL mutation or composite FVL-FII heterozygote is found in the index case. If heterozygous FVL is found it is recommended that family studies are only considered for women of child-bearing age after they have been clearly informed about the possible consequences (contraception, pregnancy, etc.). Testing for FVL mutation is debateable and should be considered on an individual case basis before prescribing oestrogen-progestogen, oral contraception to a young lady or before pregnancy if a heterozygous FVL mutation has been found.

INFORMATION

SAMPLE

- **PCaR screening test:** plasma collected into citrate, concentration 3.2% (0.109 M), 1/10 (0.5 ml per 4.5 ml of blood). Tubes containing 3.8% citrate (0.129 M) are acceptable. The blood can also be collected into a CTAD tube (citrate, théophylline, adenine, dipyridamole). No other anticoagulant may be used. The sampling conditions are the general conditions for haemostasis tests. For more information refer to the *"General pre-analytical conditions in haemostasis section"*.
- **Molecular biology testing for factor V Leiden mutation:** whole blood collected into EDTA.

ESSENTIAL INFORMATION

- **PCaR screening test:**

  Report current anticoagulants being taken: heparin can interfere with the test. The modified Dahlbäck method, which is currently used, is not influenced by anti-vitamin K treatments. PCaR testing can be invalidated by dabigatran or rivaroxaban treatment.

- **Molecular biology testing for factor V Leiden mutation:**

  This request should form part of a specific medical consultation carried out by a doctor working in a multidisciplinary team after obtaining consent from the patient.

  The test is not influenced by anticoagulant treatments.

SAMPLE STORAGE AND TRANSPORT

- **PCaR screening test:**

  – If the test is to be performed later, freeze within 2 hours of sampling

  – Plasma can be stored for 2 weeks at – 20°C and for 6 months at – 70°C

  – Transport the plasma frozen.

- **Molecular biology testing for a factor V Leiden mutation:**

  – The sample can be stored for 24 hours at room temperature (15 -20°C)

  – The purified extracted DNA can be stored for several months at – 70°C

  – Transport at room temperature or at + 4°C (if > 24 h).

ASSAY METHODS

- **PCaR screening test**

  Chronometric measurement based on measuring the APPT. The test initially developed by Dahlbäck involves measuring the patient's APPT in the presence and absence of a standardised amount of exogenous activated protein C. PCa resistance produces defective prolongation of the APTT in the presence of purified PCa. This test is relatively non-specific and cannot be used in patients being treated with anti-vitamin K agents.

  The tests used nowadays are modified versions (second generation tests) with dilution of the test plasma in factor V-deficient plasma. These are more specific for the factor V Leiden mutation and allow the abnormality to be tested in patients being treated with AVK. Most of the tests also allow

measurement of PCaR in patients treated with heparin as the reagents contain heparin inhibitors.

Other methods are available using snake venoms to activate factor V in the presence of PCa.

■ **Molecular biology testing for the 1691G > A factor V Leiden mutation**

After extracting the genomic DNA, the 1691G > A factor V Leiden (R506Q) is tested by PCR (polymerase chain reaction) amplification of a region of the factor V gene targeting the molecular abnormality. Various operating procedures are used to identify the mutation from the PCR product. These include:

– Treatment of the amplification products with restriction endonucleases (***PCR-RFLP***, *PCR-restriction fragment length polymorphism)*

– Amplification of the wild type and mutated allele form using specific *(**PCR-SSP**, PCR sequence-specific primers)* or modified primers *(**PCR-ARMS**, PCR-amplification-refractory mutation system)*

– Developing the amplification product using specific wild type and mutated allele probes (***PCR-ASO***, *PCRallele-specific oligonucleotide)*

– PCR in a homogeneous medium (or ***real time PCR***) using detection of a fluorescence signal (hydrolysis probes, tandem probes, molecular beacons, scorpion probes, fluorescent primers)

– Treatment of amplification products by primer extension (***mini-sequencing*** or *SNaPshot, Single base extension ELISA)*

– "Multiplex" methods are available to test jointly for the factor V Leiden 1691G > A mutation and the factor II 20210G > A variant.

– A molecular hybridisation method with amplification of a fluorescent signal (without PCR DNA amplification) based on Invader® technology is also available to genotype factor V and the factor II variant.

EXPRESSION OF RESULTS NORMAL EXPECTED VALUES

■ **PCaR screening test**

The result is usually expressed as a ratio of the patient's result to the result from a normal pool of normal lyophilised plasma (standardised ratio). Normal values depend on the analyser and reagents used.

■ **Molecular biology testing for factor V Leiden mutation**

This is reported as absent, or present, homozygous or heterozygous. The mutation is found in the general European population in approximately 5% of people with a decreasing north to south ratio. It is not found in Africa, Asia, Northern America or Australia.

PATHOLOGICAL VARIATIONS

– The PCaR test is difficult to interpret when a circulating lupus anticoagulant is present and in patients with factor V deficiency (titre < 50%).

Approximately 90% of cases of PCa resistance are due to the factor V Leiden mutation (confirmed by a molecular biology test). The other cases represent so-called acquired PCa resistance (factor V Leiden mutation absent): these cases of resistance may be seen in pregnancy, with oestrogen-progestogen contraception and in some cases of anti-phospholipid syndrome. They may also represent other factor V mutations (factor V Cambridge, factor V Hong-Kong, etc.).

A positive first generation PCaR test in the absence of factor V Leiden mutation is deemed to be a marker of hyper-coagulability.

– Factor V Leiden is currently the most commonly found constitutional abnormality in patients with a past personal and/or family history of deep vein thrombosis (10 to 20% of cases).

Most abnormalities found are heterozygous and increase risk of thrombosis by a factor of 3 to 5. The risk in homozygotes is increased by a factor of approximately 50. The clinical features (deep vein thrombosis and/or pulmonary embolism) occur either spontaneously or in the presence of predisposing factors (prolonged confinement to bed, oestrogen-progestogen, contraception, pregnancy, etc.), in young adults (from adolescence onward) or in slightly older people (over 40 years old).

In addition, factor V Leiden has been reported to be associated with numerous other congenital abnormalities, particularly prothrombin gene G 20210A mutation. The risk of thrombosis is higher in patients with combined deficiencies in all of these situations.

FOR FURTHER INFORMATION

■ Logiciel d'autoformation des biologistes en hémostase, CD-Rom Bioforma 2004.

■ Morange P.E., *Facteur V Leiden*, Encycl Med Biol, Elsevier, Paris, 2003.

■ Alhenc-Gelas M, Aillaud MF, Delahousse B et al. La recherche des facteurs biologiques de risque établis de maladie thrombo-embolique veineuse: état des connaissances et conséquences pour la pratique en biologie clinique. Sang Thrombose Vaisseaux 2009;21, n° spécial:12-39.

FACTOR V

DEFINITION

Factor V is a glycoprotein produced by the reticulo-endothelial system (liver) and by a small amount in platelets (20% of the circulating pool). It is a coagulation factor belonging to the prothrombin complex group: II, V, VII and X. It has a plasma half-life of 12 to 36 hours. A minimum level of 10 to 15% is sufficient to provide normal haemostasis.

Synonym: proaccelerin.

PHYSIOLOGICAL ROLE

Factor V is the enzyme cofactor for coagulation factor X. It is activated by thrombin and/or factor Xa into factor Va. Factor Va forms a complex with factor Xa in the presence of phospholipids and calcium, (prothrombinase complex) which activates prothrombin into thrombin but plays an essential role in the coagulation process as it converts fibrinogen into fibrin, amplifies its own formation and activates the protein C and TAFi systems and platelets. Factor Va is neutralised by the physiological anticoagulant activated protein C.

INDICATIONS FOR MEASUREMENT

It is a second line measurement for the diagnosis of constitutional or acquired factor V deficiency suggested by prolongation of the Quick time and the activated partial thromboplastin time.

INFORMATION

SAMPLE

Samples should be collected into citrate, concentration 3.2% (0.109 M), 1/10 (0.5 ml per 4.5 ml of blood). 3.8% (0.129 M) are acceptable. CTAD tubes (citrate, theophylline, adenine, dipyridamole) are recommended in patients receiving heparin. No other anticoagulant may be used.

The sample should preferably be taken between 7:00 and 11:00 am with the patient resting, seated, for at least 5 minutes. A light low fat snack is permitted but caffeine, tobacco and physical activity must be avoided during the hour before the sample is taken. The samples must be centrifuged promptly after the sample is taken and the test performed within 2 to 4 hours of sampling. Check that no micro-clots are present and discard haemolysed or lipaemic samples.

For more information refer to the "General pre-analytical conditions in haemostasis" section.

QUESTIONS FOR THE PATIENT

Clinical context?

Results of standard haemostasis tests?

SAMPLE STORAGE AND TRANSPORT

Store for 2 to 4 h at laboratory temperature (never store samples at + 4°C); for a few days at – 20°C (a 10 to 20% loss in activity is seen in a sample stored for 7 days at – 20°C) and for 6 months at – 70°C.

It is recommended that samples be thawed promptly in a water bath at 37°C and the sample mixed well before the test.

Transport at – 20°C if the sample is to be sent away. The plasma must be frozen at – 20°C within an hour of sampling.

ASSAY METHODS

– Routine method: functional chronometric method (measurement of activity): measurement of the Quick time in an equal volume of patient plasma diluted 1/10 and factor-V deficient reagent plasma. The time is converted into percentage activity against a calibration line from control plasma with 100% activity.

– Immunological antigen assay to distinguish quantitative from qualitative deficiency (specialist laboratory use).

– Molecular biology gene study (chromosome 1): testing for the mutation responsible for constitutional deficiency).

NORMAL EXPECTED VALUES

Results are usually expressed as a percentage of normal or in IU/ml, 1 IU/ml = 100%.

Reference values in children from 1 year old and in adults: 70 to 140% (0.70 to 1.40 IU/ml).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Activity is reduced in the newborn baby: reference value between 40 and 120%.

Activity is sometimes increased in pregnancy: reported values between 40 and 200%.

Activity is increased when coagulation is started (activation of V into Va).

PATHOLOGICAL VARIATIONS

Constitutional factor V deficiency:

Constitutional factor V deficiency is autosomal recessive in transmission and rare (severe deficiencies are very rare).

Clinically, homozygous deficiency is characterised by muco-cutaneous bleeding (bruising, haematomas, post-operative bleeding) the severity of which varies and correlates relatively well with residual platelet factor V levels. Heterozygotes are usually asymptomatic.

The diagnosis of constitutional deficiency is only made after confirming the deficiency in a second sample taken some time after the first and after excluding causes of acquired deficiency, which is far commoner. Family studies should be considered.

■ **Acquired factor V deficiency may occur in the following situations:**

– Severe liver disease (chronic hepatitis, cirrhosis): reduced factor V in these situations is an adverse prognostic indicator.

– Fibrinolysis.

– Disseminated intravascular coagulation (DIC).

– Anti-factor V autoantibodies associated with some malignancies or the use of haemostatic glues (bovine or human thrombine).

The aetiological diagnosis relies on further measurements of factors VII, IX and X:

– If all of these are normal the factor V deficiency is isolated (congenital factor V deficiency or anti-factor V auto-antibodies): note however that very occasional cases of co-existent factor V and VIII deficiencies are seen.

– If all are reduced: liver disease, defibrination syndrome or DIC.

FOR FURTHER INFORMATION

■ Logiciel d'autoformation des biologistes en hémostase, CD-Rom Bioforma 2004.

■ Aillaud M.F., *Proaccélérine*, Encycl Med Biol, Elsevier, Paris, 2003.

■ Caers J., Reekmans A., Jochmans K., et al. *Factor V inhibitor after injection of human thrombin (tissucol) into a bleeding peptic ulcer*. Endoscopy 2003; 35:542-4.

■ Samama M.M., Elalamy I., Conard J., Achkar A., Horellou M.H., *Hémorragies et thromboses : du diagnostic au traitement*, Collection «Les abrégés», Édition Masson, Paris, 2004.

FACTOR VII

DEFINITION

Factor VII is a glycoprotein produced by the liver and is a serine protease zymogen. It is a vitamin K-dependent coagulation factor belonging to the prothrombin complex group: II, V, VII and X. It has a short plasma half-life of between 4 and 6 hours. A minimum level of 10 to 15% is sufficient to provide normal haemostasis.

Synonym: proconvertin.

PHYSIOLOGICAL ROLE

Factor VII is the only coagulation factor found in the activated form (FVIIa) in trace amounts in the circulation. When tissue thromboplastin (or factor) develops on the surface of damaged, abnormal or activated vascular endothelium it binds to FVIIa initiating the extrinsic *in vivo* coagulation pathway. The tissue factor–VIIa formed activates factor X into Xa and factor IX into IXa.

INDICATIONS FOR MEASUREMENT

This is a second line measurement for the diagnosis of constitutional or acquired factor VII deficiency suggested by an isolated prolongation of the Quick time.

INFORMATION

SAMPLE

Samples should be collected into citrate, concentration 3.2% (0.109 M), 1/10 (0.5 ml per 4.5 ml of blood). 3.8% (0.129 M) are acceptable. CTAD tubes (citrate, theophylline, adenine, dipyridamole) are recommended in patients receiving heparin. No other anticoagulant may be used.

The sample should preferably be taken between 7:00 and 11:00 am with the patient resting, seated, for at least 5 minutes. A light low fat snack is permitted but caffeine, tobacco and physical activity must be avoided during the hour before the sample is taken. The samples must be centrifuged promptly after the sample is taken and the test performed within 2 to 4 hours of sampling. Check that no micro-clots are present and discard haemolysed or lipaemic samples.

For more information refer to the *"General pre-analytical conditions in haemostasis"* section".

QUESTIONS FOR THE PATIENT

Clinical context?

Results of standard haemostasis tests?

Current anti-vitamin K treatment?

SAMPLE STORAGE AND TRANSPORT

Store for 2 to 4 h at laboratory temperature (never store samples at + 4°C); for a few days at – 20°C and for 6 months at – 70°C.

It is recommended that samples be thawed promptly in a water bath at 37°C and the sample mixed well before the test.

Transport at – 20°C if the sample is to be sent away. The plasma must be frozen at – 20°C within two hours of sampling.

ASSAY METHODS

– Routine method: functional chronometric method (measurement of activity): measurement of the Quick time in an equal volume of patient plasma diluted 1/10 and factor VII-deficient reagent plasma. The time is converted into percentage activity against a calibration line from control plasma with 100% activity.

– Immunological antigen assay to distinguish quantitative from qualitative deficiency (specialist laboratory use).

– Molecular biology gene study (chromosome 13): testing for the mutation responsible for constitutional deficiency.

NORMAL EXPECTED VALUES

Results are usually expressed as a percentage of normal or in IU/ml, 1 IU/ml = 100%.

Reference values in children from 1 year old and in adults: 70 to 120% (0.70 to 1.20 IU/ml).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

– Activity is reduced in the newborn baby: normal values are between 25 and 105%.

– Activity is occasionally increased in pregnancy: reported values between 84 and 336%.

PATHOLOGICAL VARIATIONS

Constitutional factor VII deficiency:

Constitutional factor VII deficiency is autosomal recessive in transmission and very rare. It may be quantitative (antigen and activity are reduced) or more often, qualitative (antigen normal, activity reduced).

Clinically, homozygous or composite heterozygous deficiency is characterised by bleeding, the severity of which does not correlate with residual factor VII levels. Heterozygotes (who are far less rare than homozygotes) are generally asymptomatic (factor VII levels approximately 30%).

The diagnosis of constitutional deficiency is only made after confirming the deficiency in a second sample taken some time after the first and after excluding causes of acquired deficiency, which is far commoner. Family studies should be considered.

NB: factor VII levels may vary depending on the thromboplastin used (human or animal).

- **Acquired factor VII deficiencies:**

  Because of its short half-life, factor VII is the first of the coagulation factors to fall in the following situations:

  – Anti-vitamin K treatment.

  – Deficient vitamin K intake.

  – Malabsorption (abnormal absorption of vitamin K), sprue.

  – Hepatocellular insufficiency.

  – Fibrinolysis; disseminated intravascular coagulation (DIC).

  – Other situations: anti-factor VII autoantibodies, treatment with some cephalosporins, particularly latamoxef or cefoperazone.

  – Very rare cases of isolated, transient non-constitutional factor VII deficiency are reported. The mechanism of these transient deficiencies is unknown and several hypotheses have been forwarded: they are usually seen in patients with severe infection.

- **The aetiological diagnosis** is based on further measurements of vitamin K-dependent factors (II, IX, X) and factor V.

  – If all are normal the factor VII deficiency is isolated (the APTT is not prolonged): constitutional deficiency, anti-factor VII autoantibodies, transient deficiency or early treatment with anti-vitamin K.

  – If factors II, IX and X are reduced and factor V is normal: this may be produced by anti-vitamin K treatment, hypovitaminosis K (deficient intake or malabsorption) or moderate hepatocellular insufficiency.

  – If factors II, IX, X and V are reduced: the cause may be severe hepatocellular insufficiency, defibrination syndrome or DIC

  Finally, some authors suggest that factor VII is a prothrombotic risk factor.

FOR FURTHER INFORMATION

- Logiciel d'autoformation des biologistes en hémostase, CD-Rom Bioforma 2004.
- Aillaud M.F., *Proconvertine*, Encycl Med Biol, Elsevier, Paris, 2003.
- Samama M.M., Elalamy I., Conard J., Achkar A., Horellou M.H., *Hémorragies et thromboses : du diagnostic au traitement*, Collection «Les abrégés», Édition Masson, Paris, 2004.

FACTOR VIII

DEFINITION

Factor VIII is a glycoprotein synthesised almost entirely by the liver (very limited renal synthesis) and present in many tissues (liver, kidney, spleen and lungs). It appears however, to be absent from the vascular endothelium (unlike Von Willebrand factor). Factor VIII circulates in plasma bound to von Willebrand factor which protects it from rapid proteolysis. It therefore has a half-life of 10 to 16 hours. The free form of factor VIII is present at very low concentrations and has a very short half-life (2 hours for the form not bound to von Willebrand factor). The minimum level required to provide normal haemostasis is 30%.

Synonym: Anti-haemophilia A factor, FVIII and FVIII:C.

PHYSIOLOGICAL ROLE

Factor VIII is an enzymatic coagulation co-factor. It is activated by factor Xa or thrombin into factor VIIIa which complexes with factor IXa in the presence of phospholipids, activating factor X into Xa.

INDICATIONS FOR MEASUREMENT

This is a specific measurement for the diagnosis of factor VIII deficiency (constitutional or acquired haemophilia A, von Willebrand's disease and very rarely constitutional FV and FVIII deficiency) suggested by an isolated increase in the activated cephalin time and/or by haemorrhage. It can also be used to monitor haemophiliac patients treated with factor VIII replacement. FVIII may also be measured as part of a thrombophilia screen (2nd line).

INFORMATION

SAMPLE

The reference anticoagulant is sodium citrate at a recommended concentration of 0.109 M (3.2%). The ratio of anticoagulant/blood is 1 volume per 9 volumes of blood. It is recommended that the tube be filled >90% although 80% is acceptable.

Blood can also be collected into a CTAD (citrate, theophylline adenine, dipyridamole) tube. No other anticoagulants must be used.

The sample should preferably be taken between 7:00 and 11:00 am from a fasting patient. Coffee, smoking and alcohol must be avoided during the hour before the sample is taken; a light low fat snack is permitted.

The sample must be centrifuged promptly after being taken at 2000-2500 g for 15 minutes (thermostatically controlled centrifuge at room temperature 18-22°C).

Confirm that no microclots are present and discard haemolysed or lipaemic samples.

For further information refer to the *"General haemostasis preanalytical conditions"* page.

QUESTIONS FOR THE PATIENT

Clinical context of bleeding (chronic or sudden onset symptoms)? Family history of bleeding?

Results of recent haemostasis tests?

Oral contraception (this increases factor VIII levels)?.

SAMPLE STORAGE AND TRANSPORT

– Factor VIII is a very labile, fragile sample and can be stored for a maximum of 2 hours at room temperature (never store the sample at +4°C); beyond this, freeze the sample promptly.

– Store for up to 2 weeks at –20°C and for up to 6 months at –70°C

– The plasma must be thawed quickly at 37°C in a water bath (for no more than a few minutes) and never in an oven/incubator.

– Transport: plasma must be frozen within 2 hours of the sample being taken.

ASSAY METHODS

– The method used in everyday practice is chronometric assay (measurement of FVIII:C activity): measurement of the coagulation time of an equal volume of the patient's plasma diluted 1/10 and factor VIII-deficient reagent. The time measured is expressed as a percentage activity against a calibration curve constructed from control plasma with 100% activity.

NB: Circulating lupus anticoagulant can interfere with the assay. In this case factor VIII should be measured with higher dilutions of the test plasma: for circulating lupus anticoagulant the measurement becomes normal on dilution (diluting the effect of the circulating antibody).

– Immunoassay (generally ELISA) (FVIII:Ag).

– Molecular biology gene studies (locus Xq28): testing for the molecular abnormality responsible.

NORMAL EXPECTED VALUES

The results are usually expressed as a percentage (and more rarely in IU/ml: 1 IU/ml=100%).

Normal values: 50 to 150% (0.50 to 1.50 IU/ml) in adults and children.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

– At birth: values are between 50 and 200%.

– Pregnancy: normal values may rise to 500% (physiological increase in FVIII to compensate for bleeding at childbirth).

– Blood group O: lower values to approximately 40%.

– Physical exercise (increase up to 250%).

PATHOLOGICAL VARIATIONS: REDUCED

Constitutional factor VIII deficiency: Constitutional haemophilia A

Constitutional factor VIII deficiency or haemophilia A is a sex-linked inherited coagulation disorder affecting 1/5000 male births characterised by 'interiorised' haemorrhage mostly in the muscles and joints (haemarthroses and subcutaneous haematomas) and also haematuria and occasional CNS haemorrhage. The genetic abnormalities are carried by the F8 gene on the X chromosome (Xq28). The molecular abnormalities involved are deletions, point mutations, inversions (inversion in intron 22 = 50% of severe haemophilia A patients).

The laboratory deficiency is variable, and defines several types of haemophilia:

– Severe haemophilia A: level of factor VIII < 1%

– Moderate haemophilia A: level of factor VIII ≥1% and < 5%

– Mild haemophilia A: level of factor VIII ≥5% and ≤30%.

The severity of bleeding is directly related to circulating factor VIII levels.

– Severe haemophilia: initial events when the child begins to walk, such as multiple bruising, forehead and buttock haematomas or haematomas after vaccination, bleeding from tongue injuries, frequent recurrent spontaneous episodes or due to minimal trauma ("disproportionate haematomas").

– Moderate haemophilia: haemorrhages beginning later, less frequent and often post-traumatic.

– Mild haemophilia: bleeding is rare and only provoked (surgery when the diagnosis is not known).

The diagnosis of constitutional deficiency is only made after rechecking the deficiency on a second sample taken later and after excluding other causes of deficiency (particularly von Willebrand's disease). Family screening is normally carried out.

Female haemophilia A carriers have average factor VIII levels of 50% normal, although depending on the degree of inactivation of the X gene carrying the abnormality, the FVIII level may vary (approximately 30% of carriers are in the 30-49% range). Female haemophilia A carriers must be identified in order to be managed when they undergo surgery (risk of provoked bleeding if FVIII level < 50%) and in pregnancy (risk of transmission to the child with antenatal diagnosis in carriers of the severe haemophilia A molecular abnormality). These carriers must give birth in a specialised unit. Haemophilia A carrier status is only formally defined by molecular biology methods (testing for the abnormality found in the family).

It should be noted that haemophilia A is an almost exclusively male disease, although occasional cases of genuine female haemophilia A occur (2X carriers of the abnormality or cytogenetic abnormalities with loss or inactivation of the normal X and expression of he disease X, X/0) or X/autosomal translocation.

Von Willebrand Diseases (1, 2A, 2B, 2M, 2N, 3)

In type 1 von Willebrand Disease (partial von Willebrand factor deficiency) and type 2 von Willebrand's Disease (qualitative von Willebrand factor deficiency), the factor VIII deficiency depends on the level of von Willebrand factor antigen (proportional reduction as factor VIII is protected by von Willebrand factor).

Factor VIII deficiency is more severe in type 2 von Willebrand disease (total quantitative deficiency of von Willebrand factor with traces of approximately 5-8% FVIII) and in type 2N von Willebrand's disease (reduced affinity of von Willebrand factor for factor VIII and average FVIII level of 12-15%).

Acquired haemophilia A

This involves sudden-onset acquired FVIII deficiency with no past personal or family history of bleeding. The mechanism is due to the development of anti-FVIII antibodies (IgG, A or M). Acquired haemophilia A usually occurs in elderly people (incidence peak with 15 cases/1000000/year after the age of 65 years old) and post-partum, from a few days to approximately 6 months after childbirth. Acquired haemophilia A in the elderly usually has a poor outcome. The immunological profile of the anti-FVIII auto Ab differs depending on the type of acquired haemophilia (the profile is different in the elderly to that seen post-partum).

Combined FVIII and FV deficiency

This is not due to an abnormality of the FVIII and FV genes but to a rare joint abnormality due to a mutation in the chromosome 18 LMAN-1 or chromosome 2 MCFD2 gene. The abnormality causes a reduction in FV and FVIII transport (the factors are sequested in the cells and cannot be secreted into the circulation). FV and VIII levels are generally moderately reduced in the disorder (levels are usually 5 to 30%). Symptoms are mild (mostly muco-cutaneous). This combined deficiency is one of the rare haemorrhagic diseases (1 case per million rising to 1 case per 100,000 in Iran).

PATHOLOGICAL VARIATIONS: INCREASE

- Reactive increase (inflammation and liver disease).
- Chronic primary increase in factor VIII (> 150%): permanent increase independent of inflammation associated with an increased risk of the venous thrombosis. It is seen in approximately 6 to 8% of people in the general population and in 10 to 15% of patients who have had a deep vein thrombosis. The increase appears to be hereditary and is a risk factor for DVT, and particularly for recurrences. The molecular abnormality has not yet been identified and the role of the increase in FVIII and its possible therapeutic management are still uncertain. It is not appropriate to request this measurement first line in thrombophilia investigations (GEHT 2009 recommendations).

FOR FURTHER INFORMATION

- Aillaud M.F., *Facteur VIII : anti-hémophilique A*, EMC Hématologie, Elsevier, Paris, 2003.
- Samama M.M., *Hémorragies et thromboses : du diagnostic au traitement*. Collection «Les abrégés», Édition Masson, Paris, 2009.

FACTOR X

DEFINITION

Factor X is a glycoprotein produced by the liver and is a serine protease zymogen. It is a vitamin K-dependent coagulation factor belonging to the prothrombin complex group: II, V, VII and X. It has a short plasma half-life of between 36 and 48 hours. A minimum level of 10 to 15% is sufficient to provide normal haemostasis.

Synonym: Stuart factor.

PHYSIOLOGICAL ROLE

Factor X is involved in the classical coagulation pathway. It is activated into factor Xa by tissue factor VIIa complex or by factor VIIIa-IXa complex in the presence of phospholipids. Generation of factor Xa leads to the formation of prothrombinase (FXa, FVa, phospholipids and calcium), which activates conversion of prothrombin into thrombin. Factor Xa is neutralised by TFPI *(Tissue Factor Pathway Inhibitor)* and by antithrombin.

INDICATIONS FOR MEASUREMENT

This is a second line measurement for the diagnosis of constitutional or acquired factor X deficiency, suggested by a prolongation of the Quick time and activated partial thromboplastin time.

INFORMATION

SAMPLE

Samples should be collected into citrate, concentration 3.2% (0.109 M), 1/10 (0.5 ml per 4.5 ml of blood). 3.8% (0.129 M) are acceptable. CTAD tubes (citrate, theophylline, adenine, dipyridamole) are recommended in patients receiving heparin. No other anticoagulant may be used.

The sample should preferably be taken between 7:00 and 11:00 am with the patient resting, seated, for at least 5 minutes. A light low fat snack is permitted but caffeine, tobacco and physical activity must be avoided during the hour before the sample is taken. The samples must be centrifuged promptly after the sample is taken and the test performed within 2 to 4 hours of sampling. Check that no micro-clots are present and discard haemolysed or lipaemic samples.

For more information refer to the *"General pre-analytical conditions in haemostasis"* section".

QUESTIONS FOR THE PATIENT

Clinical context?

Result of standard haemostasis tests?

Current treatment with anti-vitamin K agents?

SAMPLE STORAGE AND TRANSPORT

Store for 2 to 4 h at laboratory temperature (never store samples at + 4°C); 2 weeks at – 20°C; months at – 70°C.

It is recommended that samples are thawed promptly in a water bath at 37°C and the sample mixed well before the test.

Transport at – 20°C if the sample is to be transported. The plasma must be frozen at – 20°C within 2 hours of sampling.

ASSAY METHODS

– Routine method: functional chronometric method (measurement of activity): measurement of the Quick time in an equal volume of patient plasma diluted 1/10 and factor X deficient reagent plasma. The time is converted into percentage activity against a calibration line from control plasma with 100% activity.

– Immunological antigen assay to distinguish quantitative from qualitative deficiency (specialist laboratory use).

– Molecular biology gene study (chromosome 13): testing for the mutation responsible for constitutional deficiency.

NORMAL EXPECTED VALUES

Results are usually expressed as a percentage of normal or in IU/ml, 1 IU/ml = 100%.

Reference values in children and adults: 70 to 150% (0.70 to 1.52 IU/ml).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

– Activity is reduced in the newborn baby: reference values are between 10 and 70%

– Activity is occasionally increased in pregnancy: values reported are between 70 and 200%.

PATHOLOGICAL VARIATIONS

Constitutional factor X deficiency:

Severe homozygous or composite heterozygous deficiencies are very rare. Heterozygous deficiencies are characterised by bleeding occurring after invasive procedures or serious injury. PPSB treatment is indicated to maintain factor X concentration levels > 25 %.

The diagnosis of constitutional deficiency is only made after confirming the deficiency in a second sample taken some time after the first and after excluding causes of acquired deficiency, which is far more common. Family studies should be considered.

Acquired factor X deficiencies are seen in:

– Treatment with anti-vitamin K agents

– Deficient vitamin K intake

– Malabsorption (abnormality of vitamin K absorption)

– Hepatocellular insufficiency

– Fibrinolysis

– Disseminated intravascular coagulation (DIC)

– Amyloidosis: severe isolated factor X deficiency may reveal or complicate primary amyloidosis or amyloidosis secondary to myeloma

– Others: circulating anti-factor X autoantibodies.

The aetiological diagnosis relies on further measurements of vitamin K dependent factors (II, VII, IX) and factor V:

– If all are normal the factor X deficiency is isolated (congenital deficiency, amyloidosis, anti-factor X auto-antibodies)

– If factors II, VII and IX are reduced and factor V normal: this may be produced by anti-vitamin K treatment, hypovitaminosis K (deficient intake or malabsorption) or moderate hepatocellular insufficiency

– If factors II, VII, IX and V are reduced: the cause may be severe hepatocellular insufficiency, defibrination syndrome or DIC.

FOR FURTHER INFORMATION

■ Aillaud M.F., *Facteur X: Stuart*, Encycl Med Biol, Elsevier, Paris, 2003.

■ Samama M.M., Elalamy I., Conard J., Achkar A,. Horellou M.H., *Hémorragies et thromboses : du diagnostic au traitement,* Collection «Les abrégés» Edition Masson, Paris, 2004.

FACTOR XI

DEFINITION

Factor XI is a glycoprotein produced by the liver and is a serine protease zymogen.

It has a plasma half-life of 40 to 80 hours. A minimum level of 30 to 45% is sufficient to provide normal haemostasis.

Synonyms: Rosenthal factor, Plasma thromboplastin antecedent (PTA).

INTRODUCTION

Factor XI is involved in the contact phase, which initiates the intrinsic coagulation pathway. It is activated by factor XIIa into factor XIa which itself activates factor IX in the presence of calcium ions.

INDICATIONS FOR MEASUREMENT

It is a second line measurement for the diagnosis of constitutional or acquired factor XI deficiency suggested by an isolated prolongation of the activated partial thromboplastin time.

INFORMATION

SAMPLE

Samples should be collected into citrate, concentration 3.2% (0.109 M), 1/10 (0.5 ml per 4.5 ml of blood). 3.8% (0.129 M) are acceptable. CTAD tubes (citrate, theophylline, adenine, dipyridamole) are recommended in patients receiving heparin. No other anticoagulant may be used.

The sample should preferably be taken between 7:00 and 11:00 am with the patient resting, seated, for at least 5 minutes. A light low fat snack is permitted but caffeine, tobacco and physical activity must be avoided during the hour before the sample is taken. The samples must be centrifuged promptly after the sample is taken and the test performed within 2 to 4 hours of sampling. Check that no micro-clots are present and discard haemolysed or lipaemic samples.

For more information refer to the *"General pre-analytical conditions in haemostasis"* section.

QUESTIONS FOR THE PATIENT

Clinical context?

Results of standard haemostasis tests?

Current anticoagulant therapy affecting the APTT (heparin): in practice the plasma dilutions used for measurement make interference from anticoagulation negligible.

Where applicable, Ashkenazi Jew ethnicity? More than 50% of cases of constitutional deficiency.

SAMPLE STORAGE AND TRANSPORT

Store for 2 to 4 h at laboratory temperature (never store samples at + 4°C); 2 weeks at – 20°C; 6 months at – 70°C.

It is recommended that samples are thawed promptly in a water bath at 37°C and the sample mixed well before the test.

Transport at – 20°C if the sample is to be transported. The plasma must be frozen at – 20°C within 2 hours of sampling.

ASSAY METHODS

– Routine method: functional chronometric method (measurement of activity). Measurement of the activated partial thromboplastin time in an equal volume of patient plasma diluted 1/10 and factor XI deficient reagent plasma. The time is converted into percentage activity against a calibration line from "control plasma" with 100% activity.

NB: the presence of a circulating antibody can interfere with measurement. In this case factor XI should be assayed with higher dilutions of the test plasma. Correction of the deficiency at higher dilutions indicates a circulating antibody, the inhibitory effect of which reduces within increasing plasma dilution. These circulating antibodies can be a lupus anticoagulant or a specific anticoagulant against factor XI (acquired "deficiency", *cf. interpretation of results*).

– Immunological antigen assay to distinguish quantitative from qualitative deficiency (specialist laboratory use).

– Molecular biology gene study (chromosome 4): testing for the mutation responsible for constitutional deficiency.

NORMAL EXPECTED VALUES

Results are usually expressed as a percentage of normal or in IU/ml, 1 IU/ml = 100%

Reference values in children and adults: 70 to 120% (0.70 to 1.20 IU/ml)..

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

– Activity is reduced in the newborn baby: reference values are between 20 and 70%.

– Activity is occasionally reduced by 10 to 30% in pregnancy.

PATHOLOGICAL VARIATIONS

Constitutional factor XI deficiency:

Constitutional factor XI deficiency is rare in the general population, although is seen more commonly in the Ashkenazi Jew population. Homozygote or composite heterozygote deficiencies are characterised by bleeding of variable severity (Rosenthal disease). There is no close relationship between the severity of the deficiency and the expression of bleeding due to the inherited deficiency. Bleeding is usually moderate, late but prolonged, and occurs after injury or surgery (dental extraction, adenoidectomy). This risk of bleeding is debated in heterozygotes where it is always less and usually associated with an invasive procedure. Residual factor levels do not predict risk of bleeding. The diagnosis of constitutional deficiency is only made after confirming the deficiency in a second sample taken some time after the first and after excluding causes of acquired deficiency, which is far more common. Family studies should be considered.

- **Acquired factor XI deficiencies** are associated with anti-factor XI auto-antibodies in plasma which are occasionally seen in patients who have been given factor XI, or in auto-immune diseases (systemic lupus erythematosus), neoplasia, blood dyscrasias or in psoriasis.

FOR FURTHER INFORMATION

- Aillaud MF. *Facteur X: Stuart*, Encycl Med Biol, Elsevier, Paris, 2003.
- Samama M.M., Elalamy I., Conard J., Achkar A., Horellou M.H., *Hémorragies et thromboses : du diagnostic au traitement*, Collection «Les abrégés» Edition Masson, Paris, 2004.

FACTOR XII

DEFINITION

Factor XII is the glycoprotein produced by the liver and is a serine protease zymogen. It has a plasma half-life of 50 to 70 hours.

Synonym: Hageman factor.

PHYSIOLOGICAL ROLE

Factor XII is involved in the contact phase, which initiates the intrinsic coagulation pathway. When activated in contact with a negatively charged surface it becomes able to activate prekalikrein into kalikrein (a reaction which is greatly amplified by high molecular weight kininigen or HMWK) and then activates factor XI into XIa in the presence of HMWK. The factor XIIa that is formed also activates factor XII into XIIa amplifying the reaction.

Factor XIIa can also activate plasminogen into plasmin and therefore the fibrinolysis pathway. It also stimulates angiogenesis.

INDICATIONS FOR MEASUREMENT

It is a second line measurement for the diagnosis of constitutional (heterozygotes are relatively common, homozygotes are very rare) or acquired factor XII deficiency suggested by a prolonged activated partial thromboplastin time.

INFORMATION

SAMPLE

Samples should be collected into citrate, concentration 3.2% (0.109 M), 1/10 (0.5 ml per 4.5 ml of blood). 3.8% (0.129 M) are acceptable. CTAD tubes (citrate, theophylline, adenine, dipyridamole) are recommended in patients receiving heparin. No other anticoagulant may be used.

The sample should preferably be taken between 7:00 and 11:00 am with the patient resting, seated, for at least 5 minutes. A light low fat snack is permitted but caffeine, tobacco and physical activity must be avoided during the hour before the sample is taken. The samples must be centrifuged promptly after the sample is taken and the test performed within 2 to 4 hours of sampling. Check that no micro-clots are present and discard haemolysed or lipaemic samples.

For more information refer to the *"General pre-analytical conditions in haemostasis"* section.

QUESTIONS FOR THE PATIENT

Clinical contexts?

Results of standard haemostasis tests?

Current anticoagulation treatment affecting the APTT (heparin): in practice, the plasma dilutions used for the assay make interference from anticoagulation negligible.

SAMPLE STORAGE AND TRANSPORT

Store for 2 to 4 h at laboratory temperature (never store samples at + 4°C); 2 weeks at – 20°C; 6 months at – 70°C.

It is recommended that samples are thawed promptly in a water bath at 37°C and the sample mixed well before the test.

Transport at – 20°C if the sample is to be transported. The plasma must be frozen at – 20°C within 2 hours of sampling.

ASSAY METHODS

– Routine method: functional chronometric method (measurement of activity). Measurement of the activated partial thromboplastin time in an equal volume of patient plasma diluted 1/10 and factor XII deficient reagent plasma. The time is converted into percentage activity with reference to a calibration line from "control plasma" with 100% activity.

NB: the presence of a circulating antibody can interfere with measurement. In this case factor XII should be assayed with higher dilutions of the test plasma. Correction of the deficiency at higher dilutions indicates a circulating antibody, the inhibitory effect of which reduces within increasing plasma dilution. These circulating antibodies can be a lupus anticoagulant or a specific anticoagulant against factor XII (acquired "deficiency", *cf. interpretation of results*).

– Immunological antigen assay to distinguish quantitative from qualitative deficiency (specialist laboratory use).

– Molecular biology gene study (chromosome 5): testing for the mutation responsible for constitutional deficiency.

NORMAL EXPECTED VALUES

Results are usually expressed as a percentage of normal or in IU/ml, 1 IU/ml = 100%.

Reference values in children from one year old and adults: 52 to 160% (0.52 to 1.64 IU/ml).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Activity is reduced in the newborn baby: reference values are between 22 and 70%.

PATHOLOGICAL VARIATIONS

Constitutional factor XII deficiency:

Constitutional factor XII deficiency is usually quantitative and is autosomal recessive in transmission. Factor XII levels of < 1% in homozygotes and between 15 and 80% in heterozygotes. The deficiency is not associated with any bleeding features even in homozygotes. It may however carry an increased risk of thrombosis (venous or arterial) because of the involvement of factor XII in the Fibrinolysis system.

The diagnosis of constitutional deficiency is only made after confirming the deficiency in a second sample taken some time after the first and after excluding causes of acquired deficiency, which is far commoner. Family studies should be considered.

- **Acquired factor XII deficiencies** are associated with anti-factor XII autoantibodies in plasma.

FOR FURTHER INFORMATION

- Aillaud M.F., *Facteur X: Stuart*. Encycl Med Biol, Elsevier, Paris, 2003.
- Samama M.M., Elalamy I., Conard J., Achkar A., Horellou M.H., *Hémorragies et thromboses : du diagnostic au traitement*, Collection «Les abrégés» Edition Masson, Paris, 2004.
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FACTOR XIII

DEFINITION

Factor XIII or fibrin stabilisation factor (FSF) is the zymogen of a transglutaminase. It is a high molecular weight tetramer formed from 2 alpha sub-units, which act as supports for its activity and are produced by the bone marrow cells and placenta and 2 beta sub-units (carrier protein) produced by the liver. It has a plasma half-life of 3 to 7 days. Factor XIII activity levels of 1 to 10% are sufficient to provide normal haemostasis.

PHYSIOLOGICAL ROLE

Factor XIII is activated by thrombin and is involved in the final phase of fibrin formation to stabilise the fibrin clot (it makes the fibrin polymer insoluble by forming covalent bonds). Activated factor XIII is also involved in tissue repair and healing processes by binding of collagen to fibronectin.

INDICATIONS FOR MEASUREMENT

It is a second line measurement for the diagnosis of constitutional factor XIII deficiency suggested by severe bleeding occurring from birth, contrasting with normal usual haemostasis tests (Quick time, activated partial thromboplastin time and fibrinogen). The only highly suggestive test is the thromboelastogram.

INFORMATION

SAMPLE

Samples should be collected into citrate, concentration 3.2% (0.109 M), 1/10 (0.5 ml per 4.5 ml of blood). 3.8% (0.129 M) are acceptable. CTAD tubes (citrate, theophylline, adenine, dipyridamole) are recommended in patients receiving heparin. No other anticoagulant may be used.

The sample should preferably be taken between 7:00 and 11:00 am with the patient resting, seated, for at least 5 minutes. A light low fat snack is permitted but caffeine, tobacco and physical activity must be avoided during the hour before the sample is taken. The samples must be centrifuged promptly after the sample is taken and the test performed within 2 to 4 hours of sampling. Check that no micro-clots are present and discard haemolysed or lipaemic samples.

For more information refer to the *"General pre-analytical conditions in haemostasis"* section.

ESSENTIAL INFORMATION

– Personal and familial clinical context.

– Result of standard haemostasis tests.

SAMPLE STORAGE AND TRANSPORT

Store for 2 to 4 h at laboratory temperature (never store samples at + 4°C); 2 weeks at – 20°C; 6 months at – 70°C.

It is recommended that samples are thawed promptly in a water bath at 37°C and the sample mixed well before the test.

Transport at – 20°C if the sample is to be sent away. The plasma must be frozen at – 20°C within 2 hours of sampling.

ASSAY METHODS

– Quantitative method: measurement of the transglutaminase activity factor XIII using a chromogenic method.

– Qualitative functional method: study of clot solubility in a solution of 5M urea or 1% monochloracetic acid.

– Assay of antigen by an Elisa immunological method.

– Molecular biology gene studies.

NORMAL EXPECTED VALUES

Results are usually expressed as a percentage of normal or in IU/ml, 1 IU/ml = 100%.

Factor XIII activity: 50 to 140%.

Factor XIII antigen: plasma concentration = 20 to 30 mg/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

– Activity is reduced in the newborn baby: reference values are between 30 and 100%. Values rise towards other levels at around 6 months.

– Activity is reduced during pregnancy: the values found are between 30 and 70%.

PATHOLOGICAL VARIATIONS

Constitutional factor XIII deficiencies:

Constitutional factor XIII deficiencies are autosomal recessive in transmission. Homozygotes are very rare. Severe homozygotes or composite heterozygotes are associated with bleeding in specific places (when the umbilical cord drops off, extensive subcutaneous haematomas) and occurs several hours after an injury. Delayed healing and scars of abnormal appearance have also been described.

The diagnosis of constitutional deficiency is made after confirming the reduced factor XIII activity in a second sample and after excluding causes of acquired deficiency. Family studies should be considered.

The deficiency is said to be:

– Major when factor XIII activity is < 1%

– Minor when factor XIII activity is between 1 and 20%.

– Moderate when factor XIII activity is between 20 and 40%.

Factor XIII activity is "borderline" when it is between 40 and 55%.

- **Acquired factor XIII deficiencies can be seen in:**
  - Autoimmune diseases (rheumatoid purpura and coeliac disease);
  - Anti-factor XIII autoantibodies;
  - Promyelocyte leukaemias;
  - Disseminated intravascular coagulation (DIC).

FOR FURTHER INFORMATION

- Aillaud M.F., *Facteur XIII*, Encycl Med Biol, Elsevier, Paris, 2003.
- Samama M.M., Elalamy I., Conard J., Achkar A., Horellou M.H., *Hémorragies et thromboses : du diagnostic au traitement*, Collection «Les abrégés» Edition Masson, Paris, 2004.

FAECAL CALPROTECTIN

DEFINITION

Calprotectin is a 36.5 kDA calcium and zinc-binding protein. It is present in neutrophil granules and represents 5% of total protein and 60% of the cytosolic proteins. It is also synthesised by monocytes and macrophages. It takes part in regulating mucosal immune responses by reacting with zinc-dependent metalloproteases which are required to activate pro-inflammatory cytokines such as TNFα. It is also believed to be involved in the regulation of apoptosis by inhibiting zinc-dependent enzymes.

INTRODUCTION

The diagnosis and monitoring of inflammatory diseases rely on imaging, although the areas affected may be difficult to access and the disease may be diffuse. The recent development of faecal markers overcomes this disadvantage because of their ability to reflect the whole damage to the gastro-intestinal tract. Faecal calprotectin concentrations reflect the extent of inflammatory infiltration of the intestinal mucosa and measurement has been proposed for a few years as a marker of organic disease of the gastro-intestinal tract in adults and children (inflammatory, infectious and neoplastic disease). The Positive Predictive Value (PPV) and Negative Predictive Value (NPV) at a threshold of 50 µg/g in stool samples are 76% and 89% respectively for the differential diagnosis of functional and organic damage. Highest concentrations are found in patients suffering from inflammatory bowel disease (Crohn's disease, ulcerative colitis or UC, indeterminate inflammatory colitis etc.). Faecal calprotectin concentrations in these patients correlate with the stage of the disease and the response to treatment.

INDICATIONS FOR MEASUREMENT

Faecal calprotectin measurement is requested to identify an inflammatory gastro-intestinal process in suggestive clinical situations. It is therefore requested to confirm or exclude the presence of organic disease, to assess the level of inflammation of the intestinal mucosa in patients with organic small or large bowel disease and to monitor the response to treatment. Measurements form part of the monitoring for risk of relapse in Ulcerative Colitis.

Measurements may be performed in the paediatric population, although the threshold value defined in adults is not reached before the age of 4 years old (*cf*. normal values).

INFORMATION

SAMPLE

Calprotectin is measured on a stool sample.

Radiological examinations with bowel opacification and colonoscopy preparations which alter the digestive ecosystem must be avoided for eight days before and during the stool collection. Care must be taken during the collection period to avoid substances which may interfere with analysis, such as laxatives, including paraffin oil, bowel cleansers and agents which delay bowel transit. In severe diarrhoea, the daily evacuation rate must be known in order to take this into account when interpreting the result.

SAMPLE STORAGE AND TRANSPORT

Calprotectin is highly resistant to intestinal proteolysis and is stable for one week at room temperature, allowing it to be sent at room temperature. The sample should then be stored at + 4°C if the measurement is to be performed within a week or frozen at– 20°C if the measurement is to be performed later. It is stable for 6 months at – 20°C.

ASSAY METHODS

Faecal calprotectin is assayed by an ELISA sandwich method after extracting faecal proteins.

NORMAL EXPECTED VALUES

The result is expressed in µg/g of faeces. Normal values are less than 50 µg/g of faeces. The threshold of 50 µg/g can be used after the age of 4 years old in a paediatric population. Faecal calprotectin concentrations are physiologically higher in younger children.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Day-to-day faecal calprotectin variations are minor, except in severe diarrhoea, due to exogenous sample dilution which can produce a falsely low result. In this case, the result should be re-checked on a new sample once the consistency of faeces has returned to normal.

PATHOLOGICAL VARIATIONS

Normal calprotectin concentrations in a patient with gastro-intestinal symptoms suggest functional bowel disorders. Conversely, a rise in faecal calprotectin indicates organic disease. This may include infectious, neoplastic or inflammatory disease. In the latter case, faecal calprotectin concentrations reflect the extent of the inflammatory process in the intestinal mucosa. Highest faecal concentrations are associated with active inflammatory bowel diseases. Conversely however, lower concentrations remaining mostly above normal values are seen in diseases during remission.

Calprotectin concentrations over 150 µg/g of stool in quiescent UC suggests that patients should be monitored more carefully because of a risk of relapse. The faecal calprotectin level which predicts risk of relapse in Crohn's Disease is more controversial.

FOR FURTHER INFORMATION

- ANAES, *Indications des examens de selles chez l'adulte*, Gastroenterol Clin Biol, 2003; 27: 627-642.
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- Rugtveit J., Fagerhol M.K., *Age-dependent variations in fecal calprotectin concentrations in children*, J Pediatr Gastroenterol Nutr 2002; 34: 323324.

FAECAL CHYMOTRYPSIN ACTIVITY

DEFINITION

The purpose of a determination of faecal chymotrypsin activity is to evaluate faecal proteolytic activity of pancreatic origin. In contrast to measurement of elastase, it involves measuring proteolytic activity; the result will therefore be sensitive to enzyme replacement therapy (pancreatic extracts), the only treatment for pancreatic exocrine insufficiency. The measurement is performed in order to reveal any pancreatic exocrine insufficiency or to monitor proper compliance with enzyme replacement therapy.

INTRODUCTION

Several pathologies are associated with the development of pancreatic exocrine insufficiency, in both adults and children. Diagnosing these pathologies is traditionally based on finding morphological changes which should instigate a search for a functional abnormality in pancreatic secretion. Secretin-pancreozymin stimulation tests continue to be the "reference examination", although they are no longer routinely used because of their invasive nature, especially in children. An indirect diagnostic alternative is available by measuring pancreatic enzymes in the stools, since these enzymes are resistant to digestive and bacterial proteolysis.

INDICATIONS FOR MEASUREMENT

A measurement of faecal chymotrypsin activity is prescribed in two situations:

– To evaluate pancreatic exocrine function. It will be required if poor digestion of pancreatic origin is suspected (chronic pancreatitis, mucoviscidosis, pancreatic surgery, etc.).

– To verify compliance with treatment by a patient suffering from pancreatic exocrine insufficiency and presenting with clinical symptoms associated with greasy diarrhoea.

INFORMATION

SAMPLE

The measurement of faecal chymotrypsin activity is performed on a stool sample.

In a patient suffering from pancreatic exocrine insufficiency, it will be necessary to confirm compliance with enzyme replacement therapy. If an assessment of residual pancreatic function is required, the patient must be asked to stop all treatment at least 3 days before the stool sample is collected. If, on the contrary, a confirmation of compliance with treatment is required, the patient must be asked to make no changes to normal habits.

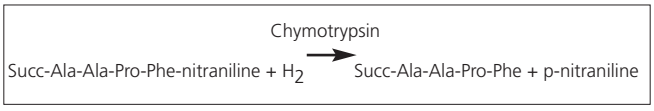
For the 8 preceding days and during sample collection, radiological examinations with digestive opacification, and preparations for colonoscopy which modify the digestive ecosystem, must be avoided. During the collection period, care must be taken to avoid products which can modify the analysis of data, such as laxatives (including medicinal paraffin), intestinal dressings or transit retarders. If the diarrhoea is severe, the daily faecal output will need to be known and taken into account in interpreting the results.

SAMPLE STORAGE AND TRANSPORT

Fecal chymotrypsic activity is stable for a week at +4° C. Stool containers must therefore be kept in a refrigerator between +2 and +8° C, if measurement is to take place within 7 days, or frozen at -20° C if measurement is to be later than this. The duration of stability at -20° C is one year.

ASSAY METHODS

Chymotrypsic enzyme activity is measured by colourimetric kinetic assay.



NORMAL EXPECTED VALUES

The results are expressed in UA per g of stool material at 25° C.

The normal value is in excess of 6 UA per g of stool material at 25° C.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Physiological variations in faecal chymotrypsin activity from one day to another are slight, except in cases of severe diarrhoea (exogenic dilution of the sample may result in a falsely low value being obtained). It is then advisable to check the result when the consistency of the sample has normalised.

PATHOLOGICAL VARIATIONS

A low concentration of faecal chymotrypsin indicates the existence of pancreatic exocrine insufficiency. The sensitivity of faecal chymotrypsin for diagnosing pancreatic exocrine insufficiency is lower than that of elastase, with the result that the activity often remains normal during minor or incipient pancreatic exocrine insufficiency. Conversely, it will be reduced in major pancreatic exocrine insufficiency. In a patient treated for pancreatic exocrine insufficiency by enzyme replacement therapy, a measurement of faecal lipids and faecal chymotrypsin will allow an evaluation of the efficacy of treatment and of compliance; faecal chymotrypsin is normalised in cases of efficacious enzyme replacement therapy, which is not the case for elastase.

FOR FURTHER INFORMATION

■ ANAES, *Indications des examens de selles chez l'adulte*, Gastroenterol Clin Biol, 2003; 27: 627-642.

■ DelMar E.G., Largman C., Brodrick J.W., Geokas M.C., *A sensitive new substrate for chymotrypsin*, Anal Biochem, 1979; 99: 316-20.

FAECAL E1 ELASTASE

DEFINITION

Measurement of faecal E1 elastase, commonly called faecal elastase, is used to assess faecal proteolytic activity originating from the pancreas. Unlike measurement of chymotrypsin, this involves an immunological assay and not a measurement of proteolytic activity. The result therefore only reflects trough pancreatic secretion and is not sensitive to treatment with replacement enzymes, which is the only treatment for exocrine pancreatic insufficiency. Measurement is therefore used to identify exocrine pancreatic insufficiency whether or not the patient has been treated with replacement enzymes. The test should be distinguished from measurement of NP elastase (neutrophil elastase) which can be used to identify inflammatory infiltration of the intestinal mucosa.

INTRODUCTION

Several diseases are associated with exocrine pancreatic insufficiency in adults and children. Conventionally their laboratory diagnosis is based on demonstrating morphological changes which trigger investigation for functional abnormalities of pancreatic secretion and testing for steatorrhoea. The secretin-pancreozymin stimulation test is still the "reference investigation" but is no longer used routinely as it is invasive, particularly in children. An indirect diagnostic alternative is measurement of pancreatic enzymes in faeces as these enzymes are resistant to gastro-intestinal and bacterial proteolysis.

INDICATIONS FOR MEASUREMENT

Faecal elastase is measured to assess exocrine pancreatic function. It should be requested if pancreatic malfunction is suspected: chronic pancreatitis, cystic fibrosis, post-pancreatic surgery, etc.

INFORMATION

SAMPLE

Elastase is measured on a stool sample. Radiological investigations with gastro-intestinal opacification and colonoscopy preparations which alter the gastrointestinal ecosystem must be avoided for 8 days before and during the collection. During the collection period, substances which can alter the results, such as laxatives, including paraffin oil, local intestinal preparations or those which delay bowel transit should be avoided. In severe diarrhoea the daily faecal output must be known in order to take this into account when interpreting the result.

SAMPLE STORAGE AND TRANSPORT

The sample can be stored for a maximum of 48 hours after the faeces are collected, at + 4°C, before being processed. Beyond this, freeze the sample at – 20°C.

ASSAY METHODS

Faecal elastase is measured by a sandwich ELISA method specific for human elastase (elastase E1). The result is expressed in µg/g of faeces.

NORMAL EXPECTED VALUES

The normal value is over 200 µg/g of faeces. The threshold of 200 µg/g applies from the end of the first month after birth in paediatric patients.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Day-to-day variations in elastase are small, except in severe diarrhoea causing exogenous dilution of the sample, which may produce a falsely low result. In this situation, the patient should be re-tested once sample consistency has returned to normal.

PATHOLOGICAL VARIATIONS

Low faecal elastase concentrations indicate exocrine pancreatic insufficiency. Concentrations between 150 and 200 µg/g reflect moderate exocrine pancreatic insufficiency and those below 15 µg/g indicates severe exocrine pancreatic insufficiency. Elastase is more sensitive than chymotrypsin activity to diagnose exocrine pancreatic insufficiency. It is therefore reduced in minor or early exocrine pancreatic insufficiency whereas chymotrypsin activity and faecal fat excretion often remain normal. As faecal elastase measurement is specific to human pancreatic elastase, it remains low in patients with pancreatic insufficiency treated with replacement enzyme therapy.

FOR FURTHER INFORMATION

- ANAES, *Indications des examens de selles chez l'adulte*, Gastroenterol Clin Biol, 2003; 27: 627-642.
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- Stein et *al., Mesure de l'élastase 1 fécale par immunoréactivité : une nouvelle approche indirecte de la fonction pancréatique*, Gastroenterol Clin Biol, 1996, 20: 424-9.

FAECAL NITROGEN EXCRETION
(FAECAL CREATININE OR CREATORRHEA)

DEFINITION

Measurement of faecal nitrogen excretion (often incorrectly called faecal creatinine) is requested to assess faecal protein loss. Faecal nitrogen excretion is defined as an increase in faecal protein output above normal values. The measurement used is that of elemental nitrogen and not purely protein nitrogen and it is more accurate to talk of faecal nitrogen excretion than faecal creatinine excretion. This investigation may be requested in combination with the measurement of faecal fat excretion to investigate and monitor malabsorption or maldigestion or in a nutritional assessment.

INTRODUCTION

Protein digestion begins in the small bowel lumen with the action of gastric and pancreatic proteases, and is continued in the jejunal enterocyte brush border and ileum with peptidases, depending on their specificity (endopeptidases, aminopeptidases, carboxypeptidases and dipeptidases). This phase is believed to result in the release of 30 to 40% of amino acids and 60 to 70% of oligopeptides formed from two to six amino acids. The intestinal epithelium absorbs these substrates in the form of free or di- and tripeptide amino acids through the specific active transporter systems. Oligopeptides are degraded secondarily by enterocyte peptidases with the result that it is almost entirely free amino acids which are transported by the portal system to the liver and then to the systemic circulation.

40 to 70% of intraluminal proteins are estimated to originate from the diet, the remainder being endogenous (enzymes and glycoproteins from salivary, gastric, pancreatic, intestinal or biliary secretions: intestinal cell sloughing and even exsudation of plasma proteins through the intestinal lumen).

INDICATIONS FOR MEASUREMENT

Measurement of faecal nitrogen excretion is used to assess the nitrogen balance which can be used to complement the investigation of gastro-intestinal function as lipid and nitrogen absorption can be dissociated. The investigation is requested in the context of investigation and monitoring of chronic diarrhoea, maldigestion or malabsorption syndrome and even exudative enteropathy, and in nutritional assessments.

Measurement of faecal nitrogen excretion is also used to identify inadequate dietary intake or even malnutrition, particularly in young children and in the elderly.

INFORMATION

SAMPLE

As applies to any faecal function studies, nitrogen excretion measurements can only be interpreted if all of the faeces passed in a given period have been collected into specific pots, taking care not to mix faeces and urine. For ease and hygiene purposes, the entire 24 hour faecal sample is collected into one or more opaque pots of adequate volume (1 litre being the usual pot volume used) and stored in a refrigerator. The date is recorded on each pot allowing daily faecal output to be measured and the average daily output to be calculated over the collection period.

There have been no specific studies on the optimal faecal collection period. It is usual to collect them over a period of three whole consecutive days also taking care to collect night faecal samples. A single day collection is probably inadequate although may be considered in people with limited spontaneous variation in bowel transit and in young children. Collections from newborn children must be made by positioning a bag around the anus in order to only collect faeces and avoid these being dehydrated by absorption on a nappy.

Radiological investigations with bowel opacification and colonoscopy preparations which alter the gastro-intestinal ecosystem must be avoided for 8 days before and during the collection. During the collection period, substances which could interfere with data analysis such as laxatives, including paraffin oil, bowel transit slowing agents and local intestinal preparations must be avoided. Any diarrhoea occurring in a context of infection must be avoided. Infectious diarrhoea may cause an increase in faecal nitrogen because of the presence of mucus, blood or leukocytes.

SAMPLE STORAGE AND TRANSPORT

The entire faecal collection (24, 48 or 72 hours) or an aliquot obtained after homogenising the 24, 48 or 72 hour sample stating the daily faecal output should be sent at + 4° to a laboratory. Samples can be stored at + 4°C if measurements are performed immediately or at – 20°C if the assays are to be performed later.

ASSAY METHODS

Nitrogen is measured after mineralising the sample by the Kjehldahl technique or by a mineral nitrogen detection technique using catharometry or chemoluminescence.

NORMAL EXPECTED VALUES

In a conventional Western diet normal faecal nitrogen excretion is between 0.8 and 2 g/24 hours in adults.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Physiological variations in bowel transit occur which justify collecting all faeces passed over a period of 3 consecutive days. The 3 day faecal collection must be carefully homogenised and assays performed on aliquot of the homogenate. Results are expressed as a mean value per 24 hours. If severe diarrhoea is present (accelerated bowel transit, motor or secretory diarrhoea) faecal nitrogen excretion may be reduced because of a drag effect.

PATHOLOGICAL VARIATIONS

Increased faecal nitrogen output is generally associated with increased fat output (steatorrhoea) in maldigestion or malabsorption. Depending on faecal output and microscopic examination results faecal drag azotorrhoea can be distinguished from malabsorption diarrhoea azotorrhoea due to endoluminal dysfunction (pancreatic enzyme deficiency) or malabsorption due to small bowel wall abnormalities (villous atrophy, infiltration or inflammation).

Increased nitrogen output may also been seen in major protein exsudation associated with chronic inflammatory disease or lymphatic obstruction (lymphangiectasia). Reduced nitrogen excretion indicates abnormal nutritional intake.

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FAMILIAL MEDITERRANEAN FEVER

DEFINITION

Familial Mediterranean Fever or Periodic Fever (FMF) is a hereditary condition transmitted as an autosomal recessive disorder. It is characterised by recurrent episodes of fever associated with inflammatory involvement of serosal surfaces and joints. It is almost exclusively confined to the population living around the Mediterranean. The importance of the condition lies in the risk of development of generalised amyloidosis. The most severe form of this involves progressive renal involvement, which terminates in renal failure. The treatment of the disease depends on regular medication with colchicine (1 to 2 mg daily).

Synonyms: Familial Mediterranean Fever (FMF); Periodic Fever; Hereditary Periodic Fever Syndromes; Mutations of MEFV gene.

INTRODUCTION

EPIDEMIOLOGY

FMF is mainly a disease of the population surrounding the Mediterranean basin, seen in Arab, Armenian, Sephardi Jews, Greeks, Turks, Lebanese, Kurds, Druze and Italians. In these populations the carrier rate of a mutation (heterozygote) is between 1/6 and 1/20, which results in a high probability of union between heterozygotes.

SYMPTOMS

In most cases the clinical manifestations appear early in childhood. The illness presents as inflammatory episodes or crises lasting 1 to 4 days. These crises are recurrent, occurring at intervals of several weeks or months, or they can even be years apart. During an acute episode, fever is almost always present (hence the name of the disease) in association with serosal inflammation, causing pain in the abdomen (peritonitis), joints (arthritis), chest (pleurisy) and muscles (myalgia). In addition, there may be erysipeloid lower limb lesions. Very often only one organ is clinically affected during an attack.

SEARCH INDICATIONS

Clinical suspicion of FMF.

Family screening (relative of a composite heterozygote or homozygous index case).

INFORMATION

SAMPLE

5 ml of whole blood in EDTA

LEGAL OBLIGATION TO ASK PATIENT

In France it is obligatory to have certification of a genetic consultation together with the request by the referring doctor (or alternatively the request from the referring doctor and the patient's informed consent) to allow a sample to be taken and the test to be performed. The result and, depending on the test, a report, are delivered only to the referring doctor.

REQUEST FORM

This should include information on the reason for genetic testing (clinical and test information), the aspects of the clinical picture that suggest FMF, the indications for the request, the family tree where an index case has been identified and the geographical origin of the subject.

SAMPLE STORAGE AND TRANSPORT

The sample is stored at room temperature or + 4°C.

The specimen is transported at ambient temperature or, if later than 24 hours from sampling, at + 4°C.

DIAGNOSTIC METHODS

Genotyping using multiplex PCR and reverse hybridisation with specific probes, sequencing, DGGE (denaturing gradient gel electrophoresis), PCR-RFLP *(Polymerase chain reaction Restriction fragment length polymorphism)*.

THE GENE

The gene responsible for this disease, named MEFV (abbreviation of MEditerranean FeVer) was discovered in 1992 to be located in a telomeric position on the short arm of chromosome 16 (in 16p13.3). In 1997 the responsible gene was simultaneously and independently cloned by an American-Israeli group *(International FMF Consortium)* and by a French group (*French FMF Consortium)*. The MEFV gene comprises 10 exons and extends over a region of 15 kb.

The gene is transcribed into a messenger RNA of 3.7 kb and is expressed exclusively in granulocytes (polymorphs). The RNA codes a 781 amino-acid protein, christened pyrin by the International Consortium and marenostrin (from the Latin *mare nostrum*) by the French Consortium. The distribution of mutations within the MEFV gene is not uniform: exon 10 is the seat of the most frequent mutations *(figure 1)*. At this stage, more than 75 mutations and/or polymorphisms have been described (MetaFMFdatabase: http://fmf.igh.cnrs.fr/metaFMF/index_us.html). Initially 4 mutations were identified in patients, mutations which were not found in healthy members of affected families or in control populations. These were missense mutations situated in exon 10 (M694V, M694I, M680I, V726A). These 4 mutations cover 80% of those found in affected subjects. Each one is associated with a different haplotype. Mutation M694V is associated with haplotype MED and is found in 90% of North African Sephardi Jews, 60% of Turks, 40% of Armenians and 30% of North African Arabs. Mutations M694I and V726A are associated with haplotypes ARA2 and ARM3 respectively. Mutation M694I is found particularly in North African Arabs, while V726A mainly involves Armenians and Druze. Finally, mutation M680I, associated with haplotype ARM2, is found predominantly in Armenians and Turks.

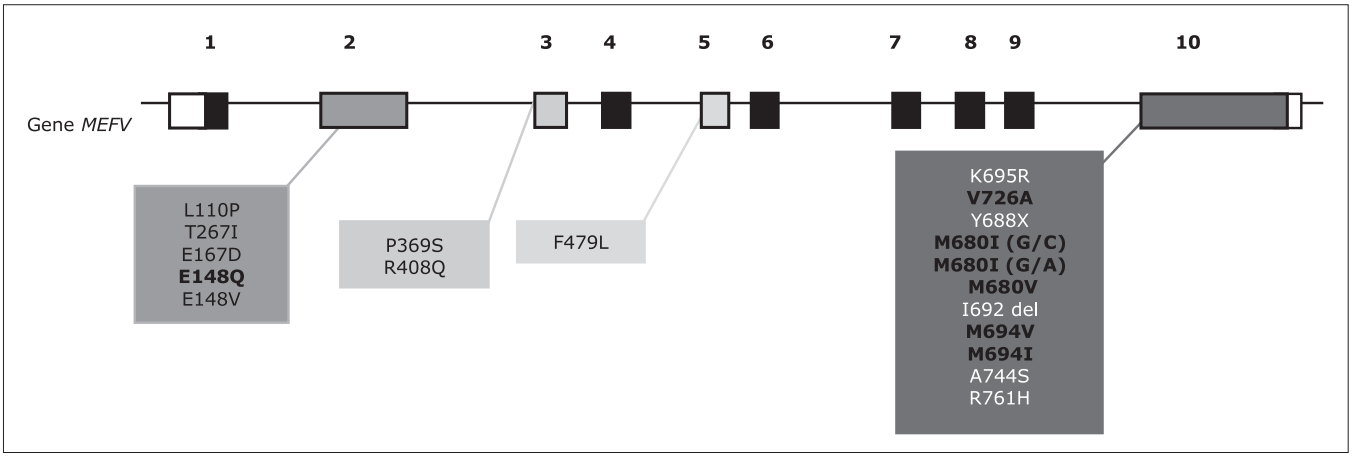

Fig 1 Diagram of MEFV gene coding pyrin/marenostrin with location of mutations (Chr. 16p13.3). The mutations labelled in bold characters are found in 85% of cases of FMF.

DIAGNOSIS

The diagnosis of FMF is essentially one of exclusion which relies on clinical and test result information after the exclusion of other diseases that can mimic FMF.

– Recurrent fever associated with episodes of inflammation.

– Familial nature of the disease.

– Member of an at-risk population.

– Presence of AA amyloidosis (also known as inflammatory amyloidosis).

– Therapeutic response to colchicine.

– Blood test results showing elevated ESR and CRP, leukocytosis, elevated SAA *(serum amyloid associated protein)*.

The importance of the molecular definition of the MEFV gene is to confirm the clinical diagnosis of the disease, but it also has a prognostic significance. The presence of 2 identical mutations (homozygote) or 2 different mutations (composite heterozygote) on each allele, confirms the diagnosis of FMF. If molecular analysis of the *MEFV* gene reveals only a single mutation, the diagnosis of FMF cannot be excluded. It is then necessary to embark on an exhaustive gene study by analysing the exons and the intron/exon junctions to try to detect another mutation. Data about the relationship between genotype and phenotype are sometimes contradictory. As the function of pyrin/marenostrin is not yet understood, the significance of mutations of the *MEFV* gene is difficult to interpret. FMF penetrance is incomplete. Indeed, studies reveal the existence of asymptomatic individuals carrying 2 mutated alleles. On the other hand, the observation of subjects carrying complex alleles (presence of 2 mutations on the same allele in the cis position) complicates interpretation of molecular analysis still further.

The severity of the phenotype decreases in the order of the following mutations: M694V, M680I, M694I, V728A and E148Q. Several studies have demonstrated that the phenotype associated with homozygous genotype M694V is more severe, with an earlier appearance of the disease and the development of renal amyloidosis. Finally, other factors have a bearing on the phenotypic expression of the disease, such as gender (penetrance is weaker in women) as well as the genotype at locus SAA1 *(Serum Amyloid Protein)*. The risk of developing renal amyloidosis is higher in the male and in those carrying a homozygotic SAA1 genotype.

DIFFERENTIAL DIAGNOSIS FROM OTHER RECURRENT HEREDITARY FEVERS

If the genetic picture is negative for FMF, one may need to consider other clinical conditions producing recurrent hereditary fever. These include hereditary fever associated with TNF receptor 1 (autosomal dominant transmission, gene TNFRS1A, involved in coding receptor 1A of tumour necrosis factor), hyperglobulinaemia D syndrome (autosomal recessive transmission, gene MVK coding mevalonate kinase) and Muckle-Wells syndrome (autosomal dominant transmission, gene CIAS1 coding cryopyrin, a protein closely resembling pyrin).

TREATMENT

Preventive treatment of affected individuals depends on taking colchicine, which forestalls the occurrence of inflammatory episodes and the development of renal amyloidosis. This treatment is only initiated after consideration of clinical features and genetic and other tests. It requires lifelong daily administration of colchicine (0.5 mg to 2 mg daily). Complete remission is found in 65% of patients.

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FDP

DEFINITION

The substrates for plasma are fibrinogen and types I and II fibrin molecules. The products are called FDP (fibrin and fibrinogen degradation products).

INTRODUCTION

The blood clot is physiologically resorbed by fibrinolysis. Plasmin, a key enzyme in fibrinolysis, is a serine-protease which degrades fibrin without destroying covalent between-chain bonds in type II fibrin (fibrin stabilised by the effect of factor XIII). At the C-terminal end of type II fibrin, plasmin releases D domain dimers (D-dimers), trimers and other polymers. Finding D-dimers indicates complete activation of coagulation prior to plasma activation. Following lysis of type 1 fibrin (or unstable fibrin) or fibrinogen, only fragments without inter-chain covalent bonds are formed. The majority of fibrinolysis occurs secondarily in the treatment of DVT or in DIC. Pure, so-called primary fibrinolytic overactivity is rare and is caused by an imbalance between the activity of plasmin and its inhibitor PAI. This is seen in lung, prostate and pancreatic surgery as these tissues are rich in plasmin activators (tPA) and are highly vascularised. Hyper-fibrinolysis therefore occasionally complicates disseminated prostate cancer, acute hepatic insufficiency, porto-caval anastamosis or extra-corporeal circulation.

INDICATIONS FOR MEASUREMENT

Specific measurement of FDP in the investigation of fibrinolysis has a role in emergency situations (DIC, diagnosis and monitoring of fibrinolysis) where a simple rapid sensitive inexpensive test is valuable.

On the other hand, measurement of D-dimers is replacing FDP as a better treat for the exclusion of deep vein thrombosis (DVT).

INFORMATION

SAMPLE

Citrated plasma.

A fasting sample is not required and patients may have a light, low fat snack.

For further information refer to the *"General preanalytical conditions in haemostasis"* page.

ESSENTIAL INFORMATION

Current treatment?

FDP rise with the thrombolytic treatments streptokinase, urokinase, rt-PA and tenecteplase. They may also rise during treatment with high dose heparin.

SAMPLE STORAGE AND TRANSPORT

Plasma can be stored for 4 hours at laboratory temperature, for 1 week at -20°C; beyond this period at – 80°C.

It is recommended that samples be thawed quickly in a water bath at + 37°C.

Transport: Samples should be centrifuged, separated and frozen within 4 hours of sampling.

ASSAY METHODS

Latex particle agglutination.

ELISA method.

REFERENCE VALUES

< 5 µg/ml.

INTERPRETATION

INTERFERENCES WITH MEASUREMENT

Rheumatoid factor in plasma may interfere with the latex particle agglutination test (false positives).

NB: A high serum FDP concentration increases the thrombin time and can interfere with chronometric measurement of fibrinogen.

PATHOLOGICAL VARIATIONS

FDP are raised in plasma whenever the fibrinolysis system is saturated.

Concentrations increase:

– In acute primary fibrinogenolysis (a rare disease)

– In fibrinolysis whether circulating (secondary to excess plasmin formation) or localised to a thrombus

– In disseminated intra-vascular coagulation (DIC): D-dimers are usually used to diagnose and monitor patients on treatment

The risk of a bleeding complication is increased by a factor of 4 in CVA treated with thrombolysis (rt-PA) if the FDP increase above 100 mg/l, 2 hours after starting treatment.

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FERRITIN

DEFINITION

Ferritin is a spherical macromolecule composed of an assembly of 24 sub-units of 2 types (H = *heart* and L = *liver*) in variable proportions, defining a central cup, within which iron is stored in the form of micelles of hydrated and phosphorylated oxides of iron. In-vivo, ferritin is present in the monocytes and macrophages of the liver, the spleen and the bone marrow. It is also found in the cytoplasm of hepatocytes, red blood cells and white cells as well as within the cells of various organs, such as the kidney, heart, lung, testis and placenta.

INTRODUCTION

Ferritin is the iron storage protein of the body. Its ability to hold iron confers on it the function of iron depot and also that of iron detoxification. The blood level of ferritin follows in parallel to that in tissues, so that measurement of the blood level gives a good reflection of easily mobilised body iron stores.

Ferritin is also an acute phase protein with its production increasing when there is macrophage activation. It is also elevated in other situations, notably during liver cell lysis or in the presence of a tumour. In these situations the serum ferritin no longer mirrors the iron stores. The red cell ferritin then becomes important because it is not affected by these phenomena. In general, the indications for its measurement are the same as those for serum ferritin, but the assay is more difficult.

INDICATIONS FOR MEASUREMENT

Principal indications: diagnosis of states of deficiency or overload of iron and monitoring of iron replacement therapy or, for overload, monitoring the effect of therapeutic removal of iron (by phlebotomy).

Minor indications: diagnostic aid in clinical situations with iron overload: hereditary caeruloplasmin deficiency or certain types of haemochromatosis. Similarly used in some situations without iron overload: Gaucher's disease, Still's disease and the hyperferritinaemia-cataract syndrome.

INFORMATION

SAMPLE

Serum (dry tube) or plasma in heparinised or EDTA tube. The patient should preferably be fasting (post-prandial changes have been described).

Discard haemolysed specimens (red cells are rich in ferritin).

QUESTIONS FOR THE PATIENT

Are you taking any medication? Iron treatment increases ferritin levels.

SAMPLE STORAGE AND TRANSPORT

Store serum or plasma for 1 week at + 4°C and 6 months at – 20°C.

Transport at + 4°C or frozen.

ASSAY METHODS

Immunoprecipitation in fluid medium (nephelometry or turbidimetry) and immunometric sandwich method.

NORMAL EXPECTED VALUES

Serum ferritin:

Example: in adult male: 20 to 200 µg/l; in pre-menopausal adult female: 10 to 125 µg/l; post-menopause: 20 to 200 µg/l.

Child:

New-born	50 to 400 µg/l
Infant < 1 month	90 to 600 µg/l
Infant 1 to 2 months	140 to 400 µg/l
Infant 2 to 6 months	40 to 220 µg/l
Infant > 6 months and children up to 15 years	15 to 80 µg/l

Red cell ferritin: adult male: 5 to 38 ag/cell (attogram; atto = 10^{-18}); adult female: 3 to 24 ag/cell.

There are two international standards for calibration of serum ferritin assay kits: the 2nd international standard derived from human spleen (NIBSC code 80/578) and the 3rd international recombinant standard (NIBSC code 94/572).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

There are variations with age and gender (see normal values given above) and a reduction in pregnancy, from the second trimester (progressive exhaustion of iron reserves).

There is also a mild reduction in subjects who exercise intensively and regularly.

PATHOLOGICAL VARIATIONS

Hypoferritinaemia

Early diagnosis of iron deficiency: A reduction in ferritin levels is a very sensitive and specific sign of iron depletion (no other condition causes hypoferritinaemia). It is the first manifestation of iron depletion in the adult, before the appearance of haematological abnormalities. Because of this, monitoring of ferritin levels aids the diagnosis and follow-up of iron deficient anaemias, as well as the management of the iron status of pregnant women, blood donors, those on chronic haemodialysis and patients with digestive tract or gynaecological bleeding.

Hyperferritinaemia

In general, hyperferritinaemia cannot be interpreted in isolation. There is a need for other measurements especially other iron tests.

Genetic haemochromatosis (HFE-1 in the great majority of cases or rarely HFE-2 and HFE-3): in the absence of a clearly suggestive clinical context, the combination of a Hyperferritinaemia (often > 1,000 µg/l) with a high degree of transferrin saturation (often > 80%) points to the diagnosis. In this situation, the degree of elevation of the ferritin level is an excellent reflection of the extent of iron overload (in the absence of associated factors which might affect this elevation, see below).

Metabolic syndrome: (ferritin usually between 600 and 1,000 µg/l): In this condition, the iron saturation of transferrin is normal and the patient shows other features associated with cardiovascular risk (hypertension, diabetes, dyslipidaemia, etc.).

Other frequent causes:

– Liver cell lysis in acute (usually) or chronic hepatitis. As well as hyperferritinaemia, there is slight elevation of serum iron and of transferrin saturation

– Muscle cell lysis in cardiac myolysis or rhabdomyolysis (peripheral muscle)

– Inflammatory syndromes where the hyperferritinaemia is usually < 500 µg/l with reduction in serum iron and transferrin saturation

– Chronic alcoholism: Hyperferritinaemia, sometimes > 1,000 µg/l because of alcohol-stimulated ferritin synthesis. In 50% of cases there is also increased serum iron. With abstention from alcohol, the serum iron returns to normal in less than a week, but the ferritin level falls slowly, taking some 3 months to achieve normality

– Post-transfusion overload.

Rare and exceptional causes:

With visceral iron overload:

– Hereditary caeruloplasmin deficiency: clear hyperferritinaemia with a tendency to anaemia, reduction in serum iron and transferrin saturation and a very low serum caeruloplasmin level.

– Mutation of the ferroportin gene: a recently identified condition with substantial iron overload, characterised by serum ferritin > 1,000 µg/l, normal or slightly elevated transferrin saturation and a tendency to anaemia.

Without iron overload:

– Gaucher's disease: ferritin level usually between 1,000 and 2,000 µg/l (without an increase in serum iron or increased transferrin saturation).

– Hyperferritinaemia-cataract syndrome: this is a familial syndrome, sometimes mimicking haemochromatosis. The eye signs are a clue to the diagnosis.

– Viral activation of macrophages: during EBV or HSV (herpes simplex) infections.

– Still's disease: massively elevated serum ferritin (> 10,000 µg/l) combined with a substantial decrease of the glycosylated fraction of the ferritin.

– Solid tumours, blood disorders and hyperthyroidism: slight hyperferritinaemia.

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FIBRINOGEN

DEFINITION

Fibrinogen is a high molecular weight glycoprotein synthesised by the liver and involved in thrombogenesis and atherogenesis.

Synonyms: Factor I, functional fibrinogen and immunological fibrinogen.

INTRODUCTION

– The protein involved in the final step of the coagulation cascade: fibrinogen is converted by thrombin into fibrin, the principal protein component of the blood clot. Its circulating half-life is 3 to 4 days. The minimum plasma concentration of fibrinogen compatible with normal haemostasis is 0.4 to 0.5 g/l.

– Acute phase protein.

– It participates in platelet aggregation.

– Concerned in the development of atherosclerosis: it encourages the accumulation of LDL cholesterol in blood vessel walls. Once it is converted into fibrin, it stimulates the proliferation of fibroblasts and smooth muscle cells.

INDICATIONS FOR MEASUREMENT

For the diagnosis of a bleeding disorder, or investigation of the aetiology of venous thrombosis by looking for hypo- or dysfibrinogenaemia.

Routine or pre-operative screening.

For the Investigation of inflammation and screening of cardiovascular risk factors.

INFORMATION

SAMPLE

Take into a citrated tube with citrate at a concentration of 3.2% (0.109 M) which becomes diluted by 1/10 (0.5 ml citrate solution for 4.5 ml of blood). Citrated tubes at 3.8% (0.129 M) are also acceptable.

If the patient has received thrombolytic treatment, the blood must be taken into a tube containing an inhibitor of fibrinolysis (aprotinin).

The blood should preferably be taken in a fasting subject in the morning between 07:00 and 11:00 hrs. Coffee, tobacco and alcohol must be avoided in the hour before sampling. A light, fat-free breakfast is allowed.

The blood must be centrifuged soon after sampling and the assay performed within 4 hours of blood sampling. The specimen should be checked for micro-clots. Haemolysed or opalescent samples should be discarded.

ESSENTIAL INFORMATION

Current treatment?

Thrombolytic therapy reduces the fibrinogen level: streptokinase, urokinase, rt-PA and tenecteplase.

SAMPLE STORAGE AND TRANSPORT

4 hours at laboratory temperature, 1 week at – 20°C or longer periods at – 80°C.

Rapid thawing is recommended at 37°C in a water bath.

Transport: frozen plasma within 4 hours of sampling.

ASSAY METHODS

FUNCTIONAL FIBRINOGEN:

Thrombin time is measured with defined conditions for the dilution of plasma, using the Von Clauss chronometric method. In the presence of an excess of thrombin and low concentrations of fibrinogen, the coagulation time is proportional to the "functional" fibrinogen. The measured time can be converted into concentration in g/l with a standard curve produced using a control plasma containing a known concentration of fibrinogen. If the fibrinogen concentration is very low or very high, it is appropriate to alter the plasma dilution so as to remain within the zone of linearity of the assay.

TOTAL FIBRINOGEN:

– Measurement of weight after treatment with a solution of thrombin and ionised calcium, which converts the fibrinogen to fibrin. It is then separated, washed and dried and the clot is weighed.

– Immunological assay: Immunoturbidimetry or quantitative radial immunodiffusion.

REFERENCE VALUES

"Functional or immunological" fibrinogen: 2 to 4 g/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

"Normal" values are lower in the young child (1.50 to 3.50 g/l). The plasma concentration of fibrinogen increases progressively though pregnancy.

PATHOLOGICAL VARIATIONS

High fibrinogen (hyperfibrinogenaemia)

– Inflammatory syndromes and cancer

– Nephrotic syndrome

– HIV infection

– Fibrinogen is a major independent cardiovascular risk factor. According to W. Koenig's meta-analysis (Thromb Haemost 2003) there is a significant association between increase in plasma concentration of fibrinogen, even when this is only modest (+ 10%), and the development of coronary artery disease. The Odds ratio between the upper and lower tertiles of the fibrinogen distribution is 1.8 (CI 95%, 1.6 to 2).

Elevation of fibrinogen is also associated with angina (stable and unstable), the occurrence of coronary artery complications after surgery, sudden death, myocardial infarction, cerebrovascular accidents and, finally, overall mortality

– Increased in smokers.

■ **Low fibrinogen (hypofibrinogenaemia)**

Acquired hypofibrinogenaemia:

– Liver failure

– Defibrination syndromes, such as disseminated intravascular coagulation (DIC) or fibrinolysis, either primary or therapeutic. Monitoring of the plasma fibrinogen concentration is useful in the follow-up of these syndromes

– The presence in plasma of a substance with anti-thrombin activity can interfere with the chronometric assay. Such agents include circulating anticoagulant, fibrin degradation products or D-dimers in large amounts and high dose heparin.

Constitutional fibrinogen deficiency:

– Afibrinogenaemia (absence of the protein) or hypofibrinogenaemia (presence in low concentration: between 0.2 and 1.2 g/l) produces a quantitative deficit (the activity and the protein level measured by weight or immunologically are reduced to the same degree).

– Dysfibrinogenaemia (qualitative deficit): the protein is present in normal concentrations (assay by weight or immunologically) but is functionally impaired (fibrinogen measured using the chronometric assay is reduced).

Constitutional dysfibrinogenaemias are usually asymptomatic. They sometimes present with a mild bleeding disorder and are associated in some 10% of cases with the development of venous or arterial thrombosis. There are rare cases of acquired dysfibrinogenaemia (hepatoma and liver cirrhosis).

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FIBRONECTIN

DEFINITION

The term fibronectin covers a group of ubiquitous adhesive glycoproteins involved in intercellular adhesion. They are present on cell surfaces, in plasma and in amniotic fluid. They are dimeric macromolecules with a molecular mass of 440 kDa, whose gene is found on chromosome 2.

INTRODUCTION

Plasma fibronectin participates in the reticulo-endothelial system's process of elimination of cellular debris and various cells (bacterial, tumour and others). It also plays a role in cell growth, scar formation and haemostasis.

INDICATIONS FOR MEASUREMENT

The measurement of plasma fibronectin has been suggested for the diagnosis and follow-up of poor nutrition and malnutrition, especially in paediatric patients.

In practice, the main reason for measuring plasma fibronectin is in the diagnosis of pre-eclampsia in pregnancy.

The measurement of foetal fibronectin is used to predict premature labour.

INFORMATION

SAMPLE

Plasma fibronectin: Preferably EDTA tube or citrated (take account of dilution due to volume of citrate).

Foetal fibronectin: Sample of vaginal or cervical secretions using a specific swab. Sampling can only be performed after 20-22 weeks of amenorrhoea. It must not be done within 24 hours after coitus or manipulation of the cervix. The result can be misleading if there is an infection (fungal or vaginitis).

This test is not valid if there has been placental separation or placenta *praevia*.

QUESTION FOR THE PATIENT

Pregnancy (determine term)?

SAMPLE STORAGE AND TRANSPORT

For plasma fibronectin the sample should be separated rapidly. Samples can be kept for a maximum of 8 days at + 4°C, or can be frozen, as long as freezing is performed within 24 hours of taking the specimen.

Transport at + 4°C or frozen.

For foetal fibronectin it is essential that the specimen is dealt with within 4 hours following sampling.

ASSAY METHODS

Immunonephelometry is the most commonly used method for assay of plasma fibronectin.

Foetal fibronectin is assayed using a rapid qualitative test. The fibronectin is detected in a membrane immunoassay with a detection threshold of 50 ng/ml. Strict conditions for reading the assay must be adhered to.

NORMAL EXPECTED VALUES

Plasma fibronectin: reference values are from 0.25 to 0.40 g/l.

Foetal fibronectin: after the 22nd week of pregnancy – concentration < 50 ng/ml.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

During normal pregnancy, plasma fibronectin rises by some 20% during the first quarter.

The presence of foetal fibronectin in vaginal and cervical secretions before 20-22 weeks of amenorrhoea is physiological: the test is therefore, not interpretable before this date.

PATHOLOGICAL VARIATIONS

Plasma fibronectin:

– In periods of poor nutrition the plasma fibronectin level falls. After correction of the nutritional deficiency, it returns to normal over several days (half-life measured at 15 – 20 hours).

– In patients with pre-eclampsia, the majority of studies show an approximate doubling of plasma concentrations, this elevation usually appearing before the development of hypertension.

Foetal fibronectin:

The presence of foetal fibronectin in vaginal or cervical secretions at levels above 50 ng/ml after the 22nd week of amenorrhoea is a risk marker of premature labour independently of uterine contractions or cervical changes.

The predictive value of the test is better in women who are in a group at risk of premature labour. In these at-risk women the predictive value of a negative test is considered to be excellent. The predictive value of a positive test, however, is poor. In an unselected population, there is uncertainty about the value of the test. It seems to be better when it is performed at an early stage (a positive test at 22-24 weeks of amenorrhoea is said to predict 50% of cases of premature labour).

FOR FURTHER INFORMATION

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- Roubille M., Mailliavin A., Biguet-Vernier B. et *al*. *Intérêt de la recherche de la fibronectine fœtale dans le dépistage du risque d'accouchement prématuré*, Ann Biol Clin 1999; 57: 93-7.

FILARIASIS

DEFINITION

The filariases are tropical parasitic diseases affecting some 150 million people in the world. The responsible organisms are viviparous nematodes known as filariae. The filariases are classified according to the location occupied by the adult worms:

– ***Lymphatic filariasis:*** Wuchereria bancrofti and Brugia malayi.

– ***Deep cutaneous filariasis:*** lLoa Loa, Onchocerciasis and Dracunculiasis.

– ***Serosal filariasis:*** Mansonella perstans and Mansonella ozzardi.

Synonyms: Wuchereria bancrofti / Bancroft's filaria. Brugia malayi / Malaysian filaria. Dracunculus medinensis / Medina worm or Guinea worm.

INTRODUCTION

EPIDEMIOLOGY

- **Lymphatic filariasis:** *Wuchereria bancrofti* is the most widespread filaria in the world, being found in Asia, Africa and South and Central America. The Pacific variety is present in Oceania. The geographic distribution of Brugia malayi is confined to Asia. Adult filariae reside in the lymph nodes and lymphatic vessels. Microfilariae are transmitted by Anopheles, *Aedes, Culex* and *Mansoni* mosquitoes. In the mosquito they turn into infective larvae over 12 to 14 days. They are then passed on to a new host during a fresh blood meal. They develop in the lymphatic system and become adult in 6 months to a year.
- **Loa Loa:** This is a specifically human cutaneous filariasis caused by the *Loa Loa* filaria. It is confined to Africa and, more particularly, to the Central African forest. It is transmitted by the Chrysops fly. The adult filariae, which can live for longer than 15 years, live in the cellular sub-cutaneous tissue.
- **Onchocerciasis:** This cutaneous filariasis is caused by *Onchocerca volvulus* and is transmitted by an insect vector, known as the Simuliid fly. It is active in tropical Africa and Central and South America.
- **Dracunculosis:** This is caused by *Dracunculus medinensis* and is also a skin infestation. It is rife in certain tropical and sub-tropical regions of Asia and Africa.
- **Serosal filariasis:** These are anthropod zoonoses, which arepathogenic in man and transmitted by Culicoid flies. The diseases are distributed in West Africa and Central and South America.

SYMPTOMS

- **Lymphatic filariasis:** The clinical manifestations are diverse. There are many asymptomatic forms in healthy carriers in endemic areas. The acute symptomatic forms include acute lymphangitic signs in the limbs, especially the lower limbs and lymphangitis of the genitalia (scrotum, orchitis and epididymitis). Chronic disease results from a progressive obstruction of the lymph vessels by filariae and can appear many years after the acute episode. Features include hydrocele, lymphatic varices, chyluria, chronic orchido-epididymitis, elephantiasis of limbs and genitalia.
- **Loa Loa:** The classical signs are localised pruritus (arms, thorax, face and shoulders), sub-cutaneous or sub-conjunctival migration of an adult worm and fleeting localised pruriginous oedema. There are sometimes neurological, renal or cardiac complications.
- **Onchocerciasis:** Symptoms are cutaneous (pruritus, filarial "scabies" and skin atrophy) and ocular (keratitis, chorioretinitis and post-neuritic optic atrophy) associated with the presence of oncocerchal cysts which enclose adult filariae.
- **Dracunculosis:** The symptoms depend on the appearance of the female worm in the sub-cutaneous tissues, causing an ulcer, then a vesicle through which the worm is slowly extruded. This orifice is usually situated on the leg.

SEARCH INDICATIONS

Diagnosis of lymphatic filariasis.

Diagnosis of Loa Loa.

Diagnosis of Onchocerciasis.

Diagnosis of Dracunculosis.

Diagnosis of serosal filariasis.

INFORMATION

SAMPLE

Venous blood: EDTA specimen for direct examination for microfilariae.

Venous blood: Dry tube for serological diagnosis.

Skin: Exsanguinated cutaneous biopsy.

Examination of the blood for microfilariae should be carried out at a time corresponding to the periodicity of the species being studied:

– Between 22.00 hrs and 04.00 hrs for *Wuchereria bancrofti* and *Brugia malayi*.

– Between 12.00 hrs and 15.00 hrs for *Loa Loa*.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

Possibility of insect bite?

Time spent in an area where filariasis is endemic?

SAMPLE STORAGE AND TRANSPORT

Blood samples can be transported at + 4°C without compromising urgency because of the time of day.

Serum can be transported at + 4°C or frozen at – 20°C.

Skin specimens should be transported within two or three hours after the biopsy procedure.

DIAGNOSTIC METHODS

PARASITOLOGICAL DIAGNOSIS

The demonstration of the adult parasite or microfilariae makes a definitive diagnosis. It is rare to find adult filariae.

However, adult worms of Loa Loa can be seen and removed when they are moving through the conjunctiva or under the skin. It is also possible to identify an adult Guinea worm (Dracunculus) when it can be felt as an indurated cord-like sub-cutaneous structure.

Onchocerca volvulus microfilariae can be seen in exsanguinated skin biopsies and also in fluid aspirate from an onchocercal nodule or in the urine. With the lymphatic filariases and Loa Loa the blood is examined for microfilariae.

– ***Direct examination of fresh blood*** can reveal microfilariae by virtue of their rapid movements.

– ***Thick and thin film preparations*** are used to identify microfilariae according to their species characteristics (size, colour or absence of colour of the sheath; size and appearance of the core...).

– ***Concentration techniques*** using saponin or the QBC® technique may be used if direct examination is negative.

– ***Microfilarial count in the blood*** is useful before the start of treatment with a microfilaricide.

■ SEROLOGICAL DIAGNOSIS

– ***Demonstration of antibodies:*** Screening should be performed using two techniques in combination:

ELISA, indirect immunofluorescence or electrosyneresis. If the tests are both positive or are discrepant, immuno-electrophoresis is necessary to confirm the diagnosis. In practice only an ELISA test is commercially available. It uses *Acanthocheilonema vitae* and it is satisfactory for the diagnosis of Onchocerciasis and Loa Loa, but poor for *W. bancrofti* and of no use for *B.malayi*. Antibody titres are very high during allergic filariasis but less so in patients with microfilaraemia. Test interpretation needs to take account of false negatives and also of cross-reaction with other helminths (Strongyloidiasis and Anisakiasis).

– ***Circulating antigens of W.bancrofti:*** This technique uses monoclonal antibodies. It is the method of choice for diagnosis of lymphatic filariasis caused by *W. Bancrofti* as well as offering a method whereby the effectiveness of therapy can be monitored by measuring the parasitic load.

TREATMENT

The microfilaricidal drugs that are in use include diethylcarbamazine, ivermectin and albendazole. They are often taken in combination. Symptomatic treatment and surgery can also be employed. Prophylaxis involves measures taken against the vectors, protection from insect bites and the elimination of microfilariae in carriers, by systematic treatment in endemic zones.

FOR FURTHER INFORMATION

■ Carme B., *Filarioses lymphatiques*, Encycl Med Chir. Elsevier Paris; 2005: p1-14.

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FLECAINIDE

DEFINITION

Flecainide is a class 1C anti-arrhythmic drug (Vaughan-Williams classification). It is supplied as 100mg divisible tablets; as delayed release capsules of 50,100,150 and 200 mg; and as an injectable solution of 40 mg in ampoules of 4 ml. It is given for prevention of life-threatening episodes of ventricular tachycardia, for symptomatic and incapacitating ventricular tachycardia itself and in supra-ventricular tachycardia. Its marked negative inotropic effect contra-indicates its use in patients with impaired left ventricular function.

The usual oral dose regime is 50 to 200 mg twice daily. The maximum dose is 300 mg total per 24 hours. The parenteral route is reserved for hospital administration: the high-dose treatment is by slow intravenous injection of 1 to 2 mg/kg given over at least 10 minutes, followed by IV infusion of 0.02 mg/kg/min for one hour, then 0.003 mg/kg/min continuing infusion.

PHARMACOKINETICS

Bioavailability (oral route)	90 to 95%
Plasma peak (Tmax)	3 hours, average
Time to steady state	3 to 5 days
Protein binding	Approximately 40%
Metabolism	Hepatic, mainly by CYP2D6; two principal metabolites identified: 2-0-dealkyl flecainide and 2-0-dealkyl lactame flecainide, inactive and excreted as glucuro- and sulpho- conjugates.
Plasma half-life	14 hours in healthy subjects. Varies with age: 20 hours at birth, 11 to 12 hours at 3 months, 10 hours at 1 year, 8 hours at 1 to 12 years. Increased in hepatic insufficiency (from 20 to 45 hours, up to 110 hours in cirrhosis) and also in heart failure and arrhythmia (12 to 30 hours).
Excretion	80 to 90% in urine: within 24 hours some 25% of the initial dose is excreted unchanged; the rest as metabolites. Excretion is reduced when the urinary pH is high.

INDICATIONS FOR MEASUREMENT

Assay is justified by the narrow therapeutic index of the drug, inter-subject variation in drug pharmacokinetics and by the known correlation between serum/plasma levels and therapeutic effect. It is indicated:

– Where the drug is therapeutically ineffective, despite being used in normal doses

– When signs of overdose appear, such as ECG changes (widening of the QRS complex), cardiogenic shock, sometimes in association with neurosensory and neuropsychiatric manifestations

– Where there are changes in flecainide metabolism:

Changes in absorption: The absorption of oral tablets is reduced if taken at the same time as food, especially with milk. In fact, assay is recommended when the feeding regime is changed in the new-born baby.

Changes in excretion, with hepatic insufficiency or heart disease.

Changes secondary to drug interactions *(see below)*.

INFORMATION

SAMPLE

Serum or plasma in EDTA or a heparinised tube. Avoid tubes with gel separator.

Sample just before the next dose (trough level), at steady state (4th or 5th day after treatment start).

QUESTIONS FOR THE PATIENT

It is mandatory that any request form for the measurement of a drug level must carry the reason for the investigation (looking for effectiveness or toxicity), the exact time of sampling and the date when treatment was started and/or of any change in dose regime. There must also be an account of the drug regime itself (dose and frequency and route of administration) as well as the age of the patient and his/her height and weight when available.

Are you taking any other medication?

Combination with fluoxetine, propranolol or cimetidine increases the excretory half-life by 10%. Combination with amiodarone increases plasma concentrations of flecainide by a factor of 2 and necessitates a reduction in the flecainide dose and careful monitoring because of the risk of over-treatment.

Combination with carbamazepine, phenobarbitone and phenytoin increases metabolism of flecainide, which can then become clinically ineffective.

SAMPLE STORAGE AND TRANSPORT

If the assay is to be delayed, freeze at – 20°C within 4 hours of collection. Store and transport at – 20°C.

ASSAY METHODS

High performance liquid chromatography.

NORMAL EXPECTED VALUES

Desirable trough level in steady state:

Adult: 0.3 to 0.8 mg/l (0.7 to 2 µmol/l); child: 0.2 to 0.5 mg/l (0.5 to 1.2 µmol/l).

FOR FURTHER INFORMATION

■ Dictionnaire Vidal®.

■ Delhotal B, Compagnon P., *Suivi thérapeutique du flécaïnide*. In: Suivi thérapeutique pharmacologique pour l'adaptation de posologie des médicaments, Collection Option/Bio, Ed Elsevier, Paris, 2004:349-54.

FLUORINE

DEFINITION

Fluorine/fluorides are halogen derivatives found in the ground in combination with calcium. Physiologically, it is essential for the normal calcification of bones and tooth enamel and is incorporated in these. Around 99% of body fluorine is bound in the skeleton. 75% of plasma circulating fluoride is not bound. It is recommended for the prevention of dental caries and for reducing the risk of osteoporosis. It is used in toothpaste, water, table salt and some nutritional products. It is also a raw material for various industries, such as aluminium, steel, pesticide and fertiliser, etc. Exposure can be by inhalation of dust or smoke and up to 99% of inhaled material can be absorbed through the lung.

INTRODUCTION

An excess of fluoride is directly toxic on tooth enamel and on the skeleton.

Acute poisoning initially presents with local effects, including irritation of the buccal and digestive tract mucosa, together with thirst and a taste of soap and salt. Later, there is abdominal pain, gastro-intestinal haemorrhage, nausea, vomiting, diarrhoea and tetany, due to hypocalcaemia. There may be, neurological manifestations such as tremor and paraesthesiae and cardiac arrhythmia. Death due to cardio-respiratory arrest can occur within 2 to 4 hours after exposure. 5 to 10g of sodium fluoride is thought to be the lethal dose.

In chronic exposure (bone fluorosis) there are spontaneous fractures and calcification of ligaments and tendons. In children, the teeth become discoloured.

Fluoride is excreted rapidly, almost exclusively via the renal route. Its half-life is split into three phases: 7 hours, 10 days and 8 years.

INDICATIONS FOR MEASUREMENT

Diagnosis and follow-up of fluoride poisoning.

INFORMATION

■ SAMPLE

Dry tube for a serum sample, or EDTA for plasma. Do not use a gel separator or a glass tube.

Urine: Collected in a plastic pot without preservative. A sample or 24 hr collection (diuresis should be specified).

To assess the risk of occupational exposure, collect:

– At beginning of work (beginning of week): measure of body fluoride load

– At end of work: assessment of exposure during the period of employment.

■ QUESTIONS FOR THE PATIENT

To evaluate exposure risk: what is the nature of the work? Date of exposure?

To evaluate clinical manifestations: is there bone damage? Are there any problems suggesting acute poisoning?

■ SAMPLE STORAGE AND TRANSPORT

The serum/plasma must be removed from the dry and EDTA tubes within 45 minutes of sampling and should be transferred to a plastic tube to avoid the release of fluoride contained within glass. The sample should be kept at room temperature.

ASSAY METHODS

Potentiometry, ionometry and fluoride specific electrode.

NORMAL EXPECTED VALUES

In non-exposed subjects: serum fluoride < 30 µg/l; plasma fluoride < 20 µg/l; urinary fluoride: 600 to 1960 µg/l (800 to 2,950 µg/ 24h) or 0.5 to 2.5 mg/g of creatinine.

Guide values in individuals who have occupational exposure: 3 mg/g of creatinine before starting work; 10 mg/g of creatinine at end of work period.

PATHOLOGICAL VARIATIONS

Any elevation above 300 µg/l in serum, or 2.5 mg/g of creatinine in urine, suggests acute poisoning or fluorosis.

FOR FURTHER INFORMATION

■ McDonagh M.S., Whiting P.F., Wilson P.M., Sutton A.J., Chestnutt I., Cooper J. et *al*. *Systematic review of water fluoridation*, BMJ, 2000; 321: 855-859.

■ *Dosage du fluor sanguin*, fiche INRS :
/www. inrs. fr/htm/nature_du_dosage_fluorures_sanguin.html.

■ *Dosage du fluor urinaire*, fiche INRS :
www.inrs.fr/INRS-PUB/ inrs01.nsf/ Intranet Objectacces Par Reference /BIO_SD_034/$File/visu.html

5 – FLUOROURACIL

DEFINITION

5-Fluorouracil (5-FU) is an anti-metabolite (anti-pyrimidine) cytostatic anti-cancer drug. It has three major anti-metabolic actions:

– It is metabolised to 5-fluorodeoxyuridine 5' monophosphate which, in the presence of 6-methylene tetrahydrofolate, blocks the methylation of uracil in thymine, thus inhibiting DNA synthesis and, therefore, cellular proliferation

– On phosphorylation it becomes a triphosphate which is incorporated instead of uracil into messenger RNA, causing errors in reading of the genetic code during protein synthesis

– It inhibits uridine phosphorylase.

5-FU is supplied as injectable solutions at 250 mg/5 ml, 500 mg/10 ml, 1 g/50 ml and 5 g/100 ml and also as capsules (combination of Tegafur, a 5-FU pro-drug, and uracil) and as a cream.

Parenteral 5-FU is used in the treatment of advanced gastro-intestinal adenocarcinoma, as adjuvant treatment after resection of colo-rectal cancer, for adenocarcinoma of the breast after local and regional treatment or after relapse. It is also given for ovarian adenocarcinoma and in epidermoid carcinoma of the pharynx and oesophagus. It is usually prescribed in combination with folinic acid.

When given as monotherapy, the usual regime is: 400 to 600 mg/m2/day, 3 to 6 days per month as an intravenous infusion (IV) over one hour. When administered in combination with other cytotoxics: 300 to 600 mg/m2/day, 2 to 5 days in cycles of 3 to 4 weeks. It is used occasionally as a slow intra-hepatic infusion or as a continuous IV infusion.

When administered orally, the dose is 300 mg/m2/day of tegafur in 3 divided doses, one hour before or after meals, in 28 day courses.

As a cream it is used in the treatment of pre-epitheliomatous keratoses, Bowen's disease and Queyrat's erythroplasia when surgery is not possible. It is also administered for genital warts.

Synonyms: Fluorouracil, 5-FU.

METABOLISM

Bioavailability	Oral administration: absorption very variable from 0 to 80%. Cutaneous route: absorption through healthy skin estimated at 6%, around 15% through damaged skin.
Diffusion	Very rapid and selective for fast growing tissues (tumour, bone marrow, intestinal mucosa) and in liver, brain and placenta.
Plasma peak (Tmax)	= 1 hour for 5 FU, tegafur, uracil.
Protein binding	< 30% for 5-FU; 52% for tegafur.
Metabolism	5FU: inactive in cells but activated by saturable intracellular metabolism. Thereafter very rapid hepatic metabolism into inactive products: CO2, urea, alpha-fluoro-alpha-alanine. Tegafur: slowly metabolised by oxidation to 5-FU; excess uracil (ratio of 4/1 in one of the commercial preparations) inhibits activity of dihydopyrimidine dehydrogenase (DPD, main enzyme for its catabolism), thus increasing 5FU concentration, especially in tumour tissue.
Plasma half-life (T1/2)	5FU: 10 to 20 minutes; tegafur: 11 hours.
Time to steady state	1 to 2 hours (continuous infusion 5-FU).
Excretion	15% via kidneys and 60 to 80% respiratory as CO2 (8 to 12 hours).

INDICATIONS FOR MEASUREMENT

Monitoring of 5FU is appropriate for the following reasons:

– Considerable inter-subject variability in pharmacokinetics, due notably to variation in activity of DPD, the principal catabolic enzyme for 5FU.

– Recognised relationship between plasma concentration of the drug and response (effectiveness and toxicity).

– Narrow therapeutic index. 5FU can produce digestive tract toxicity: stomatitis, mucositis, diarrhoea and vomiting, etc. It can cause a variety of skin reactions, such as dermatitis, rash, etc. It may have haematological effects: thrombocytopenia, leucopenia, and, less frequently, anaemia. It can result in neurological (cerebellar ataxia), cardiac (chest pain) and ocular (lacrimal hypersecretion) effects.

INFORMATION

■ SAMPLE

Plasma in EDTA, citrated or heparinised tubes. Avoid gel separator tubes.

– When 5FU is given parenterally, sample repeatedly for one hour after the end of infusion, every 15 minutes, for example. If it is administered by continuous infusion, sample once or twice daily at any time after steady state has been achieved, that is at least two hours after starting treatment. Because of circadian variation in DPD activity, the samples should be taken at the same time of day over the duration of treatment.

– When the therapy is tegafur + uracil, sample just before the next dose (trough) and 1 hour after the dose (peak).

– Place the sample in an ice bath immediately.

■ QUESTIONS FOR THE PATIENT

It is mandatory that any request form, for the measurement of a drug level must carry the reason for the investigation (looking for effectiveness or toxicity), the exact time of sampling and the date when treatment was started and/or of any change in dose regime. There must also be, an account of the drug regime itself (dose and frequency and route of administration) as well as the age of the patient and his/her height and weight when available.

Are you taking any other drugs? Combination with alpha-interferon increases intestinal toxicity of 5FU. Cimetidine and metronidazole can increase plasma concentration of 5FU, but only after some 4 weeks of concomitant treatment (not after a single dose).

■ SAMPLE STORAGE AND TRANSPORT

5FU is very unstable and it can be kept for only a few minutes at room temperature. However, the sample is stable for several hours between + 2 and + 8° C and for days to some weeks at – 20°C.

It is vital to centrifuge the sample and to remove the plasma very quickly, then to freeze it at – 20° or at – 80° C if the assay is to be delayed.

Transport frozen at – 20° C.

ASSAY METHODS

High performance liquid chromatography with UV detection or gas phase chromatography coupled to mass spectrometry.

NORMAL EXPECTED VALUES - INTERPRETATION

It appears that 5FU is not incorporated into DNA of cancer cells when 5FU plasma concentration is less than 180 ng/l, whereas complete inhibition of cellular DNA synthesis is reported for concentration > 180 mg/l.

During treatment of patients with advanced colorectal cancer, severe toxicity is reported to appear with plasma concentrations > 300 µg/l when 5FU has been given as 8 hour infusions in combination with folinic acid.

Dose adjustment may be necessary. It is based on the measured plasma concentrations or on the area under the curve (AUC) of plasma concentration plotted against time over the first 48 hours of the infusion or over the whole period of infusion.

The importance of therapeutic monitoring was shown in a randomised study carried out in 122 patients with ENT cancer. While effectiveness of treatment was not significantly better in the group with dose adjustment based on monitoring, the amount of drug required was lower and toxicity was thus diminished.

FOR FURTHER INFORMATION

■ Dictionnaire Vidal®.

■ Rousseau A., Marquet P., Bastian G. et *al*. *Suivi thérapeutique du 5-fluorouracile*. In : Suivi thérapeutique pharmacologique pour l'adaptation de posologie des médicaments. Collection Option/Bio, Ed Elsevier, Paris, 2004: 237-48.

FOLIC ACID

DEFINITION

Folic acid is the common name for pteroylmonoglutamic acid. More generally, folates belong to the family of pteroylglutamic acids. Pteroylglutamic or monoglutamate acid, represents only 20 to 25% of the natural vitamin activity, the major part being in the form of polyglutamates, composed of 3 to 7 molecules of glutamic acid on pteroic acid.

In humans, the endogenic production of folic acid by intestinal bacteria is not sufficient to cover metabolic requirements. The main source of folic acid is therefore dietary and it is found in a large number of foods, the richest in folates being leafy vegetables (spinach, salads, cress, lamb's lettuce, etc.), calves liver, brewer's yeast, wheat germ and chocolate. NOTE: More than 50% of polyglutamates (which account for the majority of the vitamin activity) are destroyed by cooking, and they are virtually absent in preserved and frozen foods.

Folic acid can be measured in serum or erythrocytes (where concentrations are 30 to 40 times higher than in serum). Serum folates provide an instantaneous snapshot of vitamin status. Erythrocyte folates are independent of dietary intake and provide a better reflection of the body's tissue reserves.

Synonyms: Folates, vitamin B9, pteroylglutamic acid.

INTRODUCTION

Pteroylglutamic acids (polyglutamates) are ingested and then hydrolysed in the digestive tract to pteroylmonoglutamic acid, which is absorbed in the intestine by an active and saturatable mechanism. While passing through the enterocytes, pteroylglutamic acid undergoes two successive reduction reactions to produce tetrahydrofolic acid (or THF, the active form in tissues), then, after methylation, N5-methyltetrahydrofolate (N5-methyl-THF), the circulating active form and the form in which it is stored in the liver. Hepatic reserves are limited to approximately 4 months, which explains the high frequency of folate deficiency.

The other folate forms are related to THF: N5 N10-methylene THF, N5, N5-formimino THF, and N5-formyl THF (or folinic acid, the form used in therapy, which is converted *in vivo* into folic acid). These compounds are eliminated in the urine.

They have two biological functions:

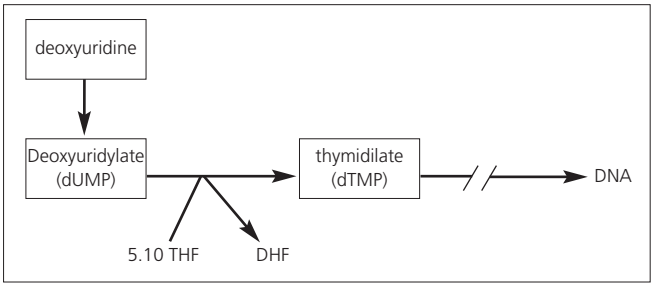
Metabolism of amino acids

THF acts as a coenzyme and is involved in the synthesis of methionine from homocysteine, the catabolism of histidine (via the conversion of formiminoglutamic acid or *FIGLU* into glutamine), the conversion of serine into glycine and the cerebral synthesis of serotonin (folic acid deficiency could engender a reduction in serotonin synthesis, resulting in behavioural disorders).

DNA synthesis

Folic acid acts as a coenzyme in the transfer of formyl or methyl groups, in particular during the synthesis of thymidylate (dTMP); dihydrofolate reductase converts DHF to THF and thus sustains the cycle of synthesis. It is involved in the synthesis of puric and pyrimidic bases and thus contributes to the creation of cellular nucleic acids.

Folates are therefore involved in cellular reproduction, particularly in all tissues which are renewed rapidly (haematopoietic tissue, intestinal epithelium, etc.).



The pathology associated with folic acid is one of deficiency. There are no symptoms linked to an excess of folates, as the folates not used by the body are excreted in urine or stored in the liver. Folate deficiency, through an abnormality in DNA biosynthesis, preferentially damages rapidly-renewable cell lines, especially cells of medullary origin, generating haematological disturbances which can sometimes be severe.

The onset of a folate deficiency occurs according to the following chronological sequence:

1. Reduced folate concentration in serum
2. Reduced folate concentration in erythrocytes
3. Hyper segmentation of polynuclear blood cells
4. Blood macrocytosis with medullary megaloblastosis.

In biological terms, haematological manifestations predominate, with normochromic macrocytary anaemia. The reticulocyte count is usually normal. There are often associated neutropaenia and moderate thrombopaenia.

In blood smears

– Anisocytosis, anisochromia, Howell-Jolly bodies

– Hyper segmentation of polynuclear cells (more than 3 % of polynuclears have 5 lobes)

– Giant platelets .

In bone marrow

– Erythroblastosis

– Marked basophilia

– Medullary megaloblastosis

– Asynchronous nucleocytoplasmic maturation.

INDICATIONS FOR MEASUREMENT

Investigation for folate deficiency, particularly when suggested by haematological disturbances such as normochromic macrocytic anaemia. However, this indication is generally late in appearing, or even absent when iron deficiency is present at the same time as folate deficiency (a common situation in the elderly). When investigating a macrocytic anaemia, folic acid measurement is combined with that of vitamin B12, a second exogenic factor necessary for nucleic acid synthesis.

If relevant, monitoring of certain antifolate treatments (methotrexate, etc.).

INFORMATION

SAMPLE

Serum folic acid: Non-haemolysed serum.

Erythrocytic folic acid: Full blood EDTA.

QUESTIONS FOR THE PATIENT

Are you taking any medicines? Many "antifolate" drugs reduce folate concentrations in the blood: trimethoprim, pyrimethamine, methotrexate, triamterene, nitrofurantoin, isoniazid, etc.

Barbiturates, phenytoin, cholestyramine and salazosulfapyridine reduce the digestive absorption of folates.

SAMPLE STORAGE AND TRANSPORT

Serum folates: Centrifuge the sample rapidly (reject haemolysed samples); storage of serum < 3 hours at ambient temperature, < 12 hours at +4° C. For longer times, freeze to -20° C.

Erythrocytic folates: Similar storage periods and temperatures; vitamin C should be added to the haemolysate, since reduced forms oxidise.

ASSAY METHODS

Competition methods in liquid media, using a radioactive or luminescent tracer. These methods do not use an antifolate antibody, employing instead a folate protein inhibitor (generally extracted from cows' milk).

NORMAL EXPECTED VALUES

These may vary slightly depending on the measurement technique and the laboratory.

Indicative values: Serum folates > 3.40 ng/ml

Erythrocytic folates 130 to 600 ng/ml.

Folate concentration in cerebrospinal fluid is 3 to 4 times greater than in serum i.e. > 10 ng/ml approximately.

Conversion factor: 1 ng/ml = 2.27 nmol/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Serum concentrations fall during pregnancy, due to capture for foetal requirements (which is equivalent to an increase in the mother's requirements). Folate deficiency is believed to be responsible for increased maternal morbidity (spontaneous miscarriages, premature childbirth, etc.) and for neural tube closure abnormalities in the foetus. Supplementation has been proposed but is not systematic in France.

PATHOLOGICAL VARIATIONS

Reduced serum folate concentrations reflect a deficiency, which can arise in various situations:

Increased requirements

– Pregnancy, breast feeding: Deficiency is common, above all at the end of pregnancy and in cases of multiple pregnancy

– Repeated congenital, acquired or haemorrhagic haemolytic anaemia with hyper-regeneration of erythrocytes

– Serious acute infection.

Intake insufficiency

– Malnutrition (poverty, senility), anorexia

– Alcoholism

– Prolonged cooking of food consumed (hydrolysis)

Absorption insufficiency

– Coeliac disease, Crohn's disease

– Resection of the small bowel, gastrectomy

Increased losses

– Haemodialysis

Drug treatment

See above ("Questions for the patient")

NB: A reduction in serum folate concentrations (sometimes, but not always, combined with a reduction of folates in the cerebrospinal fluid) has been observed in patients presenting neurological disorders (peripheral neuropathy, behavioural, sleep or memory problems, dementia) with no iatrogenic implications.

Elevated serum folate concentrations are observed after folic acid or folinic acid treatment. The excess of folates is not pathological (elimination through urine or storage in the liver).

Erythrocytic folates

When a deficiency is present, the reduction in erythrocytic folate concentrations occurs after that of serum folates. The reduction may affect erythrocytic values only (normal serum folates) in cases of an isolated vitamin B12 deficiency (artificial deficiency with plasmatic accumulation of methyl THF).

Elevated erythrocytic folate concentrations may be observed in cases of severe reticulocytosis or after blood transfusions.

TREATMENT OF DEFICIENCIES

Treatment consists of the oral administration of 5 to 15 mg/day of folic acid or 5 to 25 mg/day of folinic acid for approximately 2 to 3 months, although this should be continued indefinitely if the cause of the deficiency can not be eliminated (gastrectomy, drug treatment, etc.).

FOR FURTHER INFORMATION

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FORMIC ACID

DEFINITION

Formic acid (or methanoic acid) is the main metabolite of methanol. This strong, corrosive organic acid is in widespread use in industrial applications, notably in the textiles, tanning, organic synthesis and paper industries. Its use is strictly regulated by occupational health and safety legislation (Threshold Limit Value in France = 5 ppm, i.e. 9 mg/m^3).

INTRODUCTION

Formic acid is responsible for almost all of the toxic effects of methanol, notably metabolic acidosis, encephalopathy and optic nerve damage. Methanol is metabolised in the liver by alcohol dehydrogenase to formaldehyde which is then converted to formic acid by aldehyde dehydrogenase. Formic acid is the precursor for many amino acids, lipids and carbohydrates. It can also be oxidised giving carbon dioxide and water. From the toxicological perspective, formic acid can be absorbed via the digestive tract, lungs and skin. The proportion of formic acid that remains unmetabolised rises with the amount absorbed. Formic acid is excreted slowly in the urine and build-up is a possibility with repeated exposure.

INDICATIONS FOR MEASUREMENT

Formic acid is usually assayed in the urine.

In the blood, a methanol assay is far more informative than a formic acid assay— notably for the investigation of suspected methanol poisoning and to monitor the efficacy of treatment of methanol poisoning (antidote). It is also used to monitor workers exposed to high concentrations of formaldehyde but, in this context, this assay lacks sensitivity and is of limited value.

INFORMATION

SAMPLE

For occupational exposure, urine should be collected into a plastic recipient at the end of the work shift.

QUESTIONS FOR THE PATIENT

It is important to know about any recent drinking because alcohol inhibits the excretion of formic acid in the urine.

SAMPLE STORAGE AND TRANSPORT

Urine: 3 days at +4 °C.

ASSAY METHODS

Urine assay: Gas phase chromatography.

REFERENCE VALUES FOR FORMIC ACID IN THE URINE

General population: < 23 mg/g creatinine.

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FREE HAEMOGLOBIN

DEFINITION AND SYNONYMS

Haemoglobin is a pigment synthesised in red blood cells during maturation of the erythroblast cell line in the bone marrow. The spleen filters, binds and destroys damaged red blood cells. During this physiological cycle of red blood cells, free haemoglobin is not released into plasma, although in some pathological circumstances (intravascular haemolysis), free haemoglobin can be found in plasma.

INTRODUCTION

Haemoglobinaemia and haemoglobinuria are markers of intravascular haemolysis.

In intravascular haemolysis, lysis of red blood cells releases free haemoglobin into the circulation. Free haemoglobin is immediately bound to haptoglobin; the haptoglobin-haemoglobin complex is removed by the hepatic reticulo-endothelial system (binding iron and converting haem into unconjugated bilirubin). Haptoglobin becomes saturated once the plasma haemoglobin exceeds 1.5 g/l and haemoglobinaemia then develops. Some of the plasma haemoglobin is reabsorbed and catabolised in the kidney (proximal convoluted tubule) and iron is bound by haemosiderin and ferritin. Haemosiderin is then removed in urine. Haemoglobinuria develops if the renal reabsorption capacity is exceeded.

Various laboratory signs may give a strong diagnostic indication of intravascular haemolysis.

Lifespan of red blood cells	Moderate haemolysis 20-40 days	Severe haemolysis < 20 days
Plasma bilirubin	↑ increased unconjugated bilirubin *Rising but staying below 70-85 µmol/l (or 4-5 mg/l)*	
Met haemoglobinaemia	↑	
Plasma/serum	Pink/Red colour	
Serum haptoglobin	↓ - Absent	Absent
Haemoglobinaemia	↑	↑↑
Plasma LDH	↑	↑↑
Urine haemosiderin	Variable	Present
Haemoglobinuria	Absent	Present

From Rosse W « Anémie hémolytique », in Harrison "Internal Medicine", Ed. McGraw Hill Arnette, New York 1995.

INDICATIONS FOR MEASUREMENT

Investigation of haemolytic anaemia, particularly in intravascular haemolysis. When combined with a measurement of bilirubin it may be used to differentiate intra- from extravascular haemolysis.

INFORMATION

SAMPLE

The blood sample is collected into a dry or heparinised tube, preferably fasting to avoid lipaemia. The aim is to avoid in-vitro haemolysis, which could produce false positive results and the sampling procedure must therefore be scrupulous.

– Choice of needles with sufficient flow rate (18 Gauge)

– Light tourniquet

– Sampling into an initial dry tube and then into the analysis tube

– Gently mixing the tube to mix the anticoagulant.

The urine sample is taken into a pot from a single urine pass. The analysis must be performed promptly.

QUESTIONS FOR THE PATIENT

– Family history of red blood cell disease (thalassaemia or sickle cell anaemia)?

– Medicines taken or exposure to toxins?

– Context of measurement (infection, heart disease, dysimmune status)?

Confirm vigorous exercise has not been taken before the sample, which can produce "march" haemolysis.

SAMPLE STORAGE AND TRANSPORT

The sample must be centrifuged promptly after sampling to avoid any risk of *in-vitro* haemolysis. Plasma or serum can be stored at between + 2°C and + 8°C, for a few days.

ASSAY METHODS

The historical method is spectrophotometric with benzidine, which makes use of the peroxidise activities of haemoglobin. False positives occur due to interference with the assay method from increased serum bilirubin, increased turbidimetry and methaemoglobinaemia.

Haemoglobin concentrations can also be measured directly by absorption spectrometry at an absorbance peak of 415 nm. To identify haemoglobin specifically, the second order derivation of the absorbance spectrum is used. In order to obtain a consistent form of haemoglobin, free haemoglobin (reduced and oxygenated haemoglobin) is converted into cyanmethaemoglobin. Potassium ferricyanide and potassium cyanide or Drabkin's reagent is used for this.

NORMAL EXPECTED VALUES

< 50 mg/l of plasma.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Increased haemoglobinaemia occurs after vigorous exercise, particularly in marathon runners/walkers.

PATHOLOGICAL VARIATIONS

Intravascular haemolysis produces a marked rise in haemo-globinaemia. Concentrations of between 50 and 150 mg/l must be interpreted with caution because of the possibility of *in-vitro* haemolysis.

In acute intravascular haemolysis, haemoglobin appears in plasma and in urine once haptoglobin is saturated (concentrations exceeding 1.5 g/l). Haemoglobin is found at lower concentrations (approximately 0.25 g/l) in chronic haemolysis.

Increased haemoglobinaemia may arise from several causes. Extra cellular causes (toxins, immune and mechanical) are distinguished from cellular causes (globular) specific to the structure of the red blood cell. Haemoglobinaemia is high in acute haemolysis and may be lower in slow intravascular haemolysis as the removal system compensates for the release of haemoglobin (cellular or autoimmune anaemia).

Context	Origin	Aetiology	Haemoglobinaemia
Infectious	*Toxic*	*Plasmodium falciparum* C. perfringens septicaemia Haemolytic, uraemic syndrome	**+ to + +**
Direct lysis	*Toxic*	***Burns:*** membrane denaturation. ***Toxins:*** venoms, fungi, copper poisoning.	**+ to + +**
Immuno-allergy	*Immune*	Drugs inducing haemolytic antibodies	**+ + +**
Auto-immunity	*Immune*	**Cold agglutinin disease:** viral or mycoplasmin infections, lymphoma, paroxysmal cold haemoglobinuria **Warm auto-antibody disease:** viral infections, drugs, lymphomas, lupus, tumours, post-transfusion	**+ to + +** **Positive Coombs test**
Schizocytes	*Mechanical*	**Materials:** aortic/cardiac valves and prostheses, extracorporeal circulation; **Fibrin deposition:** microangiopathy, adenocarcinoma, eclampsia, DIC, thrombotic thrombocytopenic purpura (TTP)	**+ + +** **except** **+ DIC**
Splenomegaly	*Mechanical*	Due to excess red blood cell filtration	**Variable**
Oxidising drugs	*Cellular*	G6PD deficiency	**+ + +** **during attack**
Red blood cell fragility	*Cellular*	**Constitutional abnormalities:** Thalassemia Sickle cell anaemia **Acquired abnormalities:** Acanthocytosis (cirrhosis and dyslipidaemia), paroxysmal nocturnal haemoglobinuria (increased complement sensitivity), lead poisoning	**Low** **Variable**

■ IATROGENIC VARIATIONS

Oxidising drugs: anti-malarials (dapsone), sulphonamides (sulfamethoxazole), nitrofurane, doxorubicin and nalidixic acid.

Drugs causing immuno-allergies (positive Coombs test in the presence of the drug): some drugs cause antibody formation against the drug- red blood cell complex: ampicillin, cephalosporin, cisplatin, erythromycin, streptomycin and tetracycline, etc.

Other drugs have also been incriminated: quinine and its derivatives, rifampicin, triamterene, diclofenac, probenecid, sulindac, sulphonamides and tolbutamide, etc.

POUR EN SAVOIR PLUS

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FREE IMMUNOGLOBULIN LIGHT CHAINS OR "BENCE-JONES PROTEINS"

DEFINITION

"Bence Jones proteins" are formed from monoclonal immunolglobulin (Ig) free light chains (FLC), Kappa (κ) or Lambda (λ) isotype of a molecular weight (RMM) of 23,000 Da. They can be found in blood and/or urine, in which case the term Bence-Jones proteinuria is used. The FLC may or may not polymerise into dimers (particularly FLC λ) or even multimers (reaching high molecular weights of up to 900 kDa). They can also bind to other proteins (albumin, α-1 and α-2-globulins). These specific physico-chemical features make the methods available to test for and quantify them in serum and/or urine difficult (large variability in electrophoretic mobility and/or antigen recognition).

NB: the term "Bence-Jones proteins" is used when the FLC are MONOCLONAL and polyclonal FLC are not "Bence- Jones proteins".

Synonyms: free kappa or lambda light chains.

PATHOPHYSIOLOGY

During Ig synthesis by plasmocytes, light chains (κ or λ) are produced in excess (+ 40%) compared to heavy chains (μ, α, γ, δ, ε) in order to allow correct conformation of the complete Ig molecule. FLC κ and FLC λ are produced in a ratio of two to one. FLC κ monomers are removed rapidly from blood over 2 to 4 hours, whereas the FLC λ dimmers or multimers are removed over 3 to 6 hours. Serum ultimately contains more FLC λ than FLC κ, despite greater production of FLC κ.

Physiologically, FLC are present in small amounts; because of their low RMM they are filtered by the glomerulus and then reabsorbed and metabolised in the proximal tubule and only a minimal amount (1-10 mg) is ultimately removed in urine.

In some pathological situations (renal insufficiency, haematological diseases), the FLC accumulate in blood or are removed to a greater extent in urine because of saturation of the reabsorption mechanisms. The two types of FLC accumulate in renal insufficiency: in blood dyscrasias with monoclonal FLC secretion, only one of the 2 isotypes accumulates. The FLC produced in excess then precipitate in the distal tubule causing or worsening renal insufficiency. Excess circulating FLC occasionally have damaging effects by being deposited in tissues causing a high risk of multi-organ complications (AL, amyloidosis, FLC deposition diseases, neuropathy etc.).

SEARCH INDICATIONS /INDICATIONS FOR MEASUREMENT OF Ig FLC

– Diagnosing and monitoring monoclonal gammopathies: multiple myeloma (particularly free light chain myeloma and non-secretory myeloma), AL amyloidosis, Randall's disease (LC deposition diseases).

– The utility of measurement is currently debated in monitored patients with serum monoclonal gammopathy of undetermined significance (MGUS): the free kappa/free lambda ratio may be a marker of progression to malignant gammopathy (?); it is also debated in monitoring FLC and Waldenström's macroglobulinaemia.

INFORMATION

SAMPLE

Blood sample: into a dry tube (serum). A fasting sample is preferable.

Urine sample: 24-hour urinary collection (record urine output) or spot urine sample.

QUESTIONS FOR THE PATIENT

Context of measurement: known disease, monitoring treatment?

SAMPLE STORAGE AND TRANSPORT

- **Urine:** urine can be stored for several days at + 4°C and for several years at – 20°C. An antiseptic (sodium azide) is added to provide any growth of bacteria.
- **Serum:** can be stored for 4 days at + 4°C and for 3 months at – 20°C.

DETECTION/ASSAY METHODS

– Serum and urine protein electrophoresis

A monoclonal component is suspected on serum or urine protein electrophoresis (performed on agarose gel or by capillary), from the presence of a peak (narrow band), which is monoclonal in appearance. This method may be ineffective, however, in testing for FLC because of low concentrations (often under 2 g/l), their migration features (they may run from the α-globulins to the γ-globulins) and from their ability to bind other proteins. FLC (serum or urine) are quantified by densitometry: by integrating the peak when it can be integrated. They may be expressed as the Bence-Jones/total proteinuria ratio in urine.

– Serum and/or urine immunofixation

Immunofixation (IF) is a more sensitive method which can be used to both characterise (identify the whole Ig or FLC isotype) and confirm the monoclonal nature of the abnormality which is detected (or not detected) on electrophoresis. In order to determine whether the free chains are "free" it is essential that the IF is performed with an immune serum able to bind to total "bound and free" light chains and another immune serum able to bind only to free light chains (in this case the immune serum binds to an epitope which is only accessible when the light chain is free). The total anti-light chain immune serum is more sensitive than the anti-FLC immune serum. Urine IF can be performed on concentrated urine increasing the sensitivity of the method at very low urine FLC.

Other methods can be used to confirm the presence of FLC, particularly in serum with the same sensitivity as IF: immunoelectrophoresis (reading is relatively subject particularly at very low concentrations) and immunosubtraction.

– Nephelometric measurement

Since about 10 years, a new tool, nephelometry has been available for the diagnosis and monitoring of FLC monoclonal gammopathies. This assay uses a reagent comprising monospecific anti-FLC κ and anti-FLC λ polyclonal antibodies; these antibodies recognise an epitope which is only accessible if the light chains are free and which is masked if the light chain is bound to a heavy chain. Serum measurement offers excellent sensitivity of 1.5 mg/l for FLC κ and 3.0 mg/l for FLC λ. A monoclonal FLC isotype is then suspected if the FLC κ/FLC λ ratio is very abnormal although only a qualitative method such as IF can actually confirm that it is monoclonal. It should also be noted that a large increase in polyclonal FLC (e.g. in renal insufficiency, autoimmune disease and chronic inflammatory diseases, etc.), the presence of low concentrations of monoclonal FLC may be masked. Measurements and the ratio must therefore be interpreted in a clearly described clinical and laboratory context.

The role of urinary measurement is more debatable: the test still offers excellent sensitivity, although depends on collection conditions (clear urine transported under correct conditions). Considerable inter and intra-individual variability is seen in the correlation between serum and urine measurements because of the impact of renal function on FLC excretion (many factors can produce renal dysfunction in malignant blood dyscrasias). Serum measurements therefore appear to correlate better with disease activity than urine measurements.

Sensitivity of different methods to detect, identify and assay Ig FLC.

Method	Serum (mg/l)	Urine (mg/l)
Electrophoresis	500-2000	10 (if concentrated)
Immunofixation	150-500	5-30 (if concentrated)
Nephelometry	2-3	0.5 (if urine is clear)

NORMAL EXPECTED VALUES

– Electrophoresis / IF / IEP / immunosubtraction: no abnormality detectable.

– Nephelometry: for reference for the Freelite® from The Binding Site test

In serum:

Free Kappa Light Chains: 3.3 to 19.4 mg/l.

Free Lambda Light Chains: 5.7 to 26.3 mg/l.

In urine:

Free Kappa Light Chains : < 10 mg/l.

Free Lambda Light Chains: < 5 mg/l.

Reduced glomerular filtration rate causes an increase in serum κ and λ FLC because of an increase in their ½ life up to 2 to 3 days.

– In people with normal renal function: twice as much FLC κ is produced, although they are excreted approximately 3 times faster than FLC λ. Because of this the R κλ is approximately 2/3 or 0.66.

– In patients with renal dysfunction, FLC κ are raised more as the excretion is reduced. Because of this, the R κλ ratio is increased over 1 (average values in the region of 1.8).

New standards for R κλ have been adapted for patients with multiple myeloma and renal insufficiency allowing myeloma to be diagnosed in the presence of renal insufficiency.

Reference values for the κλ ratio in serum

Disease	R κλ reference interval	Specificity
With renal insufficiency	0.26 – 1.65	93%
Without renal insufficiency	0.3 – 3.3	99%

INTERPRETATION

Interpretation (Bradwell AR, *Serum Free Light Chain Analysis; 5th Edition*)

If R κλ normal with raised FLC κ and raised FLC λ	Polyclonal increase or renal insufficiency
If R κλ raised	Monoclonal FLC κ present
If R κλ reduced	Monoclonal FLC λ present

– Multiple myeloma: myeloma is a malignant blood disease responsible for about 15% of blood dyscrasias: it is diagnosed from a bone marrow, which shows bone marrow plasmocytosis or dystrophic plasmocytes. Myelomas are classified into stages I, II, and III by the Salmon and Durie classification (monoclonal Ig concentration, serum calcium, haemoglobin, Bence-Jones proteins and bone disease).

Whole chain Ig myelomas

These are characterised by secretion of a whole chain monoclonal Ig (light chain bound to a heavy chain, characterised and quantified by integration of the electrophoretic peak). FLC concentrations are abnormal in almost 90% of cases and the κ/λ ratio is abnormal in approximately 96% of cases. No relationship has yet been demonstrated between FLC concentrations and patient survival. The utility of routine FLC measurement is still to be proven.

FLC myeloma

These are characterised by secretion of only an Ig light chain and constitute approximately 15% of all myelomas; circulating FLC concentrations are often too low to enable quantification of an electrophoretic peak; measurement of FLC then becomes a key feature in the diagnosis and then monitoring of the disease.

"Non-secretory" myeloma

These have the specific feature of having no secreted monoclonal band: however, the immature plasmocytes almost always contain a light chain in their cytoplasma. Here again, FLC measurement becomes an essential feature and is now the best means of monitoring the disease.

– AL amyloidosis: this is caused by FLC deposition (mostly the λ isotype) in various tissues (heart, kidney, skin and nerves). The diagnosis is made histologically (Congo red staining and finding the monoclonal LC isotype in depositions). Combination of IF (serum and/or urine) with FLC measurement can be used to detect almost 99% of cases of AL amyloidosis. FLC measurement is a key feature in these situations both to diagnose and monitor the disease and to assess residual disease (a fall in circulating FLC concentrations of more than 50% after treatment is associated with improved 5 year survival: 88% vs 39%, p<0.0001).

– Randall's disease: this is also an LC deposition disease (mostly the κ isotype), which above all affects the kidney and occasionally other organs. The diagnosis is also made histologically (the deposits do not stain with Congo red, finding the monoclonal component). FLC concentrations are often very low and measurement is a useful tool to monitor the disease.

– MGUS monoclonal gammopathy of undetermined significance: this is characterised by an isolated monoclonal Ig (Ig concentration < 30g/l, no bone lesions, no Bence Jones proteinuria, normal serum calcium). The risk of progression to malignant disease is 1% per annum (an abnormal FLC κ/λ ratio increases relative risk to almost 2.5%) although the utility of measurement is still debated in the follow up of MGUS.

– Waldenström's macroglobulinaemia this is characterised by bone marrow plasmocytosis and secretion of monoclonal IgM. It is monitored mostly using IgM concentrations, Bence Jones proteinuria is usually negative or very low and the utility of FLC measurement remains to be determined.

POUR EN SAVOIR PLUS

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FRUCTOSAMINE

DEFINITION

The term fructosamines covers all the circulating glycated proteins. Protein glycation is produced by a condensation reaction between a glycosidic reducing group and a protein amine residue. This non-enzymatic binding of glucose to a protein leads to the formation of a labile Schiff base. Following an "Amadori rearrangement" a stable ketoamine or fructosamine structure emerges. The glycation of proteins is a general reaction which involves all the proteins of the body and its degree is directly proportional to levels of circulating glucose. The fructosamines, then, resemble HbA1c as retrospective and cumulative markers of glycaemic balance, which can be employed to monitor diabetic patients. However, fructosamine measurement is less valuable than that of HbA1c and covers a shorter time period of three weeks.

INTRODUCTION

In general, the circulating level of glycated proteins is a relevant marker for glucose control in diabetic patients.

The glycation of proteins, especially those of a long half-life such as collagen, plays an important role in modifying the biosynthesis of basal membranes, in particular those of the renal glomerulus and capillary blood vessels. This is recognised as underlying some of the long-term complications of diabetes.

In current practice, the monitoring of glycaemic control in diabetics depends for the most part on the determination of glycated haemoglobin (HbA1c), a retrospective marker of glycaemia over the 2 to 3 months prior to assay. The plasma concentration of fructosamines reflects more recent variations in glycaemia, for two to three weeks before measurement. In fact, this test is indicated in the monitoring of diabetic patients as a complement to, or in place of, that for glycated haemoglobin, which remains the reference standard:

– In situations where the antidiabetic treatment needs to be altered rapidly (an indication, which remains, nevertheless, unproven), especially in the pregnant woman with gestational diabetes, or when it is necessary to adjust treatment rapidly as in patients on insulin pumps and in the elderly, children or adolescents.

– When the HbA1c assay is inoperable. This applies in the following situations:

- Anaemia and/or haemolysis, which alters red cell survival, and hence the haemoglobin concentration and reduces the duration of exposure of the haemoglobin to glucose.
- Abnormal haemoglobins which interfere with HbA1c assays and alter red cell survival. This is at present the most generally accepted indication for the fructosamine assay.
- In renal failure. This indication is uncertain.

NB: Fructosamine assay is not recommended in screening for diabetes.

INFORMATION

SAMPLE

Blood for serum or plasma samples collected into EDTA (ethylene-diamine-tetraacetic acid) or lithium-heparin tubes. Fasting is not required. Discard haemolysed, icteric and lipaemic specimens.

QUESTIONS FOR THE PATIENT

Are you taking alpha methyldopa or calcium dobesilate? These drugs can increase serum/plasma fructosamine concentrations.

SAMPLE STORAGE AND TRANSPORT

Storage of whole blood specimen, < 48 hours at + 4° C.

Storage of serum or plasma: 72 hours at + 4° C or 3 months at – 20° C.

Transport: + 4° C.

ASSAY METHODS

Colorimetric using nitrotetrazolium blue.

Enzymatic method. Values are slightly lower with this technique compared with colorimetric assays.

NORMAL EXPECTED VALUES

The results depend on the assay method. In the euglycaemic healthy adult, for example, they lie between 200 and 265 µmol/l using the colorimetric method.

When there is a qualitative or quantitative protein variation, they can be presented as a proportion of total protein: normal expected values 2.8 to 3.9 µmol/g of protein.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

There is age dependency and values are slightly lower in the child, then gradually increasing into adulthood.

In pregnancy values are slightly lower.

PATHOLOGICAL VARIATIONS

The serum/plasma concentration of fructosamines is directly correlated with the glycaemic levels of the previous 2 to 3 weeks. The worse the diabetic control has been in that period, the higher will be the fructosamine level.

However, it is important to be aware that interpretation of fructosamine can be invalidated by various disease processes that alter protein metabolism, especially that of albumin. These include thyroid disease (increase of fructosamine in hypothyroidism and decrease in hyperthyroidism), inflammation, severe hepatic failure, substantial proteinuria and, for the colorimetric method, renal failure. In addition the results are not interpretable in patients with macroglobulinaemia.

Fructosamine measurement is, then, a valuable alternative to that of HbA1c, in particular when the latter is not valid.

However, it has not been clearly established that the maintenance of good glycaemic control, as reflected in circulating fructosamine levels, reduces the complications of diabetes, whereas that has been shown to be the case with HbA1c measurements.

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FSH

DEFINITION

FSH *(Follicle Stimulating Hormone)* is a glycoprotein hormone of the gonadotrophin family, secreted in a pulsatile fashion by the anterior pituitary, under the control of hypothalamically derived GnRH (Gonadotrophin Releasing Hormone, previously called LHRH) and of circulating concentrations of oestradiol, progesterone and inhibin.

It has a dimeric structure, composed of a specific β sub-unit and an α sub-unit, which is also found in LH, TSH and HCG. The beta chain confers immunological and biological specificity on the hormone. Each of the sub-units contains a carbohydrate portion, which guarantees stability in plasma and facilitates hormonal activity. Dissociation of the alpha and beta sub-units results in the loss of biological activity. The considerable variation in the carbohydrate section, together with that of sulphation and sialylation of the gonadotrophins, is at the root of the substantial heterogeneity of their circulating forms, which causes many assay problems.

Synonyms: Folliculostimulin and follicle stimulating hormone.

INTRODUCTION

In the adult, FSH is secreted in a pulsatile manner into the blood, where it has a half-life of between 2 and 30 hours and where it circulates as the free hormone without a binding protein. In the female, it acts in conjunction with LH on the ovaries to stimulate growth and maturation of the follicles and the secretion of oestrogens. In the male, FSH stimulates spermatogenesis and the secretion of testosterone.

INDICATIONS FOR MEASUREMENT

FEMALE

Investigation of reproductive function

– In amenorrhoea, measurement of FSH and LH together can distinguish ovarian hypogonadism (hypergonadotrophic hypogonadism) from hypogonadotrophic hypogonadism of pituitary – hypothalamic origin.

– In a woman with periods to assess ovarian reserve by combining with an oestradiol assay and often with that of inhibin B and/or AMH (Anti-Mullerian Hormone).

– Dynamic testing: LHRH testing to measure stimulated gonadotrophin secretion.

– In the peri-menopausal period (women above 45 years of age): In France, a group of experts reported in 2004 that measurement of FSH should not be used to diagnose the menopause or a peri-menopausal state. They felt that it was more appropriate to assess age, clinical signs and response to testing with progestogens. However, their more detailed recommendations were as follows:

- Measurement of FSH should be restricted to certain clinical situations, such as hysterectomy (measurement of FSH and oestradiol combined) or when looking for gonadotrophin deficiency in a case of amenorrhoea without menopausal symptoms. It is also useful in monitoring treatment with GnRH agonists

- FSH should not be performed to inform a decision on hormone replacement therapy

- Most experts agree that FSH measurement does not help to decide when to halt contraception. The suggested strategy is to stop oral contraception, to replace it with another contraceptive method and to monitor clinically for the appearance of amenorrhoea and climacteric symptoms.

Investigation of puberty disorders:

FSH and LH measurements: baseline and after GnRH stimulation.

MALE

– Diagnosis of the cause of hypogonadism

– Infertility with abnormalities of sperm count.

INFORMATION

SAMPLE

Serum (dry tube) or other according to the manufacturer's recommendations (avoid tubes with gel separator); sampling is preferable in the early morning. A fasting sample is not required.

Choice of day of sampling depends on the reason for the assay:

– In amenorrhoea: any day

– In patient having periods: between the 3rd and the 5th day of the cycle. When assessing ovarian reserve, sample on the 3rd day (2nd or 4th also possible). When these patients are followed-up, the measurements must always be performed in the same laboratory

– Dynamic testing: LHRH test at the beginning of the follicular phase (day 2 to day 5). Baseline measurement, then at 30, 60, 90 and 120 min after injection of GnRH.

QUESTIONS FOR THE PATIENT

– In sexually active women the presence or absence of periods needs to be noted, date of last period, usual duration and regularity of cycle; and presence of clinical signs such as acne and hirsutism. In the peri-menopausal period, duration of cycles in the past and presence or absence of hot flushes.

– Current therapy, in particular, hormonal treatment such as contraceptives and post-menopausal hormone replacement therapy.

SAMPLE STORAGE AND TRANSPORT

Centrifuge after complete clot retraction (about 30 minutes at room temperature).

Assay within hours of sampling or store according to the manufacturer's recommendations. This is usually several days at + 4° C. If storing for more than a week, freeze at – 20° C. Thawing should be followed by homogenisation in a vortex mixer followed by further centrifugation before the assay procedure.

ASSAY METHODS

Sandwich immunometric methods with monoclonal antibodies: radio-isotopic, enzymatic, chemoluminescent, fluorescent or colorimetric label.

Standardisation: There are several standards, the most commonly used being WHO hFSH 2nd IRP 78/549.

NORMAL EXPECTED VALUES

The serum concentration of FSH varies with age and, in the female, the phase of the menstrual cycle. There are substantial differences between results obtained with different immuno-assays because of the structural heterogeneity of circulating FSH. For this reason normal values must be qualified with details of the method used.

Reference values in the adult woman *(in IU/l)*

Examples:

	Follicular phase	Pre-ovulatory peak	Luteal phase	Pregnancy	Menopause
Immunochemoluminescence CS 180®	2.5 to 10.2	3.4 to 33.4	1.5 to 9.1	< 0.3	23 to 116.3
Chemoluminescence Elecsys® Roche	3.5 to 12.5	4.7 to 21.5	1.7 to 7.7		26 to 135

When the ovarian reserve is being assessed, a patient should always have assays performed in the same laboratory using the same technique, as the management of her medically assisted fertilisation depends to a large extent on the assay results. Several IVF specialist teams use an FSH threshold of 10 IU/l, believing that beyond this the pregnancy success rate is too low to embark on treatment.

Examples

In the adult male: Chemoluminescence Elecsys® Roche: 1.5 to 12.4 IU/l.

In the child: (Chemoluminescence Elecsys® Roche):

	1 to 7 days	8 to 30 days	1 to 12 months	1 to 5 years	6 to 10 years	11 to 13 years	14 to 17 years
FSH, girls (IU/l)	< 0.1 – 4.5	< 0.1 – 22.2	0.2 – 7.5	0.2 – 11	0.3 – 11.1	2.1 – 11.1	1.6 – 17.0
FSH, boys (IU/l)	< 0.1 – 4.5	< 0.1 – 22.2	1 - 5	0.2 – 2.8	0.4 – 3.8	0.4 – 4.6	1.5 – 12.9

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

– Before puberty, serum FSH and LH are low. During puberty the first (nocturnal) peaks of LH appear, followed by those of FSH.

– During the menstrual cycle, the blood level of FSH rises towards the end of the luteal phase and then normally falls from the 7th day of the follicular phase. The LH/FSH ratio is less than 2, except in the ovulatory phase, when it can rise to a value as high as 4.

– During the menopause/ "andropause", serum gonadotrophins increase from the perimenopausal period in woman. The increase in FSH precedes that of LH. In the male, a progressive fall in serum testosterone is accompanied by a concomitant increase in FSH and LH.

■ IATROGENIC VARIATIONS

The combined oral oestrogen-progestogen contraceptive pill and some progestogens taken for 15 to 20 days per month reduce the level of circulating FSH to a greater or lesser extent, as do anabolic androgens or high dose corticosteroids (or even corticosteroids administered cutaneously or by inhaler).

FSH can be reduced even more markedly, (or even fall) after about 10 days of treatment with GnRH agonists or 24 hours of GnRH antagonist treatment.

Clomiphene citrate (200 mg per day) increases the serum FSH value by approximately 50% in 6 to 10 days.

INTERPRETATION

■ DISORDERS OF THE GONADOTROPHIC AXIS

– In the female a frank elevation of serum concentrations of FSH and LH reflects primary ovarian insufficiency. In the case of primary amenorrhoea this is likely to be due to gonadal dysgenesis. In secondary amenorrhoea one is dealing with premature menopause or castration (surgical, irradiation, radiotherapy or chemotherapy). In the male, in a context of investigation of hypogonadism, an increase in serum FSH suggests a gonadal cause (Klinefelter's syndrome, anorchidism, cryptorchidism, irradiation, chemotherapy or senescence, etc.).

– In general, both in the male and the female, a clear reduction in FSH and LH usually suggests anterior pituitary hypo-function, primary or secondary. This could be due to tumour, pituitary necrosis or hypophysectomy, etc.

Interpretation of the principal abnormalities of gonadotrophin secretion

	FSH	LH
Female		
Amenorrhoea due to pituitary-hypothalamic insufficiency or psychogenic cause	↓	↓
Amenorrhoea due to ovarian insufficiency or premature menopause	↑	↑
Type I Stein-Leventhal syndrome (polycystic ovaries)	N	↑
Type II Stein-Leventhal syndrome	↓	N or ↓
Male		
Primary testicular insufficiency	↑	↑
Pituitary insufficiency	↓	↓
Isolated damage to seminiferous tubules	↑	N

■ ASSESSMENT OF OVARIAN RESERVE

If FSH is elevated and/or oestradiol low, a reduction in ovarian reserve is likely. This means that the patient's follicular potential is diminished and that the process of ovarian ageing has started. However, this process develops over several months and an abnormal value of one or other of these parameters does not mean that sterility is established.

Currently (apart from the patient's age) the best laboratory test predictors of the success of implantation of an embryo are the FSH, plasma oestradiol (E2), sometimes inhibin and AMH, and the response to stimulation testing.

For example, the test of exogenous FSH stimulation or EFORT (Exogenous FSH Ovarian Test) consists of a measure of oestradiol and FSH 3 days after injecting 3,000 IU of FSH with a repeat of the oestradiol assay at day 4.

An FSH at day 3 of < 10 IU/l and an increase in E2 of more than 30 pg/ml at day 4, define a normal response. Hypo-responders all show an FSH at day 3 of > 10 IU/l and an increase in E2 of less than 30 pg/ml at day 4.

INFERTILITY INVESTIGATION IN THE MALE

When testicular androgens are normal, an increase in serum FSH with normal LH suggests an abnormality of the germinal cell line and/or of the seminiferous tubules. With a normal serum FSH blockage of the genital tract (epididymis or vas deferens) is more likely.

FOR FURTHER INFORMATION

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GAMMA HYDROXYBUTYRIC ACID

DEFINITION

Gamma hydroxybutyric acid is a naturally-occurring product in mammals, found at trace concentrations (nanomolar) in the central nervous system and other tissues. This acid is used for medical applications as an anaesthetic and in the treatment of narcolepsy, insomnia, alcohol and opiate dependency and head injuries. It is also used for non-medical applications by body-builders (in dietary supplements) and as a psychoactive substance by drug addicts. Finally, it is used by criminals to knock out their victims with a view to rape or theft. It is easy to synthesise and is available on the black market in the form of a powder or a colourless or orange liquid which can easily be used to spike a drink. Many advertisements promote its virtues on the Internet.

The lactone derivative of GHB (gammabutyrolactone or GBL) is sometimes combined with GHB in illegally sold preparations (or sold in the United States as Renewtrient, Revivarant, Blue Nitro, GH Revitalizer, Gamma G, Insom-X and Remforce).

Synonyms:

– GHB, sodium hydroxy-4-butyrate, gamma-OH, sodium oxybate, somatomax PM, Wy-3478, NSC-84223, 4-HB.

– Names on the black market: *Liquid E, Liquid X, Nature's Quaalude, Love Potion Number 8, Easy Lay,* "date rape drug".

METABOLISM

½-life in plasma and peak concentration	Proportional to the dose administered: for a dose of 12.5 mg/kg: ½-life in plasma = 20 minutes. After the oral absorption of 25 mg/kg every 12 hours for 7 days, the peak concentration is reached in the plasma in 20-45 minutes. With a dose of 75 mg/kg, the peak concentration is reached in the plasma in 2 hours.
Metabolism	Oxidised by alcohol dehydrogenase.
Elimination	In the urine: 1-5%.

INTRODUCTION

GHB both inhibits the release of dopamine and stimulates its production resulting in marked increases in the concentration of this neurotransmitter in certain parts of the brain. GHB itself therefore acts as a neurotransmitter, inducing a state of torpor resembling the "absences" of petit mal epilepsy. It reduces glucose consumption by 30%, oxygen consumption by 25%, and cerebral blood flow by 10%.

Its effects depend on the dose ingested. After a dose of 10 mg/kg, its effects are amnesia and muscular relaxation; between 20 and 30 mg/kg, it induces a state of euphoria followed by sleep; between 30 and 50 mg/kg, it induces sleep and has an anaesthetic effect; between 50 and 100 mg/kg, it can induce sleep, respiratory depression and coma—and possibly death.

Because of its hypnotic activity, it can be dangerous when driving.

According to some experts, GHB abusers claim to feel "intoxicated" for 24-48 hours, after which they experience a "hangover". GHB also induces an amphetamine-like euphoria effect and potentiates the effects of other central nervous system stimulants such as ecstasy and amphetamines. It also interacts strongly with narcotics and alcohol. Some athletes use it as an alternative to steroids, believing it to stimulate the release of growth hormone. It has also been proposed as an alternative to tryptophan for people who want to lose weight. Finally, it induces memory gaps and has been used for criminal purposes to sedate subjects with a view to rape or theft; doses of 40-60 mg/kg are usually administered in this context.

GHB can induce adverse reactions such as sleepiness, hypotonia, dizziness, amnesia, nausea, vomiting, bradycardia, abnormal muscle movements, hypotension and hypokalaemia.

INDICATIONS FOR MEASUREMENT

To investigate the possibility that the subject has abused GHB, if there is evidence of toxicity or after a traffic accident, or that the subject might have been involuntarily administered the drug for rape or theft, for example.

INFORMATION

SAMPLE

– Heparinised plasma or blood on EDTA: The blood sample should be drawn as soon as possible after oral absorption.

– Urine: one urine sample.

QUESTIONS FOR THE PATIENT

If possible seek to understand the circumstances of the intoxication. Current medications and concomitant alcohol consumption?

SAMPLE STORAGE AND TRANSPORT

Freeze the plasma or urine at –20 °C within one hour of sampling.

ASSAY METHODS

Gas phase chromatography coupled with mass spectrometry.

NORMAL EXPECTED VALUES

– Blood: in the absence of any exogenous GHB, the physiological concentration in blood is below 6 mg/l.

– Urine: in the absence of any exogenous GHB, the physiological concentration in urine is below 10 mg/l

FOR FURTHER INFORMATION

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GASTRIN

DEFINITION

Gastrin is a polypeptide hormone derived from its precursor, progastrin, which is mainly synthesised in the G cells of the gastric antrum and the duodenum. Its secretion is under cephalic control via the vagus nerve and is also affected by the presence of food in the stomach. Its release is slowed by somatostatin C, which is produced by the antral D cells when the pH falls below 3.

PATHOPHYSIOLOGY

Gastrin has a trophic effect on the gastric and intestinal mucosa and it stimulates the secretion of hydrochloric acid from gastric parietal cells. It is measured to reveal gastrin hypersecretion, which may be secondary to G cell hyperplasia or to a gastrinoma. Gastrinomas (Zollinger-Ellison syndrome), are gastrin producing extra-gastric tumours, usually found in the pancreas or duodenum. They may be sporadic or form part of a type I multiple endocrine neoplastic syndrome (MEN I). They cause multiple relapsing ulcers, usually duodenal but sometimes gastric, which may be accompanied by diarrhoea and steatorrhoea.

INDICATIONS FOR MEASUREMENT

– Suspicion of the presence of gastrinoma (Zollinger-Ellison syndrome). Gastrin secretion can be studied in the fasting state or after stimulation with secretin (infusion of 3 U/kg in one hour; samples at baseline (t0) and then every 15 minutes during the infusion and 15 minutes after it is stopped).

– Monitoring of gastrinoma post-surgery.

INFORMATION

■ SAMPLE

Serum is preferable and blood should be collected in a dry tube. If assaying plasma, blood can be collected with any anticoagulant other than heparin. The sample should be taken after fasting for a minimum of 10 hours. Patients must stop anti-secretory treatment with proton pump inhibitors at least 2 days before the test and H2 antagonists at least 5 days before testing *(see below)*.

■ QUESTIONS FOR THE PATIENT

Are you on any treatment? Certain drugs can change blood levels of gastrin. Calcium can increase gastrin secretion, and protein pump inhibitors and H2 antagonists increase blood levels during the treatment period (with return to normal after stopping treatment).

■ SAMPLE STORAGE AND TRANSPORT

Assay straight after sampling or centrifuge, separate serum or plasma and freeze within an hour of sampling.

Transport frozen at – 20°C.

ASSAY METHODS

Competitive radio-immunoassay.

NORMAL EXPECTED VALUES

These vary according to the assay employed. For example: fasting serum gastrin: 30 to 120 pg/ml. The most frequently used standard is MRC 68/439.

In the healthy subject, a secretin test reduces blood gastrin and gastric acid secretion.

Serum gastrin rises after food and increases slightly with age.

PATHOLOGICAL VARIATION

Interpretation of hypergastrinaemia is only possible if one is supplied with clinical information and an assessment of gastric acid secretion. In general, hypergastrinaemia associated with acid hypersecretion puts the patient at risk of gastro-duodenal mucosal ulceration and/or diarrhoea. The combination of hypergastrinaemia and reduced gastric acid secretion, on the other hand, suggests that malignant proliferation of the mucosal cells of the gastric fundus is a possibility with a background of severe atrophic gastritis and/or pernicious anaemia.

Diagnosis of gastrinoma is suggested by an increase in serum gastrin combined with increased gastric acid production. The degree of the increase in fasting gastrin levels can be substantial (up to 1,000 times normal) but this is not always the case. For example, it has been shown that gastrin levels > 325 pg/ml have a specificity of 100% and sensitivity of 53% in the diagnosis of gastrinoma. However, in about 1 case out of 2 with gastrinoma, the basal gastrin level is normal.

A secretin test increases the sensitivity of diagnosis and/or can be used to confirm a diagnosis of gastrinoma. In Zollinger-Ellison syndrome the results are striking with gastrin > 270 pg/l after secretin and no inhibition of acid secretion. Antral G cell hyperplasia is the main differential diagnosis of the cause of ulcers, usually duodenal, in the presence of gastrin hypersecretion, gastric hyperacidity and steatorrhoea. In this condition and in simple ulceration, the secretin test is negative with inhibition of acid secretion and no increase in gastrin.

NB: Increased fasting gastrin levels can also be found in other situations, such as:

– gastric or duodenal ulcers (slight elevation)

– Helicobacter pylori infection (slight elevation)

– gastric fundal atrophy

– gastric cancer

– pernicious anaemia (sometimes substantial elevation)

– total vagotomy

– phaeochromocytoma

– chronic renal failure.

Post-surgical monitoring of gastrinoma: The persistence of elevated gastrin values after surgery suggests that there is residual tumour tissue or, more often, the existence of multifocal tumours.

FOR FURTHER INFORMATION

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GENTAMICIN

DEFINITION

Gentamicin is an aminoglycoside antibiotic characterised by its bactericidal action on numerous Gram positive and negative bacteria. It is used as monotherapy and also in combination with other antibiotics, notably with β-lactams for infections with sensitive organisms, especially Gram negative bacilli. Its main drawback is its nephro- and ototoxicity.

For systemic use it is supplied as:

– Injectable ampoules of 1 ml at 10 mg/ampoule, of 2 ml at 40 mg/ampoule, 80 mg/ampoule and 160 mg/ampoule.

– Beads for surgical use (chains of 30 or 60 beads with 4.5 mg/bead), carried on a multifilament stainless steel wire.

The preferred route of administration is by intramuscular injection according to two regimes:

– Two or three injections daily.

– Or more often, by single daily injection: in patients less than 65 years old with normal renal function and no neutropaenia, for treatment courses less than 10 days and only for infections with Gram negative organisms, excluding Pseudomonas and Serratia. In these situations it has been shown that effectiveness is at least as good and tolerability is sometimes better with a single daily injection.

– It is sometimes administered intravenously, by intermittent infusion over 30 to 60 minutes. It can also be given intrathecally.

The usual dose is:

– In the adult: 3 mg/kg/day

– In the child >1 year: 1 to 1.5 mg/kg every 8 hours

– In the infant (10 days to 1 year): 1.5 to 2 mg/kg every 8 hours

– In the newborn < 10 days: 2 to 3 mg/kg every 8 hours.

The aminoglycosides are concentration dependent antibiotics carrying a post-antibiotic effect, so that it is possible to space out the interval between doses without the fear of renewed bacterial growth.

PHARMACOKINETICS

Bioavailability (parenteral route)	100%
Plasma peak	30 to 60 minutes after intramuscular injection
Time to steady state	24 hours (second day of treatment)
Protein binding	Weak (0 to 3%)
Metabolism	Nil
Excretion	Renal, unchanged
Excretory half-life	– 2 to 3 hours in subjects with normal renal function – 7 to 8 hours in newborn – Increased in the elderly and with renal failure – Varies with body weight, in patients in intensive therapy and those with severe burns.

INDICATIONS FOR MEASUREMENT

Tests on plasma are usually carried out during treatment, because the therapeutic index for gentamicin is low and its pharmacokinetics is variable.

Because of the danger of toxicity (ototoxicity and nephrotoxicity, gentamicin being the most nephrotoxic of the aminoglycosides), and the severity of the infections being treated, these measurements check that the drug regime is effective without reaching toxic levels.

These tests are mandatory when treatment has to continue beyond 7 days in the following situations:

– Patient over 65 years old

– Renal failure

– Severe infection with Gram negative bacilli

– Newborn and young children

– Obese subjects.

Two measurements are necessary:

– "peak" (maximum concentration) to check effectiveness

– "trough" to check that the antibiotic is not accumulating (toxicity).

INFORMATION

SAMPLE

Serum or plasma.

Measurements must be performed at the initiation of treatment, once steady state has been achieved, that is 24 hours after start of therapy, or 24 hours after a change of dose:

– before the next dose so as to obtain a trough level

– for peak level, 30 minutes after end of an IV infusion (blood collected from the arm opposite to that used for the infusion) or 1 hour after IM injection.

QUESTIONS FOR THE PATIENT

It is mandatory that any request form for measurement of a drug level, must carry the reason for the investigation (looking for effectiveness or toxicity), the time of sampling and the date when treatment was started and/or of any change in dose regime. There must also be an account of the drug regime itself (dose and frequency and route of administration), as well as the age of the patient and his/her height and weight when available.

Treatment with other nephro- or ototoxic drugs (danger of additive toxicity).

Previous treatment with other aminoglycosides or with a loop diuretic. There is potentiation of nephrotoxicity, especially with cyclosporin, tacrolimus, amphotericin B, polymyxin and cisplatin.

SAMPLE STORAGE AND TRANSPORT

Period between sampling and arrival in the laboratory less than 3 hours; centrifuge and separate rapidly.

Storage and transport of serum/plasma for 24 to 48 hours at + 4° C; for longer time, freeze at – 20° C.

ASSAY METHODS

Mainly immunological: FPIA (fluorescein polarisation immunoassay), EMIT (enzyme multiplied immunoassay technique) and immunoturbidimetry.

NORMAL EXPECTED VALUES

Classical treatment (2 injections daily):

Trough level < 2 µg/ml. A trough concentration less than this threshold shows that the treatment regime is well adapted to the patient's ability to eliminate the drug.

Peak concentration; 5 to 12 µg/ml. Low peak concentrations are associated with failure of therapy.

Single daily dose:

Trough level < 1 µg/ml.

Peak concentration: 15 to 20 µg/ml.

Conversion factor: 1 µg/ml = 0.463 µmol/l.

Adjustment of regime according to serum concentrations of antibiotic:

This is to be done case by case taking account of the patient's condition, the severity of the infection and the route and frequency of administration.

In general, if the trough level is too high, doses should be spaced out. If, after repeating the test, the peak level is too low, the administered dose should be increased.

FOR FURTHER INFORMATION

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GLUCAGON

DEFINITION

Glucagon plays a key role in blood homeostasis of glucose. Whereas insulin reduces blood sugar, the other regulatory hormones (glucagon, adrenaline, growth hormone and cortisol), all act to increase blood glucose.

The pre-proglucagon gene on chromosome 2 codes for a polypeptide which is converted to glucagon in the pancreas and to enteroglucagon in the intestine. Enteroglucagon is split into different components. These include glicentin, a 69 amino-acid peptide which includes the 29 amino-acid sequence of glucagon, oxyntomodulin and the *glucagon-like I and II* peptides (GLP-I and GLP-2). While GLP-I is a potent stimulator of insulin release, both GLPs have a role in secretion of gastric acid.

Glucagon is a polypeptide hormone secreted by the α cells of the pancreatic islets of Langerhans. It is composed of a 29 amino-acid polypeptide chain and its molecular mass is 3,485 kDa.

In response to a lowering of blood sugar, glucagon is secreted by the α cells into the hepatic portal circulation. In the liver, glucagon binds to a specific membrane receptor of the hepatocyte. An increase in cyclic-AMP follows and this stimulates activity of kinase A. That, in turn, stimulates gluconeogenesis and glycogenolysis. There is a consequent increase in hepatic glucose production.

This increase, however, is transient. Despite the hyperglucagonaemia, the production of glucose returns to basal levels. The transient nature of the glycogenolytic response is not due to depletion of glycogen, but results from the action of insulin secreted in response to the rise in glucose. In addition, glucagon induces ketogenesis and blocks hepatic lipogenesis.

The secretion of glucagon is regulated by blood glucose levels with hypo- and hyperglycaemia having respectively a stimulatory or inhibitory effect. Stress, physical exercise and amino-acids provoke glucagon secretion, while insulin inhibits it. Insulin also has an inhibitory effect on expression of the glucagon gene and thus on biosynthesis of glucagon. In addition there is a paracrine system linking the three peptides within the pancreas.

It should be noted that glucagon has a very short half-life of approximately 5 minutes.

INDICATIONS FOR MEASUREMENT

Measurement of glucagon is recommended when investigating hypoglycaemia and diabetes.

INFORMATION

SAMPLE

In view of the fragility of the glucagon molecule, blood specimens should be taken into cooled glass tubes containing EDTA and an inhibitor of proteolysis (aprotinin). The tubes are kept in an ice bath while awaiting centrifugation, which is performed at + 4° C at around 2,000 rpm. This should be done within an hour of sampling. After centrifugation, plasma is separated and frozen at – 20° C until the assay is performed.

ESSENTIAL INFORMATION

The blood sugar must be known.

ASSAY METHOD

Glucagon is measured using competitive radioimmunoassay, the tracer being 125-iodine labelled glucagon.

Before performing the assay, the specimens must be thawed in melted ice.

RESULTS AND NORMAL VALUES

The results are expressed either in pg/ml or in pmol/l. Conversion to pmol/l is performed by multiplying pg/ml by a factor of 0.28.

Fasting plasma concentrations of glucagon lie between 60 and 200 pg/ml (15 to 55 pmol/l).

PATHOLOGICAL VARIATIONS

INCREASED GLUCAGON

- **Glucagonoma:** Glucagon secreting α cell tumours of the pancreas are very rare and slow-growing. The average age of onset of symptoms is 50 years. 80% of patients are female. Glucagonomas have occasionally been described in type I multiple endocrine neoplasia (MEN I).

  The pathophysiology results from the stimulatory effects of glucagon on glycogenolysis, gluconeogenesis and ketogenesis.

  Symptoms comprise of:

  – Necrolytic migratory erythema. This is a pathognomonic clinical feature. It is most marked on the thighs, the buttocks, the inguinal region and the limbs

  – Mucosal lesions (stomatitis and glossitis)

  – Polyuria and polydipsia

  – Weight loss

  – Venous thrombosis occurs in a quarter of cases.

  Laboratory diagnosis depends on the demonstration of hyperglucagonaemia (sometimes reaching to 500 times the normal upper limit) in association with a pancreatic mass. The severity of the symptoms is not, however, proportional to the degree of hyperglucagonaemia, because of the heterogeneity of plasma immunoreactive glucagon.

  Other blood abnormalities are: diabetes, seen in the majority of cases, hypochromic normocytic anaemia, hypoalbuminaemia and hypo-aminoacidaemia.

- **Other pathological conditions:** Plasma glucagon can be elevated in other situations, such as diabetes, pancreatitis, burns, trauma, myocardial infarct, Cushing's syndrome, and acromegaly. It is also high in some obese subjects. The mechanism may then be due to insulin resistance.

DECREASED GLUCAGON

Glucagon is low in cases of isolated absence of islet α cells, in chronic pancreatitis, during beta-blocker treatment and also during long-term sulphonylurea treatment.

A fall in glucagon secretion might be of pathogenic importance in idiopathic post-prandial hypoglycaemia. A simultaneous compensatory increase in adrenaline secretion can prevent hypoglycaemia and restore normal blood glucose. In favour of this hypothesis is the fact that low plasma glucagon concentrations have been observed in patients who have a nadir of blood glucose less than 2.8 mmol/l (0.5 g/l), after a glucose load. These results have not, however, been confirmed in other studies.

FOR FURTHER INFORMATION

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GLUCOSE 6-PHOSPHATE DEHYDROGENASE

DEFINITION AND SYNONYMS

Glucose 6-phosphate dehydrogenase (G6PD) is the first enzyme in the chain involved in glucose catabolism via the pentose phosphate shunt. It catalyses the reaction:

Glucose 6-phosphate + NAD → 6-phosphogluconate + NADPH + H^+

G6PD is produced in all nucleated cells in the body. Its role in the red blood cell lies in the regeneration of systems of protection against oxidising agents (reduced glutathione and methaemoglobin reductase), as well as in energy production. As the red cell is not nucleated, it relies for metabolic purposes on the store of enzyme built up at the erythroblast stage.

INTRODUCTION

Lack of G6PD is the red cell enzymopathy which is most widely distributed in the world. It is thought that some 420 million individuals are affected worldwide. The most affected regions are sub-Saharan Africa, countries around the Mediterranean basin, the Near and Middle East, South-East Asia, the Philippines, Malaysia, South China, India and countries where a part of the population has come from these zones at risk (United States, West Indies and South America).

G6PD deficiency is a genetic disease. The gene coding the enzyme is on chromosome X in position Xq28. Some 500 variants have been identified: some of these are associated with deficiency, while others have normal G6PD activity. It is predominantly a condition that affects males. Female heterozygotes can express low or normal G6PD activity due to the phenomenon of random inactivation of the X chromosome. Of the variants which do not cause deficiency, B+ is the one that is commonest, taking all ethnic groups together. A+ is found in 20% of Africans without deficiency. B- does cause deficiency. It is often found in Mediterranean populations and is associated with very low red cell G6PD levels at around 1% of normal. A- is mainly found in Africans and is associated with erythrocyte G6PD activity that is less severely affected (from 5 to 15% of normal activity).

The main symptom of G6PD deficiency is haemolysis, which presents three principal clinical patterns:

– *Acute haemolytic anaemia* precipitated by ingestion of certain drugs or foods (Table 1), during the course of an infection (infectious mononucleosis, viral hepatitis, viral upper respiratory tract infections, typhoid, etc.) or by diabetic acidosis. The episode, which can be very severe, resolves spontaneously but recurs whenever the responsible drug or food is taken again. The manifestations can be extremely serious, or even fatal, in particular in the child and also in the special case of ingestion of broad beans (favism), a form which has been most frequently described in patients coming from the Mediterranean basin. This has also been found in the Middle and Far East and in Africa.

– *Chronic haemolytic anaemia:*

This is a non-microcytic anaemia and is independent of any exogenous precipitating factor. It is rare.

– *Neonatal jaundice:*

The haemolytic episode can necessitate exchange transfusion. It is usually without long-term repercussions, and is seen in countries where G6PD deficiency is common, such as the Mediterranean basin and sub-Saharan Africa.

In practice G6PD deficiency is completely asymptomatic in the majority of cases, in the absence of a toxic or infective precipitant. There seems not to be a close relationship between the degree of G6PD deficiency and the severity of the symptoms. The great heterogeneity of clinical expression can be seen as reflecting the variant involved and the level of trough activity in the red cell. The WHO classification is based on the clinical picture and trough erythrocyte G6PD activity *(table II)*.

Table I: Drugs and foods known to precipitate haemolytic crises linked to G6PD deficiency

Therapeutic class	Drug	Therapeutic class	Drug
Anti-malarials	Pamaquine Pentaquine Primaquine Quinine	**Analgesics**	Acetanilide Aspirin Benorylate Diflunisal Floctafenine Noramidopyrine Phenacetin Phenazone
Sulphonamides	Sulphacetamide Sulphadiazine Sulphadoxine Sulphafurazole Sulphamethizole Sulphamethoxazole Sulphanilamide Sulphapyridine Sulphasalazine	**Miscellaneous**	Ascorbic acid Para-aminobenzoic acid Methylene blue Cyclophosphamide Dimenhydrinate Dimercaprol Diphenylhydramine Doxorubicin Hydroquinidine Hydroxychloroquine Niridazole Phenylhydrazine Quinidine Urate oxidase Vitamin K
Sulphones	Dapsone Thiazolsulphone	**Other chemical agents**	Toluidine blue Naphthalene Nitrobenzene Trinitrotoluene
Other anti-bacterial agents	Nalidixic acid Oxolinic acid Para-aminosalicylic acid Pipemidic acid Chloramphenicol Ciprofloxacin Flumequine Nitrofurantoin Norfloxacin Ofloxacin Perfloxacin Rosoxacin Sparfloxacin	**Foods**	*Vicia faba* (broad bean)

Table II: WHO classification of G6PD deficiency

Class of deficiency	Type of deficiency	Trough G6PD erythrocyte activity
Class I	Severe deficiency	1 to 2%
Class II	Intermediate deficiency	3 to 10%
Class III	Slight deficiency	10 to 40%

INDICATIONS FOR MEASUREMENT

Measurement is indicated to define a G6PD deficiency when one is suspected because of haemolysis or haemolytic anaemia with a negative direct Coombs test, especially in the presence of prolonged jaundice in the new-born baby, or for family studies.

INFORMATION

■ SAMPLE

The assay is carried out on a washed blood cell deposit. The blood should be collected on EDTA or lithium heparin. If the sample is taken in to a heparin tube and is to be transferred for assay, the results of the full blood count should be forwarded with the sample.

Venous sampling is performed under the usual conditions. There is no circadian rhythm variation.

■ QUESTIONS FOR THE PATIENT

Has the patient taken any drug or food that might cause haemolysis in a subject with G6PD deficiency (Table I)?

Does a family member have G6PD deficiency?

What are the patient's geographic origins?

Has the patient received a transfusion in the three months preceding the test?

■ SAMPLE STORAGE AND TRANSPORT

G6PD is a fragile red cell enzyme, so care must be taken to avoid in-vitro haemolysis of specimens. Measurements must be performed within four days of sampling. The sample should be stored and transported at room temperature or at + 4° C.

AVAILABLE ASSAY METHODS

These are mainly spectrophotometric methods. There are fluorimetric techniques that can be employed for rapid screening of at risk subjects, but these are currently rarely used. Molecular biological genetic analysis is confined to a few very specialised centres.

The simple blood count should be performed in parallel, including a reticulocyte count. The results should be related either to the red cell count or to the haemoglobin level.

Another enzyme can be measured in parallel (red cell pyruvate kinase or red cell ASAT) so as to calculate the G6PD/pyruvate kinase ratio or the G6PD/ASAT ratio. This facilitates correction for a high reticulocyte count which could mask a deficiency.

NORMAL EXPECTED VALUES

The normal expected values vary according to which reagent is used and to the temperature chosen for the reaction. Levels are higher in the new-born baby than in the adult. As an example, normal expected values in the adult are greater than 120 mU/109 red cells. In the new-born baby levels are above 190 mU/109 red cells. In the new-born baby the G6PD/ASAT ratio is greater than 0.55 (normal value of erythrocyte ASAT: 200 to 450 mU/109 red cells).

Where the results are expressed as U/g of haemoglobin, the reference values are greater than 5 U/g of haemoglobin. Each laboratory must define its own normal values and it is appropriate to relate the results to the reference values of the laboratory that has performed the test.

PATHOPHYSIOLOGICAL VARIATIONS

Concentrations of G6PD are high in the new-born baby and even more so in the premature. Within a few weeks levels fall to those found in the adult.

G6PD concentrations are higher when there is a reticulocytosis, as reticulocytes and juvenile red cells have a higher G6PD content than older cells.

Microcytosis artificially increases apparent G6PD activity when this is related to haemoglobin. In this situation it is preferable to express the result in mU/109 red cells.

After transfusion, it is difficult to interpret results, particularly if the transfusion was recent and in a large quantity.

FOR FURTHER INFORMATION

■ Frank J.E., *Diagnosis and management of G6PD deficiency*, Am Fam Physician 2005; 72: 1277-82

GLUCOSE TOLERANCE TEST

DEFINITION

The glucose tolerance test is a test of orally provoked hyperglycaemia used to study changes in blood glucose after an oral glucose load.

It is used to confirm or exclude impaired glucose tolerance or diabetes if the blood glucose is above the upper limit of normal.

INFORMATION

Venous samples are collected into tubes containing sodium fluoride. These should preferably be taken with a catheter needle left in-situ during the study.

The sampling conditions are very precise and must be observed:

– The investigation must be performed in the morning.

– The patient must have been fasting for 12 hours and have followed a normal carbohydrate diet (200 to 300 g of carbohydrates/day) for the 3 days before the investigation.

– The patient must be under strict rest in a relaxed environment and excessive physical effort must be avoided in the morning of the test.

– The patient must not smoke before or during the test.

– The test should not be performed around the time of an acute illness or surgery.

- Drugs that can interfere with blood glucose measurements should be stopped for several days:

  - Drugs that may cause hyperglycaemia (oestrogens, corticosteroids, diuretics, ß blockers, calcium channel blockers).

  - Drugs that may cause hypoglycaemia (aspirin, MAIO, perhexiline, quinine, disopyramide).

If the blood glucose measurements cannot be performed promptly, the blood samples should be collected into tubes containing sodium fluoride and centrifuged immediately. Plasma samples should then be stored on ice or frozen until measurements are performed.

PROTOCOL

– Blood glucose at T0

– 75 grams of glucose dissolved in 250 ml of water swallowed within five minutes in adults.

– 1.75 g of glucose/ kg bodyweight, not exceeding 75 grams, for children.

– 100 g of glucose dissolved in 250 ml of water for pregnant women.

– Blood glucoses at T+30, T+60, T+90 and T+120 minutes.

In addition to blood glucose, insulin and C-peptide can also be measured.

INTERPRETATION

The interpretative criteria defined by the WHO have been restated by the ADA (American Diabetes Association). The 2-hour value should be used to interpret the test. The criteria currently accepted for the diagnosis of diabetes or impaired glucose tolerance are shown in the table below. Interpretation of gestational diabetes is considered in a separate chapter *(cf: O'Sullivan test)*. The values shown are for plasma glucose measurements.

Blood glucose	Normal	Impaired glucose tolerance	Diabetes
Fasting T0	< 1.10 g/l < 6.16 mmol/l	> 1.10 - < 1.40 g/l > 6.16 - < 8 mmol/l	≥ 1.26 g/l ≥ 7 mmol/l
T+120 min	< 1.40 g/l < 7.8 mmol/l	1.40 - < 2,0 g/l 7.8 - < 11.1 mmol/l	≥ 2 g/l ≥ 11.1 mmol/l

The OGTT is a poorly reproducible test and is used mostly in epidemiological studies on diabetes, to study reactive hypoglycaemia and in early screening for diabetes. In the latter situation the test may need to be repeated to confirm the diagnosis or combined with other investigations such as measurement of insulin. The OGTT can only be interpreted validly if the sampling conditions are correctly observed and when it is interpreted in light of the clinical context.

FOR FURTHER INFORMATION

■ *Protocoles d'exploration en biochimie*, version 3, Collège National de Biochimie des Hôpitaux, 2004.

GLYCATED HAEMOGLOBIN

DEFINITION

Non-enzymatic glycation of proteins is a phenomenon, which has been known about for more than 40 years. The extent of the phenomenon is determined *in-vivo* mostly by blood glucose concentration. It affects all proteins, particularly those with a long half-life and notably haemoglobin (Hb). Glycated haemoglobin is the group of haemoglobin molecules, which have been altered by non-enzymatic glycation. It reflects mean blood glucose over the previous 120 days (2 to 3 months), which represents the lifespan of red blood cells. It is neither a screening test nor a diagnostic test for diabetes and its use is currently restricted to monitoring diabetes mellitus.

HbA1c is a glycated haemoglobin, formed from binding of a glucose molecule to the N- terminal end of at least one beta chain of HbA (the main haemoglobin fraction in adults, which has a protein structure consisting of two alpha chains and two beta globulin chains).

HbA1c has been validated to monitor diabetic patients.

The diagnostic use of HbA1c for diabetes mellitus has been under consideration since 1988 (R. R. Little, *Diabetes*, 1988); and this possibility is currently being studied by an international expert committee in conjunction with the ADA *(American Diabetes Association)*, EASD *(European Association for the Study of Diabetes)* and IDF *(International Diabetes Federation)*.

Synonyms: HbA1c is often referred to by the terms glycated haemoglobin or glycohaemoglobin, which are not exact synonyms as they are imprecise. The term "glycosylated haemoglobin" is totally inappropriate and should not be used (unlike glycation, glycosylation is an enzymatic mechanism involved in protein synthesis).

INTRODUCTION

The use of HbA1c as a monitoring marker for diabetes mellitus was validated in two large epidemiological trials, the DCCT *(Diabetes Control and Clinical Trial)* and the UKPDS *(United Kingdom Prospective Study of Diabetes)*. The DCCT trial conducted in type 1 diabetes demonstrated for the first time the relationship between glycaemic control as assessed by HbA1c and complications of diabetes and set a target HbA1c value to achieve for good control (< 7 %). The UKPDS study conducted in type 2 diabetes confirmed the relationship between glycaemic control and the complications of diabetes and showed that a 1 percentage point reduction in HbA1c reduced the risk of microangiopathic complications for 10 to 30% and the risk of myocardial infarction by approximately 15%.

In parallel, a huge standardisation exercise for HbA1c measurement methods was undertaken across the world resulting in the definition of technical standards and treatment objectives, expressed in % HbA1c *(cf. below)*.

For this reason, HbA1c is currently recognised as the best marker of glycaemic control to monitor diabetic patients.

INDICATIONS FOR MEASUREMENT

Monitoring diabetic patients (type 1 and 2 diabetes).

Monitoring in type 2 diabetes should be based on measurement of HbA1c every 3 or 4 months. HbA1c measurements must be performed in the same laboratory for the same patient to allow the successive results to be compared. HbA1c measurement is used in combination with self-monitoring of blood glucose to provide an objective reflection of the effectiveness of treatment. The testing interval may be temporarily changed as a result of adjustments to treatment or poorly controlled diabetes.

NB: HBA1c measurement is not currently proposed in usual practice to diagnose diabetes mellitus.

INFORMATION

SAMPLE

Venous blood preferably collected into EDTA. Tubes containing heparin, oxalate/fluoride or ACD mixture are acceptable. The measurement can also be performed on capillary blood collected into a capillary tube containing EDTA, or onto filter paper.

A fasting sample is not required.

QUESTIONS FOR THE PATIENT

Check the indication and frequency of measurement (Monitoring diabetes? The date of the last measurement?). Is the patient taking aspirin? Acetyl salicylic acid (and some ethanol derivatives) may lead to over-estimation of the result, although this interference appears to be negligible *in-vivo*.

SAMPLE STORAGE AND TRANSPORT

Store whole blood at + 20°C: for up to 3 days and at + 4°C for up to 7 days.

If the test is to be performed later, the sample must be frozen at – 20°C or – 70°C. It is stable for several months at – 70°C.

The red cells must be haemolysed before HbA1c is measured: this may be performed manually or automatically. The haemolysis reagent also removes unstable forms of HbA1c, which do not correlate with glycaemic control.

ASSAY METHODS

Methods that measure HbA1c specifically are either ion exchange chromatographic methods (mini-columns, high performance liquid chromatography, low pressure liquid chromatography), electrophoretic or immunochemical techniques.

NORMAL EXPECTED VALUES - STANDARDISATION

A first attempt of standardisation was made by the NGSP *(National Glycohemoglobin Standardisation Program)* in the 1990s. This was a pragmatic system based on comparing assay methods against ion exchange chromatography on a Bio-Rex 70 resin (unpure HbA1C). This method was long considered to be the reference method as it had been used in the two major international trials (DCCT and UKPDS). The IFCC *(International Federation of Clinical Chemistry)* method was developed in parallel and defined the reference HbA1c analyte as the glycated N-terminal hexapeptide of the β haemoglobin chain and the reference method (reverse phase high performance liquid chromatography linked to mass spectrometry or capillary electrophoresis). This is a laborious technique and not suitable for routine measurements although it does allow laboratory methods to be calibrated. It is more specific than the NGSP method although the values obtained by IFCC standardisation are 1.5 to 2% lower than those obtained with NGSP/DCCT. The relationship between the two standardisation methods is: NGSP = (0.925 x IFCC + 2.15). In order not to cause confusion, the decision was taken to express the results obtained by this method in mmol HbA1c per mole of haemoglobin and not as a percentage HbA1c against total haemoglobin. This expression produces results, which cannot be confused with NGSP/DCCT results but are numerically very different.

An international consensus was reached in 2007 to standardise measurement and expression of the HbA1c result. This is currently being introduced nationally in most countries. An equivalence table between HbA1c and mean blood glucose can be provided with these results provided that individual results are interpreted with caution.

The equivalence between the different ways of expressing HbA1c is shown below for a few key values:

NGSP (%)	IFCC (mmol/mol)	Mean blood glucose mmol/l	g/l
4.0	20	3.6	59
6.0	42	7.6	129
7.0	53	9.6	164
8.0	64	11.5	200

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

HbA1C is independent of variations in blood glucose during the day and is not affected by fasting status, physical exercise or recent sugar ingestion. It increases moderately with age (+ 0.6 % total haemoglobin between 20 and 70 years old).

PATHOLOGICAL VARIATIONS

Some pathological situations can produce incorrect results

Any change in the lifespan of red blood cells and/or haemoglobin synthesis interferes with the relationship between HbA1c and glycaemic control. Interpretation of HbA1c levels therefore becomes difficult or impossible in the following situations: intravascular haemolysis, severe haemorrhage, large blood losses (repeated venesection in haemochromatosis), and treatments that stimulate haemoglobin synthesis or recent transfusions. Similarly, haemoglobinopathies interfere with interpretation of results, firstly by creating metabolic abnormalities (haemolysis, inappropriate synthesis of Hb, different glycation kinetics and formation of unusual glycated products), and secondly measurement interferences particularly with methods using charge separation. It is often difficult to interpret results if a haemoglobin variant is present.

Finally, Hb carbamylation (binding of urea derivates to Hb) may invalidate results in patients with renal insufficiency.

In these situations, fructosamine measurement, which reflects glycaemic control during the 2 to 3 previous weeks before measurement, is an alternative.

Objectives in type 2 diabetes

Glycaemic targets in terms of HbA1c must be set individually depending on patient age, co-morbidities and psychosocial context. Clinical decision cut-offs have been validated in studies.

– The optimal target in type 1 and 2 diabetes is an HbA1c of 7%. If this target is achieved there is no need to adjust treatment unless side effects occur such as a risk of hypoglycaemia on sulphonylureas or insulin therapy.

– If the HbA1c is between 6.6 and 8% on two successive measurements, a change in treatment can be considered depending on the clinician's assessment of the balance between advantages and disadvantages of the planned treatment change.

– If the HBA1c is over > 8% on two successive measurements, a change in treatment is recommended.

NB: a discrepancy between a high HbA1c values, compared to the daily blood glucose measurements (self-monitoring of blood glucose) suggests an error in the patient's blood glucose record. Conversely, a low HbA1c should stimulate investigation for unrecognised nocturnal hypoglycaemia.

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- «Stratégie de prise en charge du patient diabétique de type 2 à l'exclusion de la prise en charge des complications.» (mars 2000) www.anaes.fr
- Gillery P., Périer C., Bordas-Fonfrède M., Hue G., Chapelle J.-P., Propositions pour l'expression standardisée des résultats d'HbA1c. Ann Biol Clin, 2009:669-671.

GLYCOSAMINOGLYCANS

DEFINITION

The glycosaminoglycans (GAGs), previously called mucopolysaccharides, consist of long unbranched polysaccharide chains, formed by repetition of the same disaccharide template. The disaccharides which compose this sequence, comprise a monosaccharide A (glucuronic acid, iduronic acid or galactose) and a monosaccharide B (N-acetylglucosamine or N-acetylgalactosamine). The GAGs produced in this way are hyaluronic acid, chondroitin-4-sulphate, chondroitin-6-sulphate, dermatane sulphate, heparane sulphate and keratane sulphate.

INTRODUCTION

In-vivo the GAGs do not exist in a free form but are combined with a central protein core, thus forming proteoglycans. The majority of proteoglycans are major constituents of connective tissue and of its fundamental substance. They are then found mainly in skin, bone, cartilage, blood vessels, cornea and heart valves. They are also secreted locally and enter the extracellular matrix on which they confer a variety of properties such as hydration, filtering capacity and resistance to compression. Other proteoglycans, principally, those containing heparane sulphate, are attached to the plasma membrane. They are involved in various cellular functions, notably multiplication, differentiation, migration and adherence.

GAGs are normally catabolised in the lysosomes. Abnormalities in catabolism can lead to overload in the lysosomes of affected tissues and to abnormal excretion in urine, both qualitatively and quantitatively. The diseases resulting from lysosomal overload are known as the mucopolysaccharidoses. They belong to the group of lysosomal storage diseases. The symptoms are extremely variable. In general there is an association of organomegaly (overload), dysmorphic features and, frequently, a neurological syndrome. These diseases usually appear in childhood. The symptoms are progressive, are sometimes delayed but always disabling.

INDICATION FOR MEASUREMENT

Screening (followed by characterisation) of mucopolysaccharidoses.

Measurement in parallel of GAGs and creatinine in urine.

INFORMATION

SAMPLE

Urine sample (first voiding of the morning) or 24 hour urine collection.

QUESTIONS FOR THE PATIENT

Check that the patient has not received heparin or polyanions, such as dextran sulphate, which can increase the urinary concentration of GAGs.

SAMPLE STORAGE AND TRANSPORT

Transfer the urine rapidly to the laboratory. If the analysis is to be deferred, freeze specimens within 4 hours of collection. Store and transport specimens frozen at – 20° C.

ASSAY METHODS

– Test using 1-9 dimethyl methylene blue (DMB): screening for mucopolysaccharidoses.

– Complete analysis of urinary GAGs: isolation and purification followed by quantification of uronic acids using the harmine method. Electophoretic separation to characterise and type the mucopolysaccharide.

NORMAL EXPECTED VALUES

These depend on the technique employed and vary with age.

Urinary concentrations of GAGs
(from Piraud et al, Clin Chem 1993)

	DMB test (mg/mmol creatinine)	Measurement with harmine (mg glucuronic acid/g creatinine)
0 to 3 weeks	3,6 – 43	22 – 80
3 weeks to 6 months	11,5 – 32	12 – 56
6 months to 1 year	7,9 – 26,9	9 – 46
1 to 3 years	5,0 – 18,0	8 – 29
3 to 7 years	5,6 – 15,4	6 – 23
7 to 15 years	3,0 – 11,6	3 – 16
15 to 20 years	0,9 – 6,0	1 – 15
Adult	1,3 – 4,0	1 – 8

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Varies with age *(see normal expected values above)*.

PATHOLOGICAL VARIATION

Increased urinary excretion of GAGs using the DMB test, the harmine method and/or the presence of abnormal electrophoretic fractions is usually found in patients with mucopolysaccharidoses *(see table)*. The electrophoretic profile indicates the type of the disease, but confirmation of the diagnosis requires the demonstration of an enzyme deficiency in white cells or cultured fibroblasts.

However, in some patients urinary GAGs concentration may be normal, particularly older subjects, in whom GAGs excretion is lower and the reading of abnormal fractions on electrophoresis can be difficult. The diagnosis can also be obscured in patients with type IV mucopolysaccharidosis, where urinary excretion is sometimes normal. The demonstration of an enzyme deficiency is necessary when there is a strong clinical suspicion, especially in the adult.

With the DMB test there can be false negatives if there is heavy proteinuria; false positives can occur in patients treated with heparin or polyanions (dextran sulphate). When results are borderline (small increase in urinary excretion of GAGs and/or faint abnormal fractions on electrophoresis), a further urine sample should be examined to confirm that the abnormality persists (possibility of interference by drug or food or presence of other lysosomal disease).

Mucopolysaccharidoses (MPS)

MPS I or Hurler's disease or Scheie's disease (mild form):

Deficiency of the enzyme alpha–L–iduronidase, leads to overload of heparane sulphate and dermatane sulphate. Autosomal recessive inheritance.

Symptoms appear in the first year of life: facial dysmorphism, hernia, hepatosplenomegaly, cardiac damage, respiratory problems, deafness, corneal opacities and severe mental retardation. In Scheie's disease the main abnormalities are cardiac and ocular; there is no mental retardation.

MPS II or Hunter's disease:

Deficiency of the enzyme iduronate 2-sulphatase, causing overload of heparane sulphate and dermatane sulphate. Inherited as X linked recessive.

The child is normal at birth. Symptoms appear progressively. They are similar to those of Hurler's disease apart from the absence of corneal opacities. Mild forms have been described.

MPS III or Sanfilippo's disease:

There are 4 sub-types (autosomal recessive transmission; heparane sulphate overload)

– MPS IIIA: deficiency of the enzyme heparane sulphamidase,

– MPS IIIB: deficiency of the enzyme N-acetyl glucosaminidase,

– MPS IIIC: deficiency of acetyl co-A; alpha glucosaminide N-acetyl transferase,

– MPS IIID: deficiency of acetylglucosamine-6-sulphatase.

The symptoms appear between 2 and 6 years and consist mainly of severe mental retardation. There are no mild forms.

MPS IV or Morquio's disease:

There are 2 sub-types (autosomal recessive transmission; keratane sulphate overload)

– MPS IVA: classical form with deficiency of the enzyme N-acetylgalactosamine-6-sulphatase,

– MPS IVB: classical form with deficiency of the enzyme beta-D-galactosidase.

Symptoms appear during the second year of life: skeletal abnormalities (spondylo-epiphysio-metaphyseal dysplasia), and neurological problems without mental retardation. Mild forms do exist.

MPS VI or Maroteaux-Lamy disease:

Deficiency of the enzyme acetyl galactosamine-4-sulphatase or aryl sulphatase B resulting in dermatane sulphate overload. Transmitted as autosomal recessive.

Similar clinical picture to Hurler's disease but without mental retardation. There are mild and very mild forms.

MPS VII or Sly's disease:

Deficiency of the enzyme beta-D-glucuronidase resulting in overload of dermatane sulphate, heparane sulphate and chondroitin sulphate. Autosomal recessive inheritance.

The clinical picture is very heterogeneous with ante-natal forms (anasarca), severe neo-natal affliction (dysmorphism, neurological problems, etc.) or very mild disease uncovered in adolescence or adulthood (thoracic kyphosis).

Other clinical situations in which urinary GAGs can be increased:

Rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, type 1 and type 2 diabetes and psoriasis.

FOR FURTHER INFORMATION

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GONORRHOEA

DEFINITION

Gonorrhoea is a sexually transmitted infectious disease caused by *Neisseria (N) gonorrhoeae* or gonococcus, a bacterium confined to humans and belonging to the genus *Neisseria* and the family of the *Neisseriaceae*. It is a highly contagious organism responsible mainly for genital infections. *N.gonorrhoeae* is a Gram negative encapsulated diplococcus and can be intra- or extracellular.

INTRODUCTION

EPIDEMIOLOGY

Gonorrhoea is a globally distributed disease, which is a particular problem in developing countries. It has increased in prevalence since 1998 and is one of the commonest sexually transmitted diseases (STDs) in the world, second only to Chlamydia trachomatis (Ct). Infection arises from sexual contact with an infected partner.

SYMPTOMS

- **Adult male:** the commonest manifestation is acute urethritis, which is characterised by a purulent urethral discharge, especially in the morning and by severe burning micturition. The incubation period is 2 to 6 days. The urethritis can be sub-acute or even chronic. There are attenuated or even asymptomatic forms. Local complications such as prostatitis, epididymitis and seminal vesiculitis are rare.
- **Adult female:** the infection passes unnoticed in 80% of women, who then become agents of infection. The classical picture is one of cervicitis including dysuria and yellowish or frankly purulent vaginal discharge. However, sometimes the symptoms are of urethritis alone. Complications are more extensive than those in the male. They include endometritis, bartholinitis and salpingitis, which can involve the peritoneum and, if untreated, lead to infertility.
- **New-born baby:** a contagious woman can infect her child during labour, producing gonococcal conjunctivitis in the new-born.
- **Male and female:** anorectal and pharyngeal involvement can occur. Disseminated forms combining dermatitis and polyarthralgia are rare and found especially in HIV positive individuals.

INDICATIONS FOR MEASUREMENT

DIRECT DIAGNOSIS

– Diagnosis of gonorrhoea in male and female

– Diagnosis of gonorrhoea in investigation of hypofertility

– Differential diagnosis of urethritis in the male and vaginal discharge in the female.

INDIRECT DIAGNOSIS

– Rare indication: diagnosis of cause of polyarthralgia.

INFORMATION

SAMPLE

The samples to be collected depend on the site of the infection, the sex of the patient, the age and sexual practices. The nature of the sample will also reflect the laboratory technique to be used (culture or PCR, *see below*).

Urine collection (first stream): for diagnosis of urethritis in male and female.

Urethral swab: to be performed some time after micturition. After cleaning the urinary meatus, 2 swabs are used (one for direct examination and the other for culture).

Endocervical swab: to be taken for women if signs of cervicitis.

Vaginal collection by patient: recommended for screening of females.

Swab from Bartholin gland area: if suspected bartholinitis.

Samples from para-urethral glands (Skene's glands), Fallopian tubes or endometrium.

Ano-rectal sampling: swabbing after cleaning the anal orifice.

Others: pharyngeal, ocular, cutaneous, joint fluid and blood cultures.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

Previous acute symptoms?

Name(s) of partner(s)?

Exploration of symptoms suggestive of complications?

Present antibiotic treatment?

SAMPLE STORAGE AND TRANSPORT

Samples for culture must be transported as rapidly as possible to the laboratory, as the gonococcus is a fragile organism. Ideally the swab specimen should be spread immediately onto culture medium. A suitable medium for transport is essential if there is to be any delay before culturing the sample. Maximum delay of 24 hours only.

For PCR the transport media are stable for several days or even weeks, when the specimens are handled at ambient temperature.

BIOLOGICAL DIAGNOSTIC METHODS

DIRECT BACTERIOLOGICAL DIAGNOSIS

- **Direct examination:**this is carried out on a Gram stained or methylene blue stained smear of urethral pus. The diplococci are seen as Gram negative coffee bean shaped intra or extracellular organisms. This is a very sensitive examination in the male but more uncertain in the female, in whom vaginal, urethral and endocervical secretions contain commensal diplococcal flora. In all cases, confirmation by culture is required.
- **Culture:** this is performed on chocolate agar with and without a VCN or VCAT type inhibitor. Culture can also be performed on clear media such as "G" medium or "gonococcal-meningococcal" medium. Whatever culture medium is employed, the culture must proceed in a CO2 enriched and highly humidified atmosphere. Gonococcal colonies are tiny

(0.5 to 1 mm in diameter) with irregular borders and a greyish appearance. They should be sub-cultured onto a non-selective medium.

– Biochemical and immunological identification: biochemical characteristics are examined in an isolated pure strain obtained after sub-culturing. In addition, it is possible to perform direct antigen studies on urethral swab specimens and on first stream urine samples.

Culture is sensitive, specific and cheap. This is currently considered to be the reference method and has the advantage of allowing isolation of the strain and study of its antibiotic sensitivity. However, it requires specific samples collected by qualified staff using suitable equipment. The conditions for transport and storage of specimens are demanding (< 24 h). Culture is also a delicate technique: it takes 24 to 48 hours or even 72 hours of incubation to isolate the organism, which means that it is not a suitable method for mass screening, because of the long period before results can be returned. In addition, it is all the more difficult when examining material from mixed infectious sites such as the pharynx, rectum and uterine cervix, by virtue of the presence of a rich and polymorphous commensal flora. Usually the bacterial infection in these sites is light. In fact, for such specimens, selective media containing vancomycin are employed, but this can, in some cases, also inhibit the gonococcus (5 to 30% of strains are said to be inhibited). Finally, culture is very difficult with vaginal samples taken by the patient and for first stream urine specimens.

- **Molecular biology:** current techniques of gene amplification (second generation) are more specific than those of the first generation (which cross reacted with *Neisseria* commensals), more sensitive than culture and suitable for large numbers. Amplification techniques can be applied to specimens from any site of collection including first stream urine and auto-sampling from the vagina. The technique can be automated, so that results can be obtained promptly.

  In early 2010 the majority of commercial kits recommend simultaneous screening for *Chlamydia trachomatis* and *N. Gonorrhoeae* at a price which is almost the same as for a screen for *Chlamydia* alone. Other kits are being developed, which will test for other pathogens simultaneously. These tests have excellent sensitivity and specificity, close to 99%.

INDIRECT DIAGNOSIS

Several techniques are available: complement fixation, haemagglutination and indirect immunofluorescence. It is possible to perform sero-typing on pure strains using monoclonal antibodies.

TREATMENT

CURATIVE TREATMENT

In France in 2007, 4.5% of strains were resistant to penicillin, 8% to tetracyclines and about 11% to either penicillin or tetracycline. These percentages are rising. The picture for fluoroquinolone antibiotics (FQ), which were introduced in the 1990's, is similar. Today more than 50% of strains have marked cross-resistance to all the FQ.

Therefore, current recommendations are that urethritis and uncomplicated cervicitis should be treated with ceftriaxone (or cefixime). Recently, strains resistant to third generation cephalosporins such as cefixime have been reported from Japan, India and Greece. Strains with diminished sensitivity to ceftriaxone have been identified in Greece and Portugal. It is necessary to monitor the development of antibiotic resistance constantly.

The recommended treatment regime is: ceftriaxone 500 mg by single intramuscular or intravenous injection. If there is a contraindication to beta-lactam antibiotics, spectinomycin can be substituted at a dose of 2 g intramuscularly. If the patient refuses parenteral administration or if this is not possible, then it is recommended that cefixime be given as a single oral dose of 400 mg.

PROPHYLAXIS

This comprises the treatment of sexual partners of infected patients and the use of condoms to avoid sexually transmitted infection. Prevention of ocular gonorrhea in the new-born baby, obligatory in France, is possible by instillation at birth of a silver nitrate based antiseptic eye lotion containing erythromycin or tetracycline.

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GROWTH HORMONE

DEFINITION

A polypeptide hormone synthesised in the anterior pituitary somatotropic cells. The predominant circulating form (approximately 90%) contains 191 amino acids and has a molecular mass of 22 kDa; variants in this structure are found, particularly another monomeric 20 kDa form and different dimers.

Synonyms: GH = *growth hormone*; somatotropic hormone and growth factor.

INTRODUCTION

Anterior pituitary GH secretion depends on two hypothalamic peptides which have antagonistic effects:

– Somatostatin, which has an inhibitory action.

– Growth hormone releasing hormone (GH RH) which has a stimulatory action.

Added to this is negative feedback regulation by IGF-I *(Insulin-like growth factor I)*; ghrelin has a stimulatory effect.

■ IMPORTANT

GH is secreted in a pulsatile manner into plasma with many peaks (6 to 10) per 24 hours; concentrations are low or even undetectable between peaks. GH also has a very short half-life (10 to 15 minutes) and as a result, ***an isolated one off GH measurement***– particularly when the patient is being investigated for deficiency– ***offers little information***. Half of the monomeric GH circulates bound to a specific carrier protein, GH-BP *(GH-binding protein)*; the action of GH is controlled by a binding stage to receptors on target tissue membranes (mostly liver).

GH acts through effectors, the major one of which is IGF1, produced in the liver and chondrocytes, in growth cartilage. Apart from its action on growth, GH stimulates gluconeogenesis and hepatic glycogenolysis, which explains the hyperglycaemia seen in acromegaly and hypoglycaemia found in GH deficiency.

INDICATIONS FOR MEASUREMENT

Because of its pulsatile secretion, GH measurements are performed in ***stimulation or suppression tests*** (pharmacological or physiological).

■ INVESTIGATION FOR GH DEFICIENCY (GHD)

The diagnosis of ***GH deficiency in children*** is important as it leads on to complex expensive treatment (recombinant GH). Children of small stature can suffer from diseases for which GH administration is ineffective. Therefore the paediatrician must exclude the endocrine and non-endocrine causes.

■ DIAGNOSIS OF GH INSENSITIVITY

This is LARON dwarfism, genetic GH resistance due to a mutation in the receptor gene.

■ DIAGNOSIS OF ACROMEGALY

Acromegaly is associated with increased GH secretion usually due to a pituitary adenoma.

NB: Increased secretion before puberty leads to gigantism.

INFORMATION

■ SAMPLE

The blood should be collected into a dry tube (serum).

Samples (baseline measurements and dynamic tests) are taken in the morning, resting, outside of periods of infection, with the patient preferably fasting since the previous evening.

NB: In dynamic tests, a venous canula is inserted 60 minutes before administering the agent used to stimulate or suppress GH secretion (a precaution intended to remove interferences between spontaneous and stress-induced GH peaks).

Comments

– It is important to be aware of blood glucose and IGF1 concentrations when interpreting the results

– Sex steroid premedication (testosterone in boys and ethinylestradiol in girls) in the prepubertal phase can correct for possible functional GH deficiency

– GH secretion is stimulated (apart from GH RH) by:
- Prolonged fasting
- Amino acids (arginine, ornithine)
- L. dopa
- Clonidine
- Glucagon
- Insulin (which causes hypoglycaemia)
- Propanolol or betaxolol
- Arginine-insulin couple
- Glucagon-propanolol couple

– GH secretion is suppressed by:
- Hyperglycaemia
- Obesity

– The 24 hour GH secretion cycle is difficult to study in practice and is performed above all when stimulation tests are equivocal or consistent.

■ ESSENTIAL INFORMATION

Suspected disease and clinical details?

Other investigations intended (MRI, etc.)?

Are you taking pegvisomant? This drug produces a large increase in serum GH concentrations (it causes GH resistance) and a fall in IGF1. Note the high dose hook effect on GH measurements in patients taking pegvisomant (it is recommended that measurement is firstly performed on undiluted serum and then on serum diluted 1/50).

■ SAMPLE STORAGE AND TRANSPORT

After centrifugation and separating into aliquots, samples should be stored at – 20°C until the time of assay (this should be performed within 4 hours after sampling). Samples are stable for several freeze/thaw cycles.

ASSAY METHODS

These are monoclonal antibody "sandwich" methods. A system which only recognises the 22 kDa form is recommended.

NB: GH is occasionally measured in urine after a pre-concentration stage (this reflects **mean** concentration although has the disadvantage that it needs to be standardised against glomerular function).

REFERENCE VALUES AND UNITS

UNITS: mIU/l

NB: Secondary calibrants used in reagent kits vary between different manufacturers. Since 2004, all GH measurements should be calibrated against the IS 98/574 recombinant GH standard.

NORMAL EXPECTED VALUES

Adults and children < 6.7 ng/ml i.e. < 20 mUI/l

In response to OGTT: Fall in 60 minutes.

TRH test: No rise (except in diabetes, depression or renal insufficiency).

PHYSIOLOGICAL VARIATIONS

GH secretion is very high during the first year of life and then falls gradually, rising again at puberty.

In adults, secretion falls with age, falling in some elderly subjects to equivalent levels to those seen in people with GH deficiency (GHD).

PATHOLOGICAL VALUES

CHILDREN

Baseline concentration < 6.7 ng/ml.

The diagnosis of GH deficiency (GHD) should be based on 2 stimulation tests, performed on different days (e.g. ornithine or clonidine or L-dopa followed by the glucagon/propanolol couple etc.).

2 tests > 6.7 ng/ml: GHD absent

1 test > 6.7 ng/ml and 1 test < 6.7 ng/ml: inconsistent response

2 tests < 6.7 ng/ml: GHD likely.

IN ADULTS WITH ACROMEGALY

Baseline concentration > 6.7 ng/ml (90% of patients).

OGTT suppression (100 g): Negative.

TRH test: Paradoxical stimulation (50% of patients).

NB1: IGFI concentrations are raised in acromegaly.

NB2: The GH RH stimulation test is performed when investigating for hypothalamic or pituitary causes for GH deficiency.

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HAEMATOLOGICAL CYTOGENETICS

DEFINITION

The purpose of haematological cytogenetics is to detect acquired chromosomal abnormalities using microscopic techniques (molecular banding and cytogenetic methods) to contribute to the diagnostic and/or prognostic management of patients suffering from malignant blood disorders. These abnormalities may be in number (more or less than 46 chromosomes), structure (change in the succession of several loci) or repair (chromosomal breakages).

Molecular cytogenetics is a field of cytogenetics developing from techniques based on DNA sequence homologies used to specifically identify all or part of one or more chromosomes. The FISH technique is a fluorescence in-situ hybridization technique.

MAIN INDICATIONS FOR HAEMATOLOGICAL KARYOTYPING AND HAEMATOLOGICAL FISH

Recurrent cytogenetics abnormalities are now part of the World Health Organisation (WHO) classification for the diagnostic and prognostic stratification of malignant blood disorders. The main cytogenetic abnormalities found in the malignant blood disorders according to the WHO 2008 classification are shown below. These abnormalities may be demonstrated by conventional cytogenetics and/or molecular cytogenetics (FISH) and/or molecular biology.

Myeloid neoplasia – chronic myeloid leukaemia

– t(9;22)(q34;q11.2); BCR-ABL1.

Myeloid/lymphoid neoplasia with eosinophilia

– Rearrangement of the PDGFRα (4q12) gene (cryptic fusion of FIP1L1-PDGFRα and another PDGFRα partner)
– Rearrangement of the PDGFRβ (5q33) gene
– Rearrangement of the FGFR1 (8p11) gene.

Myeloid neoplasia/myelodysplastic syndromes

A single cytogenetic entity has been defined in the WHO 2008 classification: myelodysplastic syndrome with isolated del(5q) deletion.

It is important to be aware that the IPSS (International Prognostic Scoring System) cytogenetics score classifies myelodysplastic syndromes into 3 cytogenetic groups:

– Good prognosis: normal kariotype, isolated loss of Y chromosome, isolated 5q deletion, isolated 20q deletion
– Poor prognosis: complex kariotype (≥3 abnormalities) chromosome 7 abnormality
– Intermediary prognosis: other chromosome abnormalities.

Acute myeloid leukaemias (AML)

– AML with t(8;21)(q22;q22); ETO-AML1
– AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
– AML with t(15;17)(q22;q12); PML-RARA
– AML with t(9;11)(p11,q23);MLLT3-MLL
– AML with t(6;9)(p23;q34);DEK-NUP214
– AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2);RPN1-EVI1
– AML7 with t(1;22)(p13;q13); RBM15-MKL1.

Acute leukaemias, ambiguous cell line

– AL with t(9;22)(q34;q11.2); BCR-ABL1 positive
– AL with MLL (11q23) gene rearrangement.

Acute lymphoblastic leukaemias (ALL)

– ALL-B with t(9;22)(q34;q11.2); BCR-ABL1
– ALL-B with t(v;11q23) with MLL rearrangement
– ALL-B with t(12;21)(p13;q22); TEL-AML1
– ALL-B with hyperdiploidia
– ALL-B with hypodiploidia
– ALL-B with t(5;14)(q31;q32); IL3-IgH
– ALL-B with t(1;19)(q23;p13.3); E2A-PBX1.

Chronic lymphoid leukaemias

– del(13q) deletion, trisomy 12, ATM deletion, p53 deletion.

Multiple myeloma

– del(13q), t(4;14), t(14;16), t(14;20) deletion, p53 deletion, hypodiploidy, t(11;14), hyperdiploidy.

Follicular lymphomas

– t(14;18)(q32;q21).

Mantle cell lymphoma

– t(11;14)(q13;q32).

Burkitt's lymphoma

– t(v;8q24) with C-MYC rearrangement.

INFORMATION

■ SAMPLE

Samples must be obtained sterile (bone marrow, blood and effusion fluids). Blood and bone marrow samples must be taken into a heparinised tube (or heparinised syringe for bone marrow samples).

For solid samples such as lymph nodes or spleen, the sample must be placed in a culture medium as soon as possible (< 30 min).

A clinical information form must accompany the sample, stating the presumed diagnosis(es) or known diagnosis(es) in follow- up, the stage of the disease, current treatment and other haematological laboratory findings.

■ SAMPLE STORAGE AND TRANSPORT

The sample must be transported to the laboratory which is performing the kariotype analysis as soon as possible at room temperature.

ANALYTICAL METHODS

KARYOTYPE ANALYTICAL METHOD

The sample is cultured sterile. Culture conditions are chosen to take account of the clinical and laboratory information provided. Cell counts/ml of sample must be performed in order to optimise the cell culture. The amount of sample used can be assessed by performing a cell viability test, particularly after long transport. It should be cultured following the recommended cell concentrations (which depend on the disease). The culture is carried out in incubators at 37°C with or without 5% CO^2. Cultures can be synchronised to improve the quality or even number of metaphases and analysed. Cells are usually blocked in the division stage (metaphase) by adding colchicine at the end of the culture. They are then placed in a hypotonic solution and then in a methanol and acetic acid fixing solution. After drying, the slides containing the test cell preparation, chromosome labelling methods are used (RHG and/or GTG labelling) to allow the chromosomes to be identified and analysed. The microscopic analysis stage can be automated (looking for metaphases). At this stage of the investigation it is essential to know the patient's clinical and laboratory findings. For a diagnosis of malignant blood disorder it is recommended that at least 20 mitoses be examined (this is necessary if no abnormality is detected) and that a minimum of 4 to 5 or even more kariotypes are classified depending on the disease and abnormalities found. An abnormality is deemed to be clonal if two mitoses carry the same abnormality, if a chromosomal structural loss or gain is found, or if 3 mitoses are seen in the case of chromosomal loss. The chromosomal formula is expressed according to an international nomenclature (ISCN 2009: *International System for Human Nomenclature 2009*).

FISH ANALYTICAL METHOD

Conventional chromosomal analysis (kariotype) can be supplemented by molecular cytogenetic analysis. The most widely used is fluorescence *in-situ* hybridization (FISH). The CGH method *(Comparative Genomic Hybridization)* is not currently used routinely for the laboratory diagnosis of malignant blood disorders. Molecular cytogenetic methods are used depending on the clinical information and/or cytogenetic findings. FISH can be performed on metaphases or on interphase nuclei.

The fluorescent *in-situ* hybridization (FISH) method with cold probes is based on the fact that DNA is a double helix molecule formed from two complementary strands. The two strands can be separated by heat and/or acid or alkaline treatment (DENATURATION STAGE). A nucleic acid probe containing a complementary sequence to the single strand of DNA can then form a specific hybrid with a single strand DNA (HYBRIDIZATION STAGE). This probe is labelled either directly or indirectly with one or more fluorochromes and the hybridization of the complementary nucleic sequences is visualised by its fluorescence after counterstaining the support (chromosomes or nuclei).

Interpretation of metaphase FISH results: In order to detect translocations or deletions using specific probes, the results must be interpreted taking account of the effectiveness of hybridization.

Interpretation for interphase FISH: It is essential that controls are prepared under the same operating conditions and that a sufficient number of nuclei (minimum 200) are counted.

The FISH formula is expressed according to an international nomenclature (ISCN 2009: International System for Human Nomenclature 2009).

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HAEMOCHROMATOSIS (HFE-1 GENE-RELATED)

DEFINITION

Hereditary haemochromatosis related to the HFE-1 gene was described initially by the term "bronzed diabetes" and is an autosomal recessively transmitted inherited disease. It is characterised by excessive iron absorption, which results progressively in generalised body iron overload. In 1975 the disease was shown to be transmitted via an autosomal recessive HLA system-related mechanism suggesting that the candidate gene is located close to the class I HLA locus (chromosome 6, at 6p21.3). The candidate gene, called HFE-1 (and initially called HLA-H) was cloned in 1996 and studies have shown that more than 90% of people with haemochromatosis are homozygous for the C282Y mutation.

Synonyms: Hereditary haemochromatosis; bronzed cirrhosis; familial haemochromatosis; idiopathic haemochromatosis; HFE or HFE-1.

THE GENE

The gene consists of 7 exons in the telemere position on the short arm of chromosome 6 (6p21.3). It codes for a protein, which is structurally very similar to HLA class I heavy chains. The three-dimensional structure of which was elucidated from cDNA and then confirmed by crystallography, and is that of the class I HLA heavy chain, formed at its end terminal by three extracellular (α1, α2, α3) domains, a transmembrane domain and a short intracytoplasmic C-terminal fragment. The α2 and α3 domains form loops stabilised by disulphide bridges *(Figure 1)*.

Similarly to the HLA class I heavy chains, the HFE molecule is expressed on the cell surface in association with β2-microglobulin; this association involves non-covalent bonds with the α3 domain of the HFE molecule. At present, more than 20 mutations and/or polymorphisms have been identified in the HFE-1 gene. Two point mutations located in exons 2 and 4 were described first:

– ***A major mutation*** involving transition of a guanine by an adenine (845G > A), responsible for a substitution in codon 282 of a cysteine molecule by tyrosine (mutation ***C282Y***). This mutation is found in the homozygous state in more than 90% of people with haemochromatosis.

– ***A minor mutation involving*** transversion of a cytosine into guanine (187C > G) responsible for substitution of a histidine by an aspartic acid in codon 63 (mutation ***H63D***). The clinical significance of this mutation is still debated. It may be a mutation predisposing to iron overload (mild haemochromatosis, the effect of which may be influenced by its co-existence with the C282Y mutation (composite heterozygotes) or other as yet unidentified genetic factors.

The C282Y mutation in the HFE protein blocks the formation of an intra-chain disulphide bridge in domain α3, compromising correct folding of domain α3 and it's binding to β2-microglobulin. This mutation causes an abnormality in presentation of the HFE protein to the cell membrane (retention in the Golgi apparatus and endoplasmic reticulum) and the HFE protein contained in the endoplasmic reticulum is degraded leading to the cells failing to express HFE on their surface.

PHYSIOLOGICAL ROLE

The HFE-1 gene codes for a protein (HFE protein), which has close structural homology with the HLA class I molecules. HFE protein is mostly expressed by duodenal crypt enterocytes (basolateral pole) and to a lesser extent by macrophages, Küpffer cells and endothelial cells. The purpose of HFE protein is to communicate information to crypt enterocytes in the duodenal villi about the body's iron status by binding to the transferrin 1 receptor (TfR1). Depending on the signals received about iron status, the crypt enterocytes differentiate into duodenal apical villous enterocytes, which facilitate or reduce dietary iron absorption. Failure to express HFE protein in C282Y homozygotes leads the crypt enterocytes to behave like iron deficient cells. Signals that remain to be identified induce the expression of proteins, which promote the absorption of dietary iron from the duodenal lumen by apical villous enterocytes, regardless of the body's requirements or iron status.

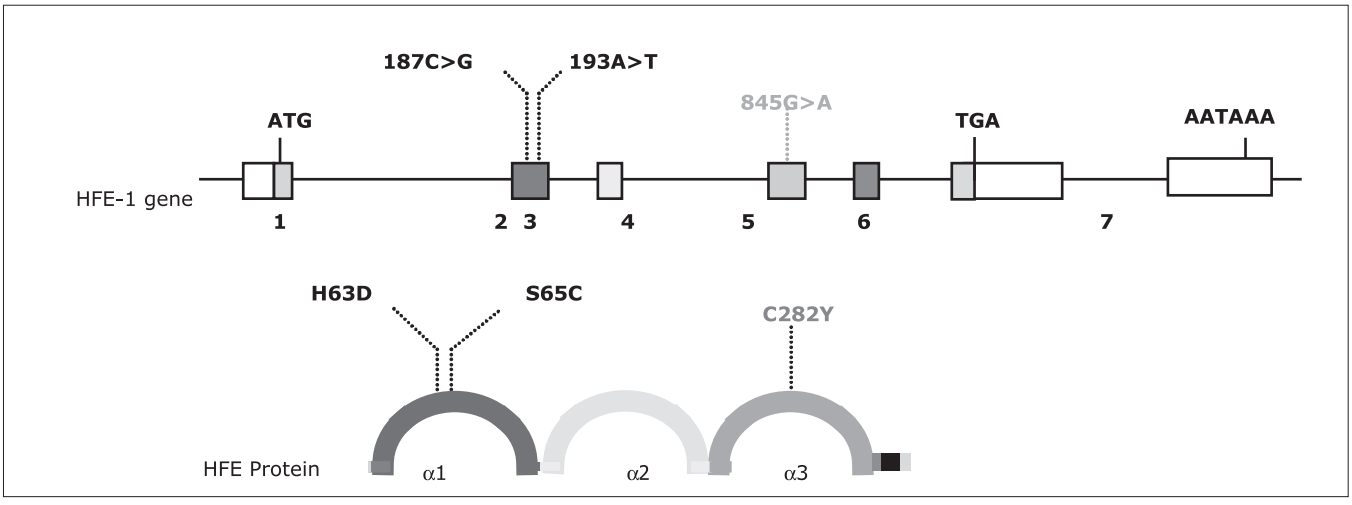

Figure 1: Diagrammatic representation of the HFE-1 gene and location of mutations C282Y and H63D *(Chr6p21.3).*

EPIDEMIOLOGY

Of the hereditary iron overload diseases; HFE-1 gene-related haemochromatosis is the most common inherited condition in Northern Europe populations. Depending on the region, 1 out of 300 to 500 people is involved and 1 out of 8 is heterozygous for the C282Y mutation.

There is disparity in C282Y homozygotes in the European population, C282Y homozygous status following a North-West/South-East gradient (1/100 prevalence in Ireland, 1/200 in Britain). The C282Y mutation responsible for haemochromatosis emerged in our regions 2000 years ago. It was initially thought to be of Celtic origin although is now attributed to the "Viking" invasions.

SYMPTOMS

The clinical features of HFE-1 gene-related haemochromatosis are usually expressed in men between 30 and 40 years old and later in women because of moderate protection from menstrual losses and pregnancies. The early signs of haemochromatosis are chronic asthenia and arthralgia (wrists, knees and phalanges). As the iron overload establishes, later signs develop including melanodermia (bronzed appearance), liver disease (hepatomegaly), insulin-dependent diabetes, hypogonadism (reduced libido) and heart disease (cardiomyopathy, dysrhythmias). It is now very rare to see late signs as the diagnosis is generally now made early *(figure 2)*.

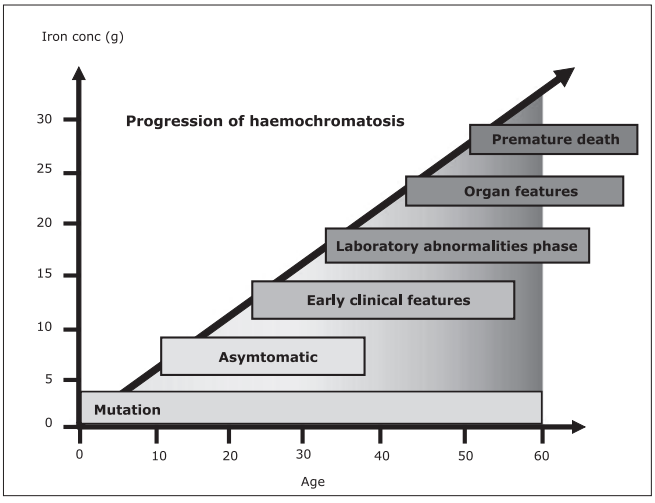

Fig. 2: Expression and clinical progression of HFE-1 gene-related haemochromatosis

DIAGNOSIS STRATEGY FOR CLINICAL SUSPICION OF IRON OVERLOAD

The initial assessment includes a clinical history and examination, with investigation of iron status, including ferritin and the transferrin saturation coefficient (CSC): if the CSC is > 45%, *and particularly* if the ferritin is raised (although this remains normal for a long time during the disease), investigation for the HFE-1 genotype is indicated.

INDICATIONS FOR INVESTIGATION OF HFE-1 GENE MUTATIONS

– Clinical and laboratory suspicion of haemochromatosis as a result of an increased transferrin saturation coefficient (> 45%)

– Family screening (relatives of a homozygous C282Y proband)

If the patient is found to be homozygous for the C282Y mutation, the diagnosis of HFE-1 haemochromatosis is made. If not, investigations for so-called minor mutations (H63D then S65C) should be triggered: interpretation of results is described below.

INFORMATION

SAMPLE

5 ml of whole EDTA blood.

LEGAL REQUIREMENTS

In France, it is essential that a certificate of genetic consultation be provided with the request from the prescribing physician (or failing this, the request from the prescribing physician and the patient's informed consent) before the sample is taken and the test is performed (decree n° **2008-321 of 4 April 2008**). Depending on the test and the result, the report may only be sent to the requesting physician (article R. 1131-19).

REQUEST FORM

The indication for the genetic test must be completed, i.e. clinical and laboratory information suggesting haemochromatosis together with a family tree if the proband has been identified.

SAMPLE STORAGE AND TRANSPORT

Room temperature if stored for < 24 h; beyond this time, store (and transport) at + 4°C.

DIAGNOSTIC METHODS

Many methods have been developed to detect the p.Cys282Tyr (C282Y) mutation and the minor mutations (p.His63Asp or H63D and p.Ser65Cys or S65C). These methods are based on PCR *(Polymerase chain reaction)* amplification of the region of the HFE gene containing the molecular abnormality. Different procedures are used to identify the mutation from the PCR amplification product. Laboratories can either use an "in-house" PCR method or commercially available kits. These include:

– Real time PCR: automated PCR allowing simultaneous analysis of DNA from several samples with fluorescence detection (hydrolysis probes, tandem probes, molecular beacons, scorpion probes, fluorescent primers).

– PCR-RFLP *(PCR-Restriction fragment length polymorphism)*: the amplification products are digested by a suitable restriction enzyme and the digestion fragments are revealed under UV light after a gel migration stage.

– PCR-SSO/ASO *(PCR-sequence specific oligonucleotide/ -allele specific oligonucleotide)*: PCR, with development of the amplified products by hybridisation of specifically labelled probes for the wild type and mutated allele.

– Allele specific PCR *(PCR-SSP: PCR-sequence specific primer)*, PCR-ARMS *(PCR*-amplification *refractory mutation system)*: PCR amplification of the wild type and mutated allele using specific or modified primers and developing the amplified products on agarose gel or capillary electrophoresis.

– PCR-DHPLC *(PCR-denaturing high-performance liquid chromatography)*: the amplified products are identified by passing through a chromatography column under denaturing conditions.

– Sequencing with the PCR amplification products.

INTERPRETATION OF TESTS FOR HFE-1 GENE MUTATIONS

- **In a symptomatic person:** testing for the C282Y mutation is of diagnostic use. Four situations can be distinguished:

The person is homozygous for the C282Y mutation (YY genotype): the diagnosis of HFE-1 gene-related haemochromatosis is made if the person is homozygous for the C282Y mutation. These patients must be treated and monitored (measurement of serum ferritin and the transferrin saturation coefficient). Family screening (parents, brothers, sisters and children) should be organised to identify any C282Y homozygotes.

The person is heterozygous for the C282Y and H63D mutations (CY/HD genotype): composite heterozygotes (C282Y/H63D) have a genotype which predisposes to moderate iron overload which is always less severe than is seen in C282Y homozygotes. Other factors responsible for iron overload cannot be excluded and must be sought (alcoholism, cutaneous porphyrias, dysmetabolic hepatosiderosis, excessive oral or parenteral iron intake). Screening of children can be offered if the partner has laboratory signs of iron overload and is also heterozygous for the C282Y gene.

Other genotypes (C282Y heterozygotes, H63D homo or heterozygotes, C282Y /S65C composite heterozygotes): no causal relationship has been demonstrated between these genotypes and iron overload. These genotypes predispose to iron overload in the presence of an additional secondary cause or genetic factor.

Testing for the C282Y mutation is negative: the diagnosis of HFE-1 haemochromatosis can be excluded. If the clinical presentation is one of iron overload, patients should be investigated for other non HFE-1 genetic factors for haemochromatosis *(see differential diagnosis)*.

- **In asymptomatic people:** C282Y mutation testing is of prognostic use, helping to predict the risk of developing clinical symptoms of the disease: C282Y homozygotes have an extremely high risk of developing the disease.

  C282Y/C282Y genotype penetrance however is incomplete and some C282Y homozygotes will never develop the disease. Considerable caution is therefore required in order to avoid alerting or in correctly reassuring people who have been tested. If an asymptomatic person is found to be C282Y homozygous, annual laboratory monitoring is indicated. If the TSE and/or serum ferritin become abnormal specific management should be instituted.

- **Family screening: genetic family screening is indicated if the proband is** C282Y homozygous *(figure 3)*.

  In this case, the likelihood in principle of siblings being homozygous is 1/4. Screening is recommended in first generation relatives of the proband (parents, brothers and sisters and children of majority age). Genetic screening is not justified from the outset in relatives of the proband, which should be preceded by phenotype screening (TSE and ferritin). Genetic screening is then recommended if phenotype screening is abnormal. Both phenotype and genetic screening are indicated in brothers, sisters and children of majority age. Genetic screening is strongly recommended particularly in females (sisters of the proband) who may be C282Y homozygous but do not have laboratory abnormalities because of the modest protection from menstruation and pregnancies.

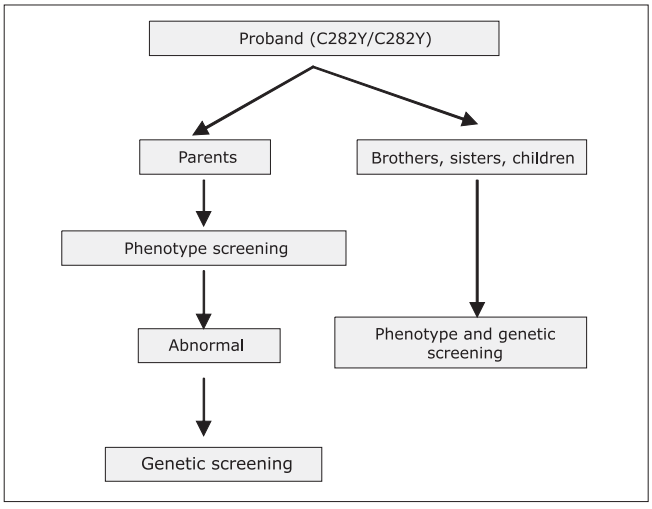

Fig.3: Family genetic screening strategy for haemochromatosis.

DIFFERENTIAL DIAGNOSIS

Other genetic abnormalities can cause the haemochromatosis phenotype in European populations. Possibilities of type 2, 3 or 4 haemochromatosis must be considered in someone with clinical features of haemochromatosis and no other cause of iron overload in whom molecular investigations for the HFE-1 gene are negative *(table 1)*.

Disease	Locus	Subject	Protein	Transmission
Haemochromatosis HFE-1	6p21.3	Adult	HFE	Autosomal Recessive
Juvenile Haemochromatosis HFE-2a	1q21	Young person	Haemojuvelin	Autosomale recessive
Juvenile Haemochromatosis HFE-2b	19q13	Young person	Hepcidin	Autosomal recessive
Haemochromatosis HFE-3	7q22	Adult	Transferrin receptor -2	Autosomale recessive
Haemochromatosis HFE-4	2q32	Adult	Ferroportin	Autosomal dominant
Haemochromatosis HFE-5	11q13	/	Ferritin (H chains)	Autosomal dominant

Table 1. Classification of the different forms of hereditary haemochromatosis.

TREATMENT

The treatment of haemochromatosis is depletion of the iron overload and is started once the ferritin is > 300 µg/l in men and > 200 µg/l in women. This is based on dietary recommendations (limiting alcohol consumption, avoiding any source of iron supplementation) and venesection: an initial phase (up to 7 ml/kg not exceeding 550 ml, 1/week; continued until the ferritin is < 50 µg/l) and maintenance phase by venesection every 2, 3 or 4 months (depending on the patient) to keep the ferritin < 50 µg/l. Must continue venepuncture if the haemoglobin falls < 11 g/dl.

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SPECIALISED MEDICAL PATHOLOGY

HAEMOGLOBIN ELECTROPHORESIS

SEARCHING FOR HAEMOGLOBIN ABNORMALITIES

DEFINITION

Haemoglobin (Hb) is a heterotetramer composed of two types of protein subunits with similar structures. Each of these subunits is linked to a haeme molecule comprised of a protoporphyrin with, at its centre, an iron atom which is capable of binding to an oxygen molecule.

Haemoglobin abnormalities are genetically-determined pathologies, some of which constitute a public health problem in vast areas of the world. These abnormalities are divided into two major groups:

– Structural abnormalities in the globin protein chains, such as haemoglobin variants S, C and E, which are among the most frequent. However, more than 900 haemoglobin variants have so far been listed.

– Globin chain synthesis abnormalities, responsible for thalassaemias and hereditary persistence of foetal haemoglobin (HPFH).

INTRODUCTION

■ ABNORMAL HAEMOGLOBINS

The commonest and most serious haemoglobinopathy is drepanocytosis (sickle-cell anaemia), which in most cases is due to a single point mutation of the homozygotic state of the betaglobin chain and is responsible for Hb S [beta 6 (A3) GAG>GTG Glu>Val]. Sickle-cell anaemia affects the black populations of Africa, the West Indies and the United States of America, and is also the leading genetic disease in France. The Hb S structural modification encourages the polymerisation of its deoxygenated form, the fibrous polymers causing the red cell to deform into the shape of a sickle. The deformation is triggered by deoxygenation, dehydration, fever and acidosis. It is responsible for normochromic and normocytic anaemia which is often well tolerated and painful vaso-occlusive episodes. Drepanocytosis patients are also particularly susceptible to infections.

The combination of an Hb S in the heterozygotic state with another haemoglobinopathy can also be responsible for a major drepanocytic syndrome. This may be Hb D-Punjab [β121(GH4) Glu > Gln] or Hb O-Arab [β121(GH4) Glu > Lys], which stabilise the Hb S polymer, or Hb C [β6(A3) Glu > Lys], which encourages dehydration of the erythrocyte and increases its concentration of Hb S.

S, C, D-Punjab and O-arab haemoglobins in the heterozygotic state are not responsible for any biological or clinical abnormality. Hb E in the heterozygotic state, which is very common in Asiatic populations, is responsible for microcytic anaemia.

■ THALASSAEMIAS

Thalassaemias are caused by a partial or total deficit in the synthesis of one or more globin chains. These are alpha- and beta-thalassaemias, as well as delta-, gamma- and delta-beta-thalassaemias.

HAEMOGLOBIN ELECTROPHORESIS SEARCHING FOR HAEMOGLOBIN ABNORMALITIES

Alpha-thalassaemias are widespread in the populations around the periphery of the Mediterranean in Africa, the Middle East and South-East Asia. They are the result of deletions (in most cases) or single-point mutations (more rarely) of one or more of the four alpha-globin genes. Three clinical states of increasing seriousness may be observed: these are alpha-plus thalassaemia and the alpha thalassaemic trait (1 or 2 alpha genes affected, resulting in hypochromia or microcytosis), haemoglobinosis H (three inactive or absent alleles), characterised by an Hb H labile fraction and chronic haemolytic anaemia, and Bart's haemoglobinosis (four inactive or absent alleles), characterised by *in-utero* mortality between the 5th month of pregnancy and birth in a scenario of *hydrops fetalis* with significant maternal morbidity.

Beta-thalassaemias are found in the populations of the Mediterranean periphery and the Middle East and constitute the commonest type in France. Three types of beta-thalassaemia have been described: minor beta-thalassaemia or the thalassaemic trait (clinically asymptomatic but associated with microcytosis, hypochromia and pseudopolyglobulism), major beta-thalassaemia (a homozygotic form characterised by hypochromic microcytic anaemia linked to diserythropoesis and haemolysis, requiring transfusions of globular concentrates and iron chelating treatment, resulting in hepatosplenomegaly and retarded height and weight development), intermediate beta-thalassaemia (encompassing composite homozygotic and heterozygotic forms), causing variable degrees of anaemia and requiring no or merely occasional transfusions, hypersplenism, cholelithisasis, extra-medullary centres of haematopoeisis, thrombotic complications and progressive iron overload.

INDICATIONS FOR MEASUREMENT

Systematic screening for haemoglobinopathy:

- A non-Caucasian pregnant woman.
- A non-Caucasian new-born baby.
- Partner of a pregnant woman who carries a haemoglobinopathy.

Screening for haemoglobinopathy in the presence of evocative clinical and/or biological signs: anaemia, microcytosis, polyglobulism, erythroblastosis, morphological abnormalities of red cells, urolithiasis, etc.

In the pre and post transfusion monitoring of patients carrying a haemoglobinopathy (major drepanocytic syndrome).

INFORMATION

Various haemoglobin exploration techniques are applied to whole blood EDTA (5 ml of blood collected in a tube with a purple stopper).

The whole blood must be stored at +4° C and delivered to the laboratory as soon as possible (< 48 – 72 hours).

■ QUESTIONS FOR THE PATIENT

– Geographical origin?

– Family history?

– Currently pregnant? Specify the term.

– Recent transfusions (less than 3 months)?

– Recent or current iron treatment (duration of treatment)?

ASSAY METHODS

The following techniques are available for haemoglobin investigation:

– Electrophoresis on polyacrylamide gel.

– Electrophoresis on agar citrate.

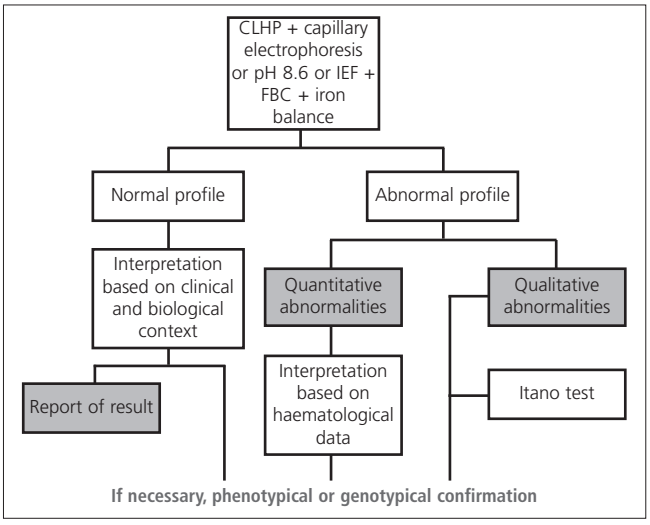
– High performance liquid chromatography (HPLC) for precise quantification of minor haemoglobin fractions (Hb A2 and foetal Hb or Hb F).

– Capillary electrophoresis.

– Isoelectric focussing (IEF).

Electrophoretic techniques do not provide a precise quantification of the various Hb A2 and Hb F haemoglobin fractions. Furthermore, an isolated electrophoretic technique used for screening may fail to recognise a haemoglobin abnormality. A working group from the French Society of Clinical Biology has stated that a precise identification of abnormal haemoglobins should make use of a number of methods. We should also not forget that any haemoglobin investigation must be accompanied by a full blood count and an iron level (and if appropriate a measurement of vitamin B12 and folates).

The general investigative strategy may be as follows:



N.B.: Looking for drepanocytes by Emmel's test (for deformation) is a low-sensitivity technique.

NORMAL VALUES

The normal majority haemoglobin in adults is Hb A.

The normal values for the minor fractions Hb A2 and foetal Hb, accurately measured by HPLC, vary with age and allow a diagnosis of quantitative haemoglobin abnormalities:.

Age *	Hb A2	Hb F
New-born baby	< 1 %	> 70 %
15 days	0.5 – 1 %	60 – 70 %
1 month	0.8 – 2.4 %	20 – 40 %
6 months	2.4 – 3.0 %	5 – 10 %
1 year	2.8 – 3.3 %	< 3. 0 %
Adult	< 3.3 %	< 1.0 %

* personal data from Dr A. FRANCINA

Hb F may persist physiologically, at less than about 3%, until the age of 5 years.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

The level of Hb F may rise during pregnancy, particularly towards the end, due to foetal erythrocytes passing into the maternal bloodstream.

PATHOLOGICAL VARIATIONS

QUANTITATIVE ABNORMALITIES

A normal level of Hb A2, with normal Hb F

Is compatible with:

– Alpha-thalassaemia (heterozygotic alpha-plus thalassaemia), with a normal haematological balance, resulting from an abnormality in only one of the four alpha genes, confirmed by genomic investigation.

– Beta-thalassaemic trait masked by iron deficiency, in the presence of microcytosis, hypochromia and iron deficiency.

Elevated Hb A2, with normal Hb A

– Hb A2 between 3.6 % and 7.0 %, combined with Hb F below 5.0% and pseudopolyglobular microcytic anaemia, means a heterozygotic beta-thalassaemia or beta-thalassaemic trait (in the absence of iron deficiency).

– Hb A2 higher than normal, but ≤ 3.6%, may be found in the following situations:

- Antiretroviral treatments (anti-HIV)
- Viral hepatitis B and C
- Hyperthyroidism
- Dyserythropoieses
- Vitamin B12 and folate deficiencies
- Trisomy 21

Reduction of Hb A2, with normal Hb A

– With normal Hb F and absence of microcytosis:

- Heterozygotic delta-zero thalassaemia

– With moderately elevated Hb F and microcytosis:

- Heterozygotic delta-beta thalassaemia

- With normal Hb F and microcytosis:

- Iron deficiency
- In the absence of iron deficiency: minor alpha-thalassaemia (two inactive or absent alpha genes)

Elevated Hb F, with no modification of Hb A2

– Hb F below 10 %:

- Haemolysis
- Treatments, such as hydroxyurea, erythropoietin, butyrates, etc.
- Haemopathies, such as juvenile myelomonocytic leukaemia, Fanconi anaemia, Blackfan-Diamond syndrome

– Hb F in the range 15 to 30% in most cases:

- Heterozygotic HPFH

Absence of Hb A

– Hb A2 normal or slightly elevated, Hb F > 90 %:

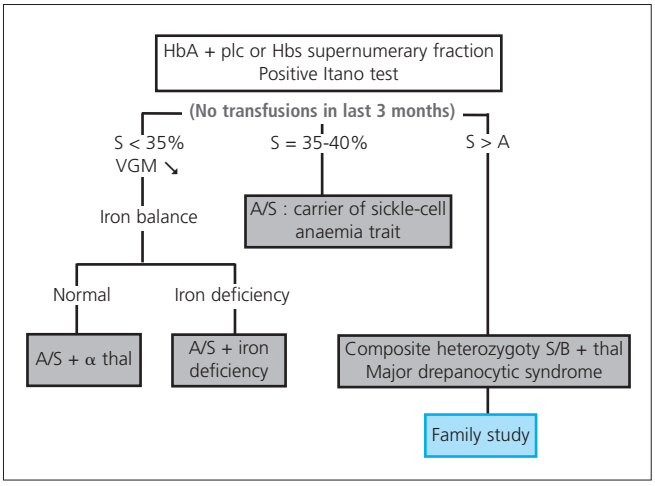
- Homozygotic beta-thalassaemia.

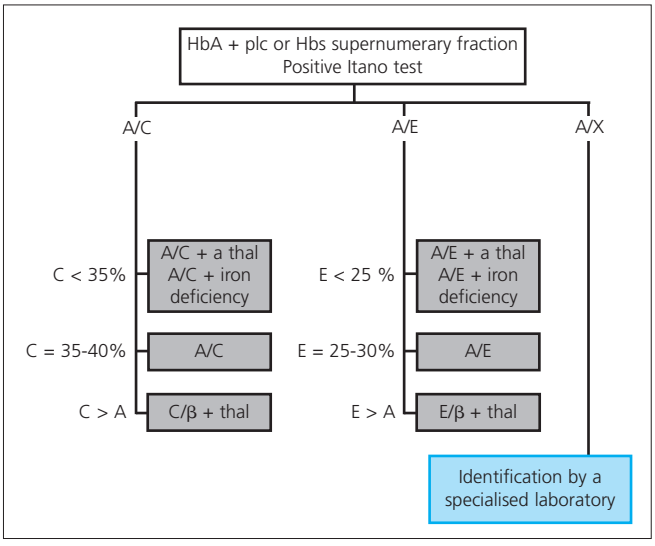
– Hb A2 nil, Hb F > 90 %:

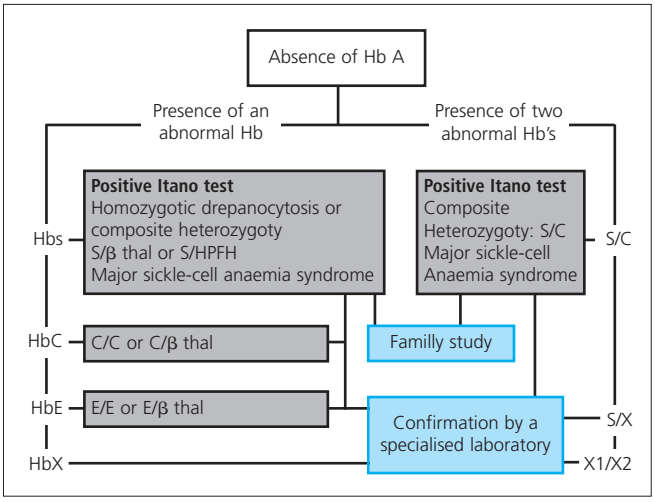
- With a scenario of thalassaemia: homozygotic delta-beta-zero thalassaemia
- In the absence of symptoms: homozygotic HPFH

QUALITATIVE ABNORMALITIES

The most frequent haemoglobin variants (Hb S, Hb C and Hb E) are revealed by using at least three techniques. A diagnosis of these haemoglobinopathies or the associated abnormalities (haemoglobin variant and thalassaemia) can be reached by following the algorithms below:







Remark

The presence of a heterozygotic haemoglobinopathy modifies the result of an HbA1c assay. On the one hand, the true HbA1c level is reduced in these patients and, on the other, the glycation of abnormal haemoglobin and the lifetime of the erythrocytes containing the abnormal haemoglobin are not known. If the HbA1c measurement can be used, the patient is then also the control and the clinician can only interpret the variation in level. It seems preferable, however, to monitor the glycaemic balance by serum assay of fructosamine. A fortiori, in diabetic patients carrying a homozygotic haemoglobinopathy (absence of Hb A), the measurement of HbA1c is impossible.

FOR FURTHER INFORMATION

■ J. Bardakdjian-Michau, J.L. Dhondt, R. Ducrocq, groupe de travail SFBC « *Recommandations dans le domaine des diagnostics des hémoglobinopathies* » (responsable: F. Galactéros), Bonnes pratiques de l'étude de l'hémoglobine, Ann Biol Clin 2003, 61: 401-409.

■ F. Kaddari-Himeur, N. Couprie, R. Ducrocq, groupe de travail CNBH, *Recommandations pour le diagnostic des anomalies de l'hémoglobine en pratique quotidienne*, panneau affiché, 37ème colloque national des biologistes des hôpitaux en 2008.

■ Szymanowicz, A. Bernay, C. Lornage, *Etude comparative du dosage de l'hémoglobine glyquée par trois méthodes: HPLC sur G7® (Tosoh Bioscience) et D10® (Biorad), et immunoturbidimétrie sur Intégra 800® (Roche)*, IBS 2009, 24: 272-280.

HAEMOPEXIN

DEFINITION

Haemopexin is a 60 kDa glycoprotein formed from a single 439 amino acid polypeptide chain. It runs in the β1 region on serum protein electrophoresis. It is a transporter for haem, for which it has extremely high affinity. Haemopexin keeps haem in a soluble form.

INTRODUCTION

Haemopexin is mostly synthesised by liver parenchymal cells. It has a half-life of 7 days in normal people, whereas the half-life of the haem-haemopexin complex is 7 to 8 hours.

Its main biological role is to keep extracellular haem in a soluble form. The main sources of extracellular haem are haemoglobin from lysis of erythrocytes, myoglobin, enzymes with a prosthetic group such as peroxidises and cytochromes released during cell injury.

Because it binds tightly to haem, haemopexin acts as an anti-oxidant, as the haem-haemopexin complex has lower peroxidise activity than free haem.

Haemopexin plays an important role in iron homeostasis. Liver parenchymal cells express haemopexin receptors which bind haem-haemopexin complexes. Following endocytosis these complexes are degraded in the cytoplasm by a haem-oxygenase. The haemopexin is then released back into the circulation and the iron obtained from the haem can be re-used.

INDICATIONS FOR MEASUREMENT

– Monitoring chronic and/or severe haemolytic syndromes when capacity of haptoglobin to bind haemoglobin has been exceeded.

– Monitoring haemolytic syndromes in people with haptoglobin phenotypes Hp2-2 or Hp2-1M. Haemoglobin binding capacity to haptoglobin is reduced in these people.

INFORMATION

SAMPLE

The sample may be taken either into a dry tube without anticoagulant or additive or into a tube containing lithium or sodium heparin.

QUESTIONS FOR THE PATIENT

Suspected disease?

Current treatment?

SAMPLE STORAGE AND TRANSPORT

Samples can be stored at +4°C for at least 72 hours. They are stable for at least 6 months at -20°C and for several years at -70°C if they are frozen within 24 hours of sampling. Transport at + 4 °C if < 72 h; beyond this time, freeze.

ASSAY METHODS

Haemopexin is assayed by immunonephelometry.

NORMAL EXPECTED VALUES

Methods are not standardised against an international standard and values may vary depending on the assay method. As an indication, in adults serum concentrations are between 0.4 and 1.5 g/l (unchanged in pregnancy). Concentrations in children are approximately 20% lower than in adults.

PATHOLOGICAL VARIATIONS

Reduced haemopexin concentrations

Haemolytic syndromes

The fall in haemopexin concentrations seen in haemolytic syndromes is proportional to the severity of haemolysis. The fall is only seen when haptoglobin binding capacity for haemoglobin is exceeded, i.e. once the plasma concentration of haem derivatives exceeds 6 mg/l. This is seen, for example, in severe haemolytic syndromes associated with homozygous thalassaemia or sickle cell anaemia. Haemopexin concentrations fall slowly and are lower than haptoglobin concentrations. When the haptoglobin concentration is very low, haemopexin may be used to monitor the progress of the haemolysis, as even in severe haemolysis; haemopexin is reduced but remains measurable.

Haemorrhagic pancreatitis

Haptoglobin concentrations are normal in this situation in which red blood cell lysis in the peritoneal cavity results in release of haemin, which binds to haemopexin, into the circulation.

Chronic hepatocellular failure

Due to a reduction in protein synthesis.

Active porphyrias

Due to porphyrin overproduction.

Severe gastro-intestinal malabsorption

Pre-eclampsia

Increased haemopexin concentration

This is extremely rare.

Inflammatory response

Haemoplexin rises moderately in the inflammatory response (rarely to more than twice the normal concentration). It is therefore more straightforward to monitor a patient suffering from both haemolysis and an inflammatory syndrome by measuring haemopexin than by measuring haptoglobin.

Diabetes

Melanoma

Breast cancer

Long term phenobarbital treatment

Neuromuscular diseases (Duchenne muscular dystrophy, dermatomyositis, polymyositis).

FOR FURTHER INFORMATION

■ Delanghe J.R., Langlois M.R., *Hemopexin : a review of biological aspects and the role in laboratory medicine*, Clin Chim Acta, 2001; 312: 13-23.

HAEMOPHILUS INFLUENZAE

DEFINITION

Haemophilus influenzae is a bacterium responsible for pathogenic community-acquired or opportunistic infections in human beings. It is a commensal bacterium of the upper respiratory tract in human beings and is occasionally found in the gastro-intestinal tract and vaginal mucosa. *Haemophilus influenzae* is a Gram negative, immobile, polymorphic, encapsulated or unencapsulated, aerobic and facultative anaerobic coccobacillus. It belongs to the *Pasteurellaceae* family and *Haemophilus* genus. There are 8 biotypes (I to VIII) within the species. The 6 capsule types (a to f) of which serotype b is the most common are distinguished by the polysaccharide nature of the capsule.

INTRODUCTION

EPIDEMIOLOGY AND PATHOPHYSIOLOGY

Pharyngeal carriage occurs in young children and falls with age. This generally involves unencapsulated biotype strains II and III. Purulent *H. influenzae* meningitis is due to the type I biotype, serotype b and usually affects children under 5 years old with a peak incidence under the age of 1 year old. Human-to-human transmission of encapsulated strains occurs through salivary droplets and also from direct contact with a patient's or carrier's secretions.

The capsule is the main virulence factor of strains which have one. This capsule protects the bacterium against phagocytosis and lysis (capsule type b is most protective against host defences). Conversely, the virulence of unencapsulated strains is multi-factorial: lipopolysaccharides (LPS), pili and adhesins, external membrane proteins, immunoglobulins A and proteases.

SYMPTOMS

Haemophilus influenzae is the most pathogenic, causing invasive features of meningitis, epiglottitis, pneumonia, pericarditis and arthritis as the type b encapsulated form. Acute non-invasive features include ENT infections: acute childhood otitis media, sinusitis, bronchitis and acute superinfection of chronic bronchitis. These are mostly caused by unencapsulated strains. Some invasive features however are caused by unencapsulated strains.

SEARCH INDICATIONS

Diagnosis of *H. influenza* purulent meningitis.

Diagnosis of acute systemic infections other than *H. influenzae* meningeal infections.

Diagnosis of acute non-invasive *H. influenzae* infections.

Differential diagnosis with other bacterial or viral meningitis.

INFORMATION

SAMPLE

Blood cultures are indicated in invasive infections.

CSF collected into 3 sterile tubes by lumbar puncture in meningitis.

Joint puncture fluid (hip, knee and shoulder) for arthritis.

Pus collected by swabbing the middle ear meatus in adult sinusitis.

Protective respiratory samples in bronchopulmonary infections.

Other possible samples are serum, urine, vaginal secretions, various sources of pus, etc

QUESTIONS FOR THE PATIENT

Age?

Symptoms?

Immune status?

Epidemiological context (return from a journey, etc.)?

Current antibiotic treatment?

SAMPLE STORAGE AND TRANSPORT

H. influenzae is a relatively resistant bacterium which tolerates a delay to sample culture of a few hours. CSF however must be transported to the laboratory without delay.

DIAGNOSTIC METHODS

DIRECT EXAMINATION

Performed fresh or after staining samples. This shows polymorphic, Gram negative, slightly filamentous coccobacillae.

CULTURE

This is performed in chocolate + polyvitex agar in a normal or CO2-enriched atmosphere. Culture also requires growth factors in culture medium, blood factors X and V. Colonies grow in 24 to 48 hours and vary in appearance depending on whether or not the strain is encapsulated. Unencapsulated colonies are smooth, mostly small and greyish coloured; encapsulated strains are larger and mucoid.

IDENTIFICATION

This is based on demonstrating the requirement for factors X and V and is supplemented by biological features (catalase +, oxidase +, indole, urease and ornithine decarboxylase (ODC) which are used to determine the biotype and by antigenic features which can be used to determine the capsule type.

MOLECULAR BIOLOGY

PCR with specific primers is used to establish the molecular capsule type.

TESTING FOR SOLUBLE CAPSULE ANTIGENS

This can be performed in CSF, serum, blood cultures, urine and pleural fluid. The method used is agglutination of latex particles, sensitised by antibodies against the type b capsule antigen.

INDIRECT DIAGNOSIS

This is performed to test for possible anti-polysaccharide antibodies to type b capsule to assess the efficacy or failure of anti-Hib vaccination.

TREATMENT

Antibiotic therapy: In France the prevalence of *H. influenzae* strain resistance to penicillins is high and the association of amoxicillin + clavulanic acid is recommended for acute non-invasive infections.

The 3rd generation cephalosporins are recommended in resistant cases in invasive infections.

Prophylaxis: This is based on anti-Hib vaccination performed in children from 3 months old and involving 3 injections at 1 month intervals with a booster at 1 year. This has considerably reduced the number of cases of meningitis in children under 5 years old.

FOR FURTHER INFORMATION

Dabernat H., *Haemophilus influenzae*, Encycl Méd Biol (Elsevier, Paris); 2004.

HAPTOGLOBIN

DEFINITION

Haptoglobin is a serum glycoprotein synthesised by the liver, which migrates in the alpha 2 region on electrophoresis. Biochemically its structure is a combination of a beta heavy chain (common) with two types of alpha chain (types 1 and 2), giving haptoglobin a genetic polymorphism. Three phenotypes are recognised: Hp 1-1 (85 kDa), Hp 1-2 (120 kDa) and Hp 2-2 (170 kDa).

The half-life of haptoglobin is 2 to 5 days depending on the phenotype.

INTRODUCTION

Haptoglobin neutralises haemoglobin produced in physiological intravascular haemolysis by forming a haptoglobin-haemoglobin complex, which is rapidly removed. These complexes have a half-life of less than 20 minutes. Metabolism by the reticuloendothelial system (liver) enables the iron to be recycled and protects the kidney against the toxic effects of free haemoglobin. After the complex is removed, haptoglobin is not recycled in the circulation. Physiological haemolysis consumes approximately 1 g of haptoglobin in this way each day.

INDICATIONS FOR MEASUREMENT

Haemolysis: Physiological haemolysis and pathologically increased haemolysis.

Inflammation: Haptoglobin is a "positive" inflammatory protein which is present in the inflammatory protein profile alongside orosomucoid and CRP (C-reactive protein).

Liver insufficiency: Haptoglobin is reduced in the same way as proteins synthesised in the liver such as albumin, prealbumin, transferrin, orosomucoid, complement fraction and coagulation factors, etc. Caution, however, is required when interpreting a result because of the possibility of co-existent haemolysis.

Hepatitis C: Haptoglobin, alpha-2-macroglobulin, bilirubin, apolipoprotein A1 and gamma GT (gamma glutamyl transferase) are used to calculate a fibrosis index (the Fibrotest®).

INFORMATION

SAMPLE

Serum or EDTA or heparinised plasma taken preferably when the patient is fasting.

Lipaemic samples should not be used.

SAMPLE STORAGE AND TRANSPORT

Plasma or serum can be stored for 1 week at + 4°C and for several months at–20°C.

Transport: + 4 °C (< 1 week).

ASSAY METHODS

Immunoturbidimetry and immunonephelometry.

Reference material: CRM 470.

REFERENCE VALUES

Haptoglobin does not cross the placenta and concentrations in new-born babies are zero because of hepatic immaturity. They then increase regularly until adulthood.

Reference values in adults: 0.30 to 2.00 g/l.

In children up to 6 months old: 0.30 to 0.80 g/l.

PATHOPHYSIOLOGICAL VARIATIONS

REDUCED

- **Intravascular haemolysis:**
  - – Immunological haemolysis (allo-immunisation)
  - – Haemolysis from infection (malaria)
  - – Haemoglobinopathy
  - – Erythrocyte membrane abnormalities
  - – Toxic or drug-induced haemolysis
  - – Mechanical haemolysis (prosthetic cardiac valve).

  Haptoglobin is a very sensitive index because of its low serum pool. 20 ml of haemolysed blood can cause a fall in the haptoglobin concentration of 1 g/l. Concentrations can fall greatly thereafter. Extravascular haemolysis does not significantly change plasma haptoglobin concentrations.

  In monitoring or diagnosing intravascular haemolysis it is important to combine a haptoglobin measurement with orosomucoid. If an inflammatory process co-exists with haemolysis, the rise produced by inflammation is inhibited by consumption of haptoglobin due to haemolysis. Concentrations may then be normal.
- **Failure of hepatic synthesis:**
  - – Acute liver disease: viral, drug-induced and toxic.
  - – Chronic liver disease: alcohol, primary biliary cirrhosis, chronic active hepatitis and autoimmune hepatitis.

  When combined with other markers in the Fibrotest® (alpha-2 macroglobulin, bilirubin, apolipoprotein A1 and Gamma GT), haptoglobin is used to calculate a fibrosis index which is proposed as an alternative to liver puncture biopsy in patients with hepatitis C. Inflammation and fibrosis in this disease activate a cytokine *(Hepatocyte Growth Factor HGF)* which reduces the synthesis of haptoglobin. The same cytokine has the reverse effect on alpha-2 macroglobulin.
- **Malnutrition**
- **Nephrotic syndrome:** protein leak
- **Congenital defects** (3% of black subjects).

INCREASED

The only cause of increased haptoglobin is inflammation (acute, sub-acute or chronic). The rise is representative of the inflammatory reaction and correlates with the rise in orosomucoid in the absence of compounding variables.

Levels expressed as % of normal values in the inflammatory protein profile follow the equation: Haptoglobin % = Orosomucoïde % X 1.3 +/- 0.2.

FOR FURTHER INFORMATION

- Kamoun P., Frejaville J.-P., Guide des examens de laboratoire. Haptoglobine sérique – 4th edition Flammarion, 2002: pp. 1375-1376.
- Thérond P., *Évaluation d'un état inflammatoire*, Cahier de formation Biochimie, tome II. Bioforma, Paris, 1994.

HCG (human chorionic gonadotrophin)

DEFINITION

Human chorionic gonadotrophin (hCG) is a glycoprotein with a molecular weight of 36 kDa. It has a dimer structure consisting of a specific β subunit and an alpha subunit which is common to FSH, LH and TSH. The beta chain of hCG has 80% homology with the LH beta chain and is responsible for its hormone activity. Each of the subunits carries a carbohydrate part which guarantees their stability in plasma and enables them to exert their hormone action. The alpha subunit is coded by a single gene, located on chromosome 6 and the beta subunit by 6 genes on chromosome 19. Expression of these genes results in different forms of combined (hCG dimer hormone) or free (free beta chain or free alpha chain) hCG, depending on pathophysiological situations. The half-life of the whole molecule is approximately 30 hours, and that of the beta chain is approximately 3 hours.

hCG acts by binding to a receptor known as CG/LH (which also binds LH), present on the surface of granulosa cells and internal theca cells in the ovary and to the surface of Leydig cells in testes.

Synonyms: hCG, chorionic gonadotrop(h)in.

INTRODUCTION

The physiological role of hCG is to support an ongoing pregnancy. It is the laboratory marker for pregnancy and is secreted by the trophoblast as early as 6 to 7 days after fertilisation, when the egg implants in the uterine mucosa. It has a luteotrophic action and stimulates the conversion of the cyclical corpus luteum to the gestatory corpus luteum, enabling oestrogens and progesterone to be synthesised at the beginning of pregnancy. After the 10th week of pregnancy the placenta takes over, particularly secreting the hormone placental lactogen.

In malignant disease, hCG is secreted by malignant placental (hydatiform moles and choriocarcinoma) and testicular germ cell tumours (arising from the ova and spermatozoa). These tumours secrete hCG in the intact form and as the free beta chain.

INDICATIONS FOR MEASUREMENT

– Diagnosis of ongoing pregnancy

– Diagnosis of early miscarriage: differentiating women who are sterile due to failure of fertilisation from fertile women in whom nidation has not occurred (spontaneous miscarriages)

– Diagnosis of extra-uterine pregnancy: an emergency measurement is performed for pelvic pain and metrorragia to distinguish extra-uterine pregnancy and salpingitis

– Screening for chromosomal abnormalities (HT21 test)

– Tumour marker: testing, diagnosing, follow up and as a prognostic indicator, mostly for malignant placental, ovarian or testicular germ cell tumours.

Trophoblast tumours (hydatiform mole and choriocarcinoma): Measurements are performed in molar pregnancy particularly in women who gain weight too quickly, with particularly heavy vomiting, anaemia, early hypertension of pregnancy or the HELPP syndrome (haemolysis, hepatic syndrome and thrombocytopaenia). This may also include women with bleeding in the first trimester or who have had an incomplete miscarriage in the first trimester. The absence of a "clear egg" foetus and the presence of a mass ("bunch of grapes") are suggestive.

Testicular tumours (seminomas and non-seminomatous tumours): Testing is used in men with cryptorchidism (even after successful reduction) or testicular atrophy (post-traumatic or mumps infection). In testicular tumours, pre-operative measurements are performed systematically with hCG, hCG beta chain and alpha-foetoprotein.

Non-trophoblastic tumours: hCG and the free beta subunit are secreted in some cases of bladder, pancreatic and breast cancer, etc.

Use of measurements of hCG and its molecular forms depending on clinical situation

(from Bidart J.M.: hCG, analytical sheet. www.corata.org).

Clinical situation	Measurement			
	Serum			Urine
	hCG	*free beta HCG*	*free alpha hCG*	*hCG*
Pregnancy				
– Normal	+			++
– Ectopic	++			(+)
Chromosomal abnormality				
– Trisomy 21	+	++		
– Trisomy 18		+	(+)	
Trophoblastic tumours: mole, choriocarcinoma	++	++		
Testicular cancers				
– Seminomas	+	++		
– Non-seminomatous (choriocarcinomas, etc.)	++	+		
Non-trophoblastic tumours: bladder etc.		+	+	
Pituitary tumour		(+)		

INFORMATION

■ SAMPLE

Serum or EDTA plasma (1 ml).

Urine: Random sample or 24 hour urine collection.

■ QUESTIONS FOR THE PATIENT

Possible pregnancy? Miscarriage?

Current treatments? hCG injections in medically assisted pregnancies? hCG can be detected up to one week after injection.

■ SAMPLE STORAGE AND TRANSPORT

Serum: Centrifuge within 6 hours of sampling for measurement of free chains.

Can be stored at room temperature for approximately 24 hours and for 1 week at +4°C. Can be stored for 1 year frozen at between –20/30°C (tumour marker).

Urine: Random sample or 24 hour urine collection. Can be stored at room temperature for 3 days and for 1 year frozen at -20° C.

ASSAY METHODS

Immunological methods, with or without radioactive labels.

Free beta chain measurement: Sandwich method using two antibodies against different epitopes of the beta chain. One antibody is immobilised on the solid phase and the other labelled with I^{125} used as a tracer. The assay range is between 0.1 and 50 ng/ml.

Measurement of total hCG: Usually on an automated analyser using an anti-beta chain hCG on the solid phase and an anti-alpha chain hCG labelled with I^{125} used as a tracer.

NORMAL EXPECTED VALUES

	hCG	hCG, free beta chain
Men, women (non pregnant)	< 2 mUI/ml	< 0,1 ng/ml
Menopausal women	< 7 mUI/ml	< 0,2 ng/ml

hCG free alpha chains (serum) ≤ 3 ng/ml.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

hCG concentrations in blood (and urine) increase progressively during pregnancy: hCG can be detected approximately 8 days after fertilisation and reaches a peak between the 6th and 10th week of the pregnancy.

For the first 10 weeks its concentration doubles approximately every 2 days. Between the 14th and 18th week of pregnancy it falls and then remains at the same level until term. It disappears rapidly after childbirth.

Serum hCG concentrations during a normal pregnancy

(from Beaudonnet A, Patricot MC. hCG. Cahier de formation hématologie-immunologie, Bioforma 1995).

NB: values shown for indicative purposes as large variations may be seen.

Term of pregnancy	Serum hCG concentrations (mIU/ml)
10th day	10
1.5 to 2 weeks	40 to 200
2 to 3 weeks	100 to 1 000
3 to 4 weeks	500 to 10 000
4 to 6 weeks	10 000 to 200 000
6 to 9 weeks	100 000 to 300 000
2nd trimester	3 000 to 50 000
3rd trimester	1 000 to 50 000

hCG changes in parallel in blood and urine. Free beta chain concentrations change in blood in parallel to hCG; alpha hCG continues to increase until the term of the pregnancy. In a normal pregnancy the ratio of the β hCG subunit to total β hCG is < 1%.

Slight rises in hCG are seen in menopausal women, hypogonadism and renal insufficiency.

INTERPRETATION

■ IN PREGNANCY

- **Early diagnosis of pregnancy:** Raised hCG can be detected in serum as early as 6 to 8 days after fertilisation (a few days later in urine). An isolated low value (5 to 25 mIU/ml) cannot confirm pregnancy. An ongoing pregnancy is established from a doubling of the hCG value in a second measurement performed approximately 48 hours later.
- **Diagnosis of early miscarriage:** Serum hCG concentrations are low for term of pregnancy in spontaneous miscarriage and one or more repeated measurement(s) every 48 hours show stable or falling values. After the miscarriage, hCG concentrations should return to below the method limit of detection. If it does not, the miscarriage is incomplete.
- **Diagnosis of extra-uterine pregnancy:** Serum hCG concentrations in ectopic pregnancies are lower than those expected for the term of the pregnancy and have a doubling time of more than 3 days. Early surgery can preserve the affected tube. Post-operatively, hCG becomes negative over 4 to 15 days (depending on the initial serum concentration).
- **Diagnosis and monitoring of trophoblastic diseases (mole and choriocarcinoma):** The change in serum hCG concentrations in molar pregnancy is anarchic with values 5 to 10 times higher than expected or even more (in a complete molar pregnancy, values may exceed > 1 million mIU/ml). Both total hCG and the beta hCG subunit are raised and the beta hCG/hCG ratio increases in proportion to the invasive nature of the tumour. Beta hCG accounts for between 0.05 and 1% of total hCG in a normal pregnancy, ranges from 1 to 5% in a molar pregnancy (benign mole) and can exceed 5% in choriocarcinoma.

  The diagnosis of a molar pregnancy is confirmed by ultrasound. After surgery for a molar pregnancy hCG becomes undetectable in blood over 3 to 12 weeks. Regular measurements of total hCG and the beta hCG subunit are used to monitor the patient until these return to normal, confirming that the trophoblast has been completely removed. Long term surveillance is used to detect development of possible choriocarcinoma. When chemotherapy has been started, treatment is also monitored by hCG measurements and any further rise in values suggests a recurrence.
- **Screening for chromosomal abnormalities:** An increased hCG for the term of pregnancy in trisomy 21 screening (HT21 test), increases the risk in the foetus (see *"Trisomy 21"* page).

■ IN MEN, TESTICULAR TUMOURS

The presence of hCG in a man is pathognomonic of tumour. Generally, testicular tumours are monitored by total hCG and beta subunit measurements combined with measurements of alpha fœtoprotein.

- **Non-seminomatous tumours (choriocarcinoma):** hCG is commonly raised and values are generally > 5000 mIU/ml. hCG and the free beta chains change in parallel.

  The kinetics of the rise varies, depending on the doubling time of the tumour, which can be a few days in rapidly growing tumours. Both serum hCG and beta chain fall as the disease regresses. Values rise again with any metastatic disease.

- **Seminomas:** hCG is less often raised (20 to 30% of cases) and to lower levels (values of generally < 2000 mIU/ml) and in a third of cases only the free beta chain is secreted. The sensitivity of detection can be increased if measurement is performed in blood taken directly from the spermatic cord veins during orchidectomy. After total tumour eradication the marker falls with a half-life of 1 to 3 days. A further rise indicates recurrence.

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HELICOBACTER PYLORI

DEFINITION - INTRODUCTION

H. pylori (Hp) are Gram-negative spiral bacilli with flagella, which make them highly mobile in the gastrointestinal mucus.

Hp infection is the most widely found bacterial infection throughout the world (50% of the population), although large geographical differences exist. It has a prevalence of > 90% in Asia, Latin America and developing countries, 50 to 70% in Eastern Europe and approximately 30% in Western countries. It has a high annual incidence in children under 5 years old (3%) and a low incidence in adults (0.3%) in all countries. All infected patients have chronic gastritis, which persists throughout life: 50% will develop atrophic gastritis, 17% will develop a peptic ulcer and 1 to 5% will develop gastric cancer. Treatment is invariably required.

The reservoir for Hp in humans and primates is the stomach; an environmental reservoir in rain-water has been suggested but is disputed.

Transmission is between human beings (within family transmission), probably orally (role of vomiting). Risk factors are close contact and low socio-economic level.

Hp has several pathogenicity factors: it secretes large amounts of a urease which neutralises gastric acidity; its flagelli allow it to move in the gastric mucus and it also has adhesins which enable it to adhere to epithelial cells and it has enzymes (superoxide dismutase and catalase) which make it resistant to phagocytosis. Its three virulence factors are the *VacA* toxin, the *Cag* pathogenicity islet and *OipA*, an external membrane protein associated with ulceration, cancer, higher bacterial density and severe inflammation.

The gene coding for the cytotoxin *VacA* is present in all strains but is not always active. The toxin induces apoptosis, causes *in-vitro* vacuolisation of cells and inhibits the activation of T lymphocytes (role in chronic progression).

The *Cag* pathogenicity islet consists of 25 to 30 genes, of which *CagA* codes for a highly immunogenic protein. Translocation of *CagA* associated with injection of Hp peptidoglycan to the infected cell activates NF *Kappa*B and induces synthesis of IL8, producing a very pronounced inflammatory response. Once *Cag* enters the cell it is phosphorylised causing cytoskeletal rearrangements, destruction of the tight junction between cells and damage.

Gastric mucosal lesions therefore occur as a result of the direct action of toxins, very severe inflammation induced particularly by the *Cag* pathogenicity islet and urease and deregulation of acid secretion.

Clinically, infected people develop acute gastritis, which develops into chronic gastritis (Hp persists throughout life in the stomach). Once colonised, if the infected person is constitutionally an acid " hypesecretor" Hp takes refuge in the gastric antrum where the pH is slightly higher and the infection develops more towards a duodenal ulcer. If the patient is an acid " hyposecretor", Hp is distributed throughout the gastric mucosa and the infection progresses more towards gastric ulceration, lymphoma or cancer. Clinical outcome therefore depends on bacterial pathogenicity factors, host genetic factors (acid secretion, IL-1β), protective (vitamin C) and worsening (salt) environmental factors and the site and duration of the infection.

Clinical features in children

Ulceration is rare but occurs (ulcers and/or erosions in 8 to 10% of children on endoscopy). Recurrent abdominal pain may suggest Hp infection, although in reality Hp infection cannot be linked to any specific gastro-intestinal disease in children.

Other non gastro-intestinal features may be suggestive:

– Iron deficiency anaemia: higher prevalence of Hp infection has been seen in children with unexplained iron deficiency anaemia and Hp eradication increases the haemoglobin concentration, whereas iron has no effect.

– Immune thrombocytopenic purpura: a raised platelet count is seen in 50% of cases after anti-Hp treatment,

– Growth retardation (contradictory studies).

Synonym: Hp.

INDICATIONS FOR MEASUREMENT

According to the 2005 Maastricht III consensus conference on Helicobacter pylori, testing for and eradicating Hp are recommended in the following situations:

– Gastric and duodenal ulcers

– First degree relatives of patients who have had gastric cancer

– Patients who have undergone a resection for gastric cancer

– Pre-malignant lesions (gastric atrophy)

– MALT lymphomas

– Patient request

– Gastro-oesophageal reflux treated by long term PPI

– Uninvestigated or investigated non-ulcerous dyspepsia

– Long term NSAID

– Ulcers bleeding on low dose aspirin

– Unexplained iron deficiency anaemia

– Idiopathic thrombocytopenic purpura.

Hp testing is recommended in children in the following situations:

– Symptoms suggestive of ulcer

– Family history of ulcer or gastric cancer

– Iron deficiency anaemia with no identified cause

– Idiopathic thrombocytopenic purpura

– Abdominal pain after excluding other causes (constipation, gluten intolerance, lactose intolerance)

– Growth retardation.

■ SAMPLE

Serology: Serum (dry tube). A fasting sample is not necessary.

Faecal Hp Ag testing: A stool sample in a sterile pot.

Labelled urea breath test: The patient should obtain the labelled urea as a ready to use kit from a pharmacy on medical prescription.

The test should be performed after stopping all antibiotic treatment for at least 4 weeks, stopping anti-secretory agents (proton pump inhibitors, anti-H2 agents) for at least 2 weeks and antacids and local gastrointestinal preparations for 24 h. The patient must be fasting from the previous night, resting without food or drink and not smoke during the test. Expired air is collected at T0 and T30 min. The detailed sampling protocol is explained in the kit.

Culture and antibiotic sensitivity profile: Biopsy, transported in physiological saline stored for 24 h at + 4°C. This can also be transported in a Portagerm® or be frozen at -80°C (as soon as the sample is taken in the endoscopy suite).

■ QUESTIONS FOR THE PATIENT

Confirm actual fasting status (food, drink and smoking) before the breath test?

Current treatment? Date when eradication treatment was stopped, in a post-eradication control?

■ SAMPLE STORAGE AND TRANSPORT

Storage of blood for serology: Store serum for up to 24 hours at + 4°C or for 1 year at – 20°C.

Labelled urea breath test: Appropriately identified tubes showing the time of the samples are stored at room temperature.

Biopsy for culture: *cf* above.

DIAGNOSTIC METHODS AND INTERPRETATION

Invasive tests use gastrointestinal endoscopy, which enables biopsies to be taken. Methods and their performance, advantages and disadvantages are summarised in table 1

Table 1: Advantages and disadvantages of invasive tests

Type	Sensitivity	Specificity	Advantages	Disadvantages
Histological examination	> 90%	> 90%	Histological diagnosis of lesions + performances	Reliability depends on the site, number and size of lesions and the histologist's experience
Culture	80 to 95%	100%	Allows antibiotic sensitivity profile to be obtained	Difficult transport conditions (< 4h at + 4°C or in special media) and culture conditions
Rapid urease test	> 80%	95%	Rapid diagnosis (< 4 h)	If negative, supplemented by histological examination plus reduced sensitivity if low density Hp
PCR	> 90%	100%	Easy transport and storage conditions, rapid result + sensitivity test to macrolides.	

Culture and antibiotic sensitivity profile

Culture requires 10 to 14 days in a microaerophilic environment on Brucella agar + 10% horse blood + antibiotics (vancomycin, co-trimoxazole and amphotericin) or on "pylori" agar (Mérieux). The antibiotic sensitivity profile is obtained in 12 days in a microaerophilic environment on Mueller Hinton agar + 5 to 10% horse or sheep blood using a very rich inoculum (Mc Farland 3).

Resistances are tested for against clarithromycin, metronidazole, amoxicillin, tetracycline and ciprofloxacin. The methods used are phenotypic (antibiotic sensitivity profile) and genotypic (PCR).

Detection of clarithromycin (Cla) resistance

The target for Cla is the ribosomal 23 S RNA and the resistance mechanism if loss of affinity for the target due to mutations in the sequence coding for 23S RNAr.

Phenotypic methods use the agar dilution method with an erythromycin disc (Ery 15 µg; GEFH recommendations: diameter > 17 mm) or a clarithromycin E-test® (good agreement between the two tests). Genotypic methods are PCR-RFLP (result in 12 to 24 h), PCR LiPA (6 h) or real time PCR (2-3 h).

Detection of metronidazole (Mtz) resistance

The genetic support for resistance is multiple: *rdxA* (NADPH nitroreductase), *frxA* (flavin oxidoreductase) and *fdxB* (NADPH ferrodoxine-*like* protein). The methods used to detect the resistance are the disc method and the E test®, although methods correlate poorly and are poorly reproducible with frequent findings of heterogeneous resistance and mixed populations. The reference method remains agar dilution (threshold MIC > 8 mg/l).

Detection of amoxicillin (Amx) and ciprofloxacin (Cip) resistance

Amx resistance is due to a change in the plp1A and resistance to quinolones to a mutation on the gyr A. Resistance is tested by discs and E Tests® (MIC threshold > 0.5 mg/l for Amx and > 1 mg/l for Cip).

Non-invasive tests

These are used when endoscopy is not required.

■ The carbon 13 labelled urea breath test

In the presence of *Hp*, labelled urea is cleaved into ammonium and bicarbonates. The bicarbonates are then converted under the action of gastric acidity to C^{13} labelled carbon dioxide which is then removed in expired air. The concentration of C^{13} labelled carbon dioxide is measured by isotope mass spectrometry coupled to gas chromatography (reference method) or by infrared spectrometry.

■ Detection of bacterial antigens (Ag) in stool samples by EIA

The first tests were based on detecting Ag with polyclonal Ab and then monoclonal Ab. The latest tests, which appear to perform better are based on immunochromatography (*Doctor-test*, monoclonal Ab).

■ Serology (serum, urine or saliva): detection of anti-H. *pylori* antibodies

There are currently major differences between the serological tests available on the market. In general, sensitivity is poor in children under 6 years old (false negatives) and serology performs less well than the other non-invasive tests.

Table 2: Advantages and disadvantages of non-invasive tests

Type	Sensitivity	Specificity	Advantages	Disadvantages
Faecal Ag testing	Variable ≈ 92%	Variable ≈ 93%	Simplicity, particularly in children and earlier test of effectiveness of treatment	Variable results in children, less specific than other tests, unpleasant for patient to collect sample, delay to performing / stopping IPP* or ATB** treatment (1 month), cost
Serology	Variable 60 to 95%	Variable 60 to 95%	No delay / stopping PPI or antibiotic treatment, simplicity	False positives (Ab fall 4 to 6 months after eradication but invariably)
Labelled urea breath test	98%	98,5%	Performance (before or after eradication), simplicity	Delay / treatment: requires PPI to be stopped for 2 weeks and ATB for 4 weeks. Cannot be used in young children.

** PPI: proton pump inhibitors*

*** ATB: antibiotics*

WHICH TESTS TO USE?

- **Diagnosis:**
  - In adults: in haemorrhage, MALT lymphoma or gastric atrophy: serology.
  - Invasive tests: endoscopy (with biopsies) performed after stopping proton pump inhibitors can be used to test for other diseases and is recommended if clarithromycin resistance is > 15% in the general population (2005 recommendations) or after 2 treatment failures.
  - Non-invasive tests: in other situations: breath test or faecal Ag test (monoclonal antibody).
- **Eradication control:**
  - Breath test: 4 to 6 weeks after stopping treatment, reliable and precise test
  - Faecal Ag testing: 1 month after stopping PPI
  - Serology: no, only for epidemiological studies.

TREATMENT

The eradication treatment for *Hp* involves triple therapy with the combination of a double dose of anti-secretory agent (omeprazole 2 x 20 mg or lansoprazole 2 x 30 mg or pantoprazole 2 x 40 mg or rabeprazole 2 x 20 mg or esomeprazole 2 x 20 mg) to increase gastric pH and two antibiotics (amoxicillin 2 x 1 g + clarithromycin 2 x 500 mg) for 7 days. Metronidazole is used in place of amoxicillin in penicillin allergy.

Factors responsible for failure are primary Cla resistance, age (< 50 years old), smoking and poor adherence (lack of patient motivation and lack of explanation). Second line treatment is a PPI, amoxicillin and metronidazole for 14 days, which provides 90% bacterial eradication. Third line treatment requires an antibiotic sensitivity profile.

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HEPARIN COFACTOR II

DEFINITION

The second heparin cofactor is a serine-protease inhibitor produced by hepatocytes, which has a plasma concentration of approximately 1 µmol/l. It inhibits thrombin (but no other coagulation or fibrinolysis protease), chymotrypsin and other enzymes belonging to the chymotrypsin group. The rate of thrombin inhibition by HCII is increased by a factor of more than 1000 by heparin, heparane sulphate or dermatane sulphate.

Synonyms: heparin cofactor II (HCII), second heparin co-factor.

INTRODUCTION

The physiological role of HCII is still poorly understood and several hypotheses have been advanced.

– HCII has been found in the intima of normal human arteries and the capacity of dermatan sulphate to stimulate HCII is reduced in atherosclerotic lesions. It may predispose to thrombosis or restenosis after endoluminal angioplasty. Japanese authors have reported that increased HCII activity may be a protective factor against atherosclerosis, particularly in the elderly.

– In pregnant women, reduced HCII activity may be associated with placental dysfunction.

– Studies on *"knock-out mice"* for the *HCII gene suggest* that HCII may inhibit thrombosis after arterial injury.

INDICATIONS FOR MEASUREMENT

An association has been found between HCII deficiency and increased risk of venous or arterial thrombosis in some studies, although has not been confirmed in others. Within current knowledge, testing for HCII deficiency is not recommended in patients with thrombo-embolic disease.

The indications for measurement are currently in research. Low plasma HCII levels appear to be a risk factor for atherosclerosis and restenosis after coronary stenting. In cardiology, HCII may be a new area of treatment to prevent these risks.

INFORMATION

SAMPLE

The sample should be taken into citrate at a concentration of 3.2% (0.109M), 1/10 (0.5 ml per 4.5 ml of blood). 3.8% (0.129 M) citrate tubes are acceptable.

A fasting sample is not required and a light low fat snack is permitted.

For further information refer to the *"General preanalytical conditions in haemostasis"* section.

QUESTIONS FOR THE PATIENT

Do you have a personal or family history of venous or arterial thrombosis?

For women: are you taking oral contraception? HCII has been described to rise by approximately 15 to 40%; are you taking hormone replacement therapy for the menopause? Some authors have reported HCII to be reduced slightly in this case.

SAMPLE STORAGE AND TRANSPORT

Can be stored for 4 hours at laboratory temperature, for 1 week at – 20°C and at – 70°C beyond this time. If the analysis is to be performed later the sample must be centrifuged, separated and frozen within 2 hours of sampling. It is recommended that samples be thawed promptly in a water bath at 37°C.

Transport: frozen at – 20°C.

ASSAY METHODS

HCII activity: chronometric assay by measurement of human thrombin inhibition in the presence of dermatan sulphate.

REFERENCE VALUES

HCII: 65 to 150%.

INTERPRETATION

PHYSIOLOGICAL VARIATIONS

Some authors have reported a rise in HCII with age of approximately 30% between the ages of 30 and 60 years old, with a fall thereafter.

HCII activity in the newborn is approximately 50% of adult values (30% in premature infants). Normal values approach those found in adults at around the age of 6 months old although remain approximately 10% less than adult values up to 16 years old.

Pregnant women: some (but not all) authors have reported a rise in HCII of approximately 40% during the third trimester of pregnancy returning to normal approximately 72 hours after childbirth.

PATHOLOGICAL VARIATIONS

Increased HCII activity:

– Nephrotic syndrome, pneumonia and haemorrhagic cerebrovascular accident: some authors report a rise of HCII of 20 to 40%.

– Venous thrombosis: transient rise of 10 to 20% in the acute phase.

Acquired HCII deficiencies are seen in the following situations:

– Severe eclampsia (HCII falls by approximately 50%)

– Disseminated intravascular coagulation (DIC)

– Acute or chronic liver disease: HCII levels are approximately 10% in fulminant hepatitis; a fall of approximately 30% in liver transplant patients

– Renal transplant patients: fall in HCII of approximately 20%

– Type 1 diabetes: some authors reported a 10 to 15% fall in HCII levels

– Chronic haemolytic anaemia (thalassaemia, SC haemoglobinopathy, pyruvate kinase deficiency)

– Various diseases: acute pancreatitis, pancreatic or gastric cancer, respiratory distress syndrome, heparin-induced thrombocytopaenia, HIV infection

– A transient fall in HCII is described in some patients following surgery.

- **Constitutional HCII deficiencies:**

The prevalence of constitutional HCII deficiencies is estimated to be approximately 1% in the general population and approximately 1.1% in people with a personal history of deep venous or arterial thrombosis. Most authors have not reported an increased risk of thrombo-embolism in people with HCII deficiency.

Constitutional HCII deficiency has been described in the literature in at least 15 families throughout the world. This is usually type 1 deficiency (parallel fall in HCII activity and antigen of approximately 50%), which is autosomal dominant in transmission. The genetic abnormalities involved are deletions and nonsense mutations (called Serpind 1 in 22q11.21). Two cases of type 2 deficiency have been reported (normal HCII antigen, HCII activity approximately 50%; genetic abnormality: nonsense mutation called HCII Oslo). One case of homozygous deficiency (nonsense mutation) has also been published (HCII activity: 10 to 15%; HCII antigen: 2 to 5%).

The proband (index case) in 7 of the 15 families had a personal history of deep vein thrombosis or pulmonary embolism although 3 of these symptomatic individuals had another thrombophilia (antithrombin or protein S deficiency or factor V Leiden). Of the 58 family members with the deficiency (excluding the proband), 10 (17%) had a personal history of thrombosis (6 venous and 4 arterial) and 1 had had a spontaneous miscarriage. The authors' conclusions from these findings were that congenital HCII deficiency was not an important risk factor for venous or arterial thrombosis but that it could contribute to increased risk if associated with another thrombophilia.

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HEPATIC DISTOMATOSIS

DEFINITION

Distomatosis is an anthropozoonosis due to the accidental presence of flatworms called flukes in human beings. The clinical classification is based on the location of the adult flukes within the body: hepatobiliary distomatosis, intestinal distomatosis and pulmonary distomatosis. ***This document will only relate to hepatic distomatosis***, which is caused by either *Fasciola hepatica (Fh)* or *Fasciola giganta (Fg)*. Infestation, the life-cycle and the clinical pictures of the 2 parasites are similar. *Fg* exists on the African continent whereas *Fh* is common in Europe, particularly in France.

Fh belongs to the Platyhelminthes branch, Trematodes class, Distoma group and *Fasciolidae* family.

– ***The adult*** is a foliated, pink-coloured hermaphrodite flatworm measuring between 2 and 3 cm long. It has an anterior cephalic cone with 2 suction pads, with one at the edge of a buccal orifice.

– ***The egg*** is ovoid, long in shape, closed and non embryonated when laid. It measures 120 to 140 microns.

Synonyms: Fasciolosis, fascioliasis, fasciolasis and *Fasciola hepatica* distomatosis.

INTRODUCTION

EPIDEMIOLOGY

Fh distomatosis is a cosmopolitan zoonosis which occurs in cattle and sheep farms as it is a disease due above all to animal faecal contamination. It also requires a relatively warm and especially moist climate. It is prevalent throughout France, with 50 to 100 cases declared annually. Infestation occurs by eating wild cress, dandelion or lamb's lettuce collected in farming areas, onto which the encysted larvae attach.

Epidemics are seasonal in nature and are seen usually in Autumn or Winter.

LIFE-CYCLE

This requires 2 hosts to be present:

An intermediary host: a freshwater mollusc (water snail).

A final host: herbivore (beef, sheep) or accidentally, human beings.

The adult flukes live in the biliary tracts of cattle and sheep. They lay eggs which are carried in bile and then excreted in faeces. The eggs embryonate in the external environment if the temperature, light, and oxygen conditions are favourable, and rupture in water releasing a swimming ciliated larva called the miracidium. The larva swims until it encounters a mollusc (water snail) which it enters. The cycle then continues inside the mollusc and the ***miracidium*** transform through several successive stages into ***cercaria*** or infectious forms.

The cercaria then leave the mollusc and swim until they attach onto an aquatic plant. They then transfer into ***metacercaria*** or infesting forms of the parasite. Animals or human beings are infested by eating the metacercaria attached to aquatic plants. The metacercaria change into small flukes in their hosts intestine, perforate the gastro-intestinal tract crossing the intestinal wall and entering the liver reaching the bile ducts where they become adult approximately 3 months after infestation.

SYMPTOMS

Incubation lasts for approximately 15 days and hepatic distomatosis develops in 2 phases:

- **The invasion phase:** or toxic infection phase occurs 2 to 4 weeks after infestation and represents migration of the small flukes into the bile ducts.

  Several clinical forms are seen from the typical acute form to rarer ectopic forms. Common symptoms include deterioration in general health (asthenia and anorexia), irregular prolonged fever, right hypochondrial pain, hepatomegaly, diarrhoea, nausea and allergic reactions (urticaria and Quincke's oedema).

- **The established disease** phase or chronic cholangitis phase develops 3 to 6 months after infestation following a transient improvement. This represents the worms becoming established in the bile ducts and its severity is directly related to the number of worms present. It presents with gastro-intestinal problems (diarrhoea, vomiting, jaundice and biliary colic) and attacks of urticaria associated with poor general health (persisting asthenia and weight loss).

  Untreated, the disease becomes chronic and may even develop to cirrhosis or can be complicated by gallstones. There are many paucisymptomatic or even asymptomatic forms of the disease.

SEARCH INDICATIONS

Diagnosis of suggestive clinical symptoms dominated by hepatobiliary problems and associated with a history of eating cress, dandelion or other wild plants.

Aetiological diagnosis of isolated hypereosinophilia.

Differential diagnosis with other helminthiases.

INFORMATION

SAMPLE

Stools for parasitology examination should be collected into a clean empty container preferably at the laboratory.

Serum for serology (Dry tube).

Others: BAL, duodenal intubation fluid, sputum, etc.

QUESTIONS FOR THE PATIENT

History of eating raw wild vegetables such as cress?

History of familial epidemics? Clinical signs and date of onset?

Current treatment?

SAMPLE STORAGE AND TRANSPORT

Serum stored at + 4°C.

Stool samples preferably collected at the laboratory; store at + 4°C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

This involves testing for eggs in stool samples but is only possible from 3 months after infestation. These tests involve:

– ***Direct fresh examination***

– ***Examination after concentration*** (Ritchie technique, etc.) as only a small number of eggs are excreted in the faeces and the test can even be negative in 35% of cases despite repeated investigations.

INDIRECT DIAGNOSIS

Serological diagnosis is essential in the invasion phase as it is the only test which can be used during this period. In addition, early treatment is more effective. It is also useful in the established disease phase when identifying eggs is difficult. The test is positive from the 3rd week after infestation. Cross-reactions occur with bilharzia and more rarely with ascaris and filaria.

The methods available are:

- **Indirect haemagglutination method (IHA):** Simple and rapid, detects IgG and IgM although rapidly becomes negative. A titre of 320 or more supports distomatosis
- **Indirect immunofluorescence (IIF):** Is performed on frozen sections of adult flukes. It is a rapid, sensitive and relatively specific method.
- **The complement fixation reaction (CFR):** Lacks sensitivity and specificity.
- **The immunoenzymatic method (ELISA):** Is very sensitive.
- **Immunoelectrophoresis:** Uses a standardised antigen. This reveals several precipitation arcs, arc 2 of which is specific for fasciolosis.
- **Electrosyneresis:** Is rapid and specific.
- **Immunoblotting (Western Blot):** One test is commercially available and offers sensitive, specific confirmation and is more objective to interpret.

NON-SPECIFIC DIAGNOSIS

- **Leukocytosis** with hypereosinophilia > 5000/mm^3. This increases according to the Lavier curve, with an early increase towards the 15th day reaching a maximum at around 10 weeks and falling very gradually to residual values which remain above normal.
- **Total and specific IgE:** Raised.
- **Liver profile:** Disturbed with hyperbilirubinaemia, increased ALP and transaminases in the established disease phase.

INTERPRETATION

The diagnosis of certainty relies on finding *Fh* eggs in stool samples or duodenal intubation fluid although is difficult and only possible in the established disease phase.

The disease is usually diagnosed in the invasion phase from the results of serological tests. The antibody titre rises during the invasion period, stabilises during the established disease phase and becomes negative after a year on effective treatment. It is very easy to interpret if the arc 2 which is specific for *Fh* on IEP or P8-9, P27-28 or P60 on Western Blot are found. If results are negative or discordant, more methods must be used and the tests repeated 2 to 3 weeks later.

TREATMENT

Drug treatment: *Triclabendazole:* 10 mg/kg in a single dose. This is a very promising drug as it is effective at all stages of the disease and also is well tolerated.

Surgical treatment: Required for biliary tract obstruction.

Prophylaxis: Livestock monitoring, delineating at risk areas, certifying cress growers and eradicating molluscs.

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- Mougeot G., *Distomatose*, Conduite des examens en parasitologie, Editor, Masson, Paris, 1995: p. 90 to 91.
- Kamoun P, Fréjaville J.-P., *Diagnostic sérologique des distomatoses*. In: Guide des examens de laboratoire, 4th Edition, Paris, Flammarion, 2002: p. 291 to 293.

HEPATITIS A

DEFINITION

The hepatitis A virus (HAV) is a *hepatovirus* in the *Picornaviridae* family. The viral particle comprises a non-enveloped icosahedral capsid 2728 nm in diameter, containing a single-strand RNA of positive polarity. The HAV genome possesses a single reading frame which codes a polyprotein, cleaved secondarily into structural and non-structural proteins. Although only one serotype has been identified, 7 different genotypes exist, some of which circulate naturally in monkeys. HAV culture is difficult, but possible, which has allowed a vaccine to be developed.

INTRODUCTION

■ EPIDEMIOLOGY

Contamination of water by the stools of infected subjects is the source of the faecal-oral cycle of HAV transmission. Human-to-human transmission also takes place. Due to viraemia in the incubation phase of the disease, HAV transmission through blood is possible, although very rare.

The most frequent cause of epidemics is the contamination of a water source, considered to be drinkable and used for irrigation. Filtering shellfish, which concentrate viral particles, may also play a part in the chain of transmission. Hepatitis A cases in France are mainly sporadic. Epidemic episodes have become very rare because of the constant improvement in hygiene conditions. Endemic hepatitis A is low in Europe, where it has now become a traveller's disease.

■ SYMPTOMS

Hepatitis A is asymptomatic in the majority of cases. The incubation period is from 15 to 45 days. In its classical clinical form, it is characterised by jaundice with a clear elevation of alanine aminotransferase activity in the serum, preceded by influenza-like symptoms. There are also anicteric and cholestatic forms, and prolonged or recurrent variations, but hepatitis A has no chronic forms. On the other hand, fulminating hepatitis may occur in rare cases, particularly if there is a further aggravating factor such as hepatotoxic medication or a pre-existing infection by HBV. The older the patient is, the greater the risk of a severe form of the disease. In children, 90% of infections are asymptomatic. Subjects with no acquired HAV immunity who travel to countries with poor hygiene standards are exposed to a risk of clinical, even fulminating, hepatitis, with this risk increasing with age.

SEARCH INDICATIONS

A diagnosis of HAV infection is indicated:

– In the event of non-B and non-C acute viral hepatitis in a subject who has visited countries with poor standards of hygiene.

– In a subject who has eaten shellfish in the previous two months.

INFORMATION

■ SAMPLE

Freshly-decanted serum or plasma.

■ SAMPLE STORAGE AND TRANSPORT

Store serum or plasma at + 4 °C. Transport must take place without interruption of the cold chain.

DIAGNOSTIC METHODS

■ DIRECT DIAGNOSIS

Direct diagnosis of hepatitis A is not routinely used:

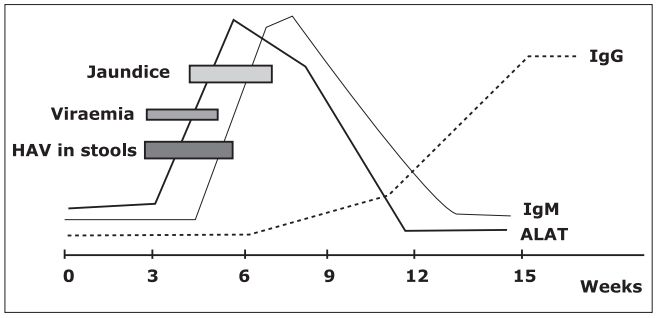
– Detection of viral particles in stools by electronic immunomicroscopy.

– Detection of the genome by RTPCR in plasma and/or stools, or in bile.

– Virus isolation by culture.

■ INDIRECT DIAGNOSIS

Anti-HAV IgM's, detectable by ELISA as soon as jaundice appears, are an excellent marker for acute hepatitis A. They persist for 8 to 12 weeks and, exceptionally, for up to 1 year in 2-4% of subjects. Measurement of total anti-HAV Ig's is used in epidemiological studies or to determine a pre-vaccination status.



Evolution of biological parameters in hepatitis A.

TREATMENT

In the absence of an active substance against HAV, treatment in the acute phase remains symptomatic, taking care to avoid any medication which might induce additional hepatotoxicity. No viral monitoring is required, although there is still a need for clinical and biological monitoring because of the (rare) risk of fulminating hepatitis.

A vaccine to prevent hepatitis A has been available for several years. To avoid unnecessary and costly vaccination, it appears logical to undertake a measurement of total anti-HAV's before vaccinating subjects more than 40 years of age, since the probability that they have already been immunised is in excess of 50%.

FOR FURTHER INFORMATION

■ ANAES, *Diagnostic et suivi virologiques des hépatites virales à l'exclusion du dépistage en cas de dons de sang, d'organes ou tissus*, 2002.

■ Buisson Y. et al., *Hépatite A*, Feuillets Biol 2000; 41/232: 2731.

HEPATITIS B

DEFINITION

Hepatitis B virus (HBV) is the agent responsible for hepatitis B in human beings and is a DNA virus belonging to the *hepadnaviridae* family (hepatotropic ***DNA*** Viruses), which also contains a *Woodchuck hepatitis B virus (WHV)* that causes hepatitis in the American groundhog and *Duck hepatitis virus (DHBV)* found in ducks. This family groups together viruses, which have an elective tropism for hepatocytes. HBV is a small, enveloped virus, the genome of which is very compact and organised as a partial two-chain circular viral DNA.

Nine sub-types of HBV have been identified, reflecting the heterogeneity of Hepatitis B Surface Antigen (HBs Ag). Once the nucleotide sequence of HBV was determined a relationship was established between sub-types and genotypes (groups A to G). Characterisation of these genotypes is of epidemiological and prognostic use.

Synonyms:

Family: *Hepadnaviridae*; Genus: Orthohepadnavirus; Hepatitis B virus (HBV).

INTRODUCTION

EPIDEMIOLOGY

HBV infection is strictly limited to human beings and can be transmitted sexually, parenterally and perinatally. Epidemiological findings however indicate that no history of exposure to these risk factors is found in a third of people infected with HBV. The infection is endemic and worldwide (approximately 400 million people carry HBV) with varying prevalence and method of transmission depending on geographical areas. In low endemic areas (North America, Australia and Western Europe) transmission is mostly parenteral and sexual. In highly-endemic areas (Africa, South-East Asia, China and Japan), mother to child transmission predominates. Whilst vaccination against hepatitis B is an effective method of prevention, infection with the hepatitis B virus is still a major public health problem. According to World Health Organisation (WHO) data, the prevalence of chronic hepatitis B infection is approximately 5% worldwide. Epidemiological studies show that following acute hepatitis B, one out of fifteen immunocompetent people will develop chronic infection. Chronic progression is an almost constant finding when infection occurs during the perinatal period (which is seen in highly endemic areas) and in the immunosuppressed. The severity of chronic hepatitis is due to its slow, silent progression, which may lead to development of cirrhosis and hepatocellular carcinoma. Progression to severe liver disease can be inhibited or delayed by anti-viral therapy.

SYMPTOMS

- **Acute hepatitis B:** After, an often asymptomatic, incubation period lasting an average of 10 weeks, the acute form is characterised by a jaundiced phase with a deranged liver profile. The acute hepatitis remains asymptomatic in 80% of adults and may not be diagnosed. Fulminant hepatitis, which is generally fatal, occurs in less than 1% of patients. Hepatic lesions in HBV infection are mostly due to recognition of viral antigens expressed on the hepatocyte surface by the host immune system. The diagnosis and prognosis of good outcome is made from the change in direct viral DNA, HBs

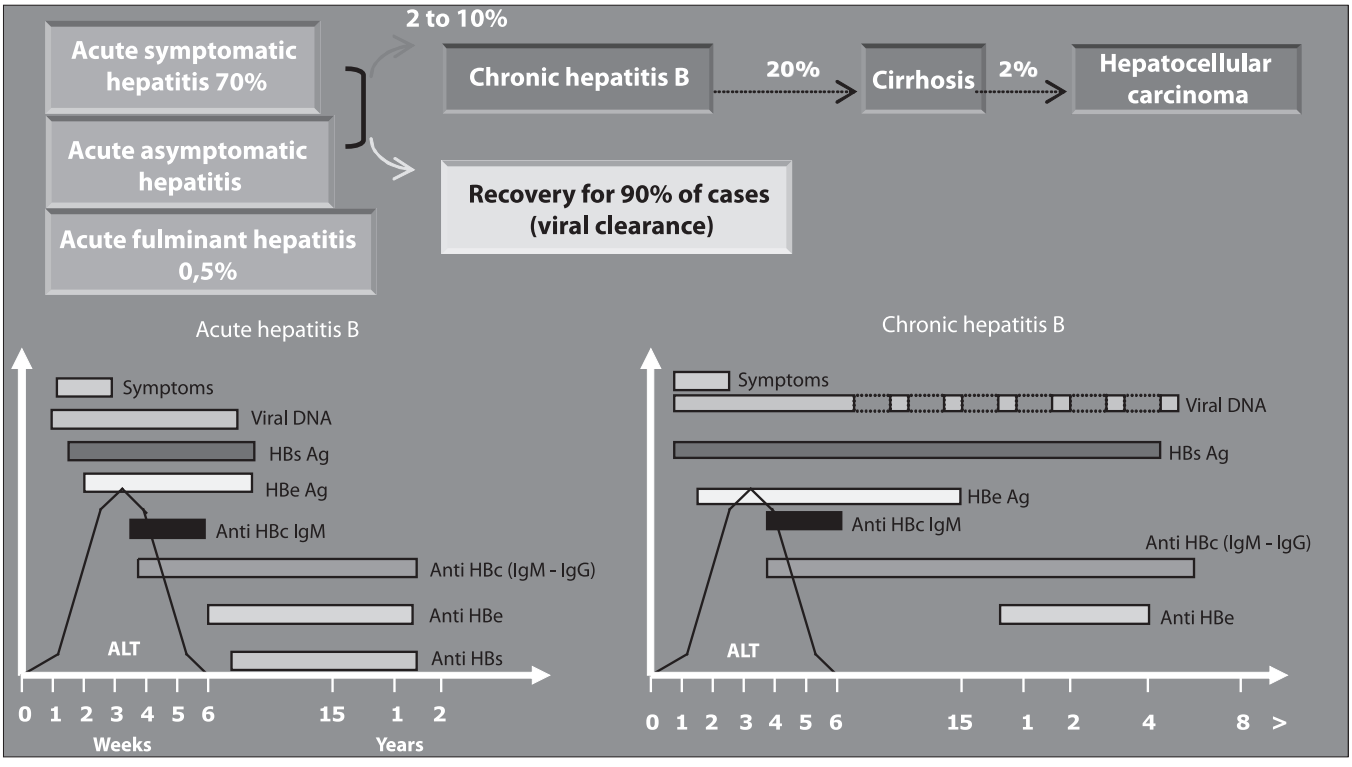


Figure 1: Serological and virological progression of hepatitis B

antigen, HBe antigen and indirect anti-HBc antibodies (IgM and IgG), anti-Hbe antibodies and anti-HBs antibodies, which are markers of the immune response *(fig. 1)*. Following acute HBV infection, approximately 5% to 10% of people are unable to develop an immune response enabling them to remove the virus. These people remain chronic HBV carriers.

- **Chronic hepatitis B:** In an initial period an ***immune tolerance phase*** develops (2 to 5 weeks or even several years in perinatal infection) with intense viral replication but no hepatic lesions (high viral load and normal or subnormal transaminases). The person then develops an immunological response (***immuno-active phase***) leading to hepatocyte lysis (raised transaminase). HBe antigen can be detected (in infection with a "wild type" virus), together with viral DNA. This period can last for several years. Over time the level of viral replication falls gradually with disappearance of HBe Ag associated with the development of anti-HBe antibodies. HBe seroconversion can select escape mutants known as "preC mutants" (see section "HBV mutants"). Following the ***non-replicative*** "post HBe seroconversion" phase the transaminases are normal and viral replication is only detectable by highly sensitive molecular techniques (replication rate < 10^5 copies/ml or 2.10^4IU/ml).

  Very occasionally, HBV immunity may be established. This is characterised by disappearance of HBs antigen and possibly development of anti-HBs antibodies.

SEARCH INDICATIONS FOR SEROLOGICAL AND VIRAL DNA

Diagnosis of acute or chronic hepatitis B.

Diagnosis of hepatitis B from transmission, following a blood exposure accident (BEA).

Diagnosis of hepatitis B in mother-to-child transmission.

Monitoring chronic hepatitis B.

Monitoring treatment of hepatitis B.

INFORMATION

SAMPLE

Serology: freshly separated serum.

HBV DNA: EDTA plasma (4 ml minimum).

SAMPLE STORAGE AND TRANSPORT

Serology: Serum can be stored between + 2 and 8°C for up to 5 days or at – 20°C if the sample is to be stored for longer than 5 days.

HBV DNA: Separated EDTA plasma, frozen at – 20°C within 4 hours of sampling.

LABORATORY DIAGNOSTIC METHODS

The laboratory diagnosis is based on tests, which measure the activity of liver disease (ALT and AST) and on detecting direct (Hbs antigen, HBe antigen, viral DNA) markers in blood or even a histological section of the liver (HBc antigen) or on indirect markers (anti-HBc IgM and IgG antibodies, anti-HBe antibodies and anti-HBs antibodies) of infection.

MARKERS AVAILABLE

HBs Ag

HBs Ag was long known as *"Australia antigen"*, and is the essential serological marker for any diagnosis of HBV infection. Its detection indicates ongoing HBV infection. The current commercially available kits use monoclonal antibodies recognising the "a" epitope of the S gene, which is common to all HBV strains. HBs Ag appears early during HBV infection and is detectable two to four weeks before the established phase of the disease, approximately two weeks before development of anti-HBc IgM and remains detectable on average for four to six weeks. Disappearance of HBs Ag indicates a good outcome of the infection. Usually this only disappears after clinical signs and serum ALT have returned to normal. Conversely, persistent HBs Ag (for more than 6 months) defines chronic progression of the infection.

Pre-S1 or pre-S2 *antigens* can be detected. Failure to detect pre-S1 antigen predicts arrest of viral multiplication. This test is a research technique.

Anti-HBs antibodies

Anti-HBs antibodies indicate:

– Arrest of viral replication, i.e. "recovery" or usually "resolution" of the infection, 2 weeks to 4 months after HBs Ag has disappeared.

– Post-vaccine protection (the only HBV marker in this situation).

All of the current immunoassay methods are quantitative and the protective cut-off is set at 10 IU/l (WHO cut off). Testing for Anti-HBs Ab is therefore only indicated to monitor vaccine immunisation status or to establish the serological status of the HBs Ag negative/total anti-HBc Ab positive person.

HBc Ag

HBc Ag can only be tested for immuno-histochemically on a liver biopsy and is not used in common practice. It can be detected in the hepatocyte nuclei during the intense viral replication phase (unlike HBs Ag which lies within the cytoplasm), although is no longer detectable in the post-HBe seroconversion phase ("non-replicative" phase).

Total anti-HBc and anti-HBc IgM

Anti-HBc IgM antibodies develop very early (approximately 2 weeks after HBs Ag). They remain at high titre throughout the entire acute phase and then become negative. They can be detected for several months (up to approximately 6 months) and are therefore often still present when HBs Ag has disappeared in hepatitis, which has resolved. If HBs Ag is discovered fortuitously, testing for IgM can distinguish old infection from acute infection, although because of the high sensitivity of the reagents, it can also be detected again if the HBV reactivates.

The IgG + IgM antibodies are tested rather than anti-HBc IgG in isolation. Total anti-HBc antibodies therefore develop very rapidly after viral contact, are non-protective and persist for very many years or even "life long" after recovery following prolonged or symptomatic infection" making them an excellent epidemiological marker of contact with HBV.

HBe Ag

HBe Ag can be used as a marker of HBV replication. It is not, however, directly associated with the virion and is only an indirect marker of replication, which correlates 80% with detection of HBV DNA. With the pre-C mutant which is currently very widespread (30 to 80% of strains depending on geographic area), HBe Ag is negative with viral loads of over $10^4/10^5$ copies /ml (or 2.10^3 to 2.10^4 IU/ml). In this situation testing for HBe Ag becomes particularly useful: negative HBe Ag when HBV DNA is present suggests (but does not confirm) infection with a pre-C mutant (this type of serological profile does not entirely correlate with infection with the pre-C mutant).

Anti-HBe antibodies

Anti-HBe antibodies normally develop to 6 to 8 weeks after HBs Ag appears in acute resolving hepatitis and marks the end of active viral replication: it is the first immunological block on viral replication and therefore a prognostic indicator of a good outcome. Conversely, its absence at 3 months with persistent HBe Ag suggests chronic progression, which should be confirmed at 6 months.

Testing for anti-HBe Ab is used to document anti-HBe seroconversion (confirming disappearance of HBe Ag), one of the aims of anti-viral treatment.

HBV DNA

The amount of serum HBs Ag present does not pre-judge the proportion of complete virions compared to defective envelope particles and as HBe Ag is not a default marker, the best marker for the presence of virions is HBV DNA. HBV DNA quantification methods now mostly use real-time PCR, which has a sensitivity of 10 to 20 IU/ml (1 IU ≈ 5 WHO standard copies: *WHO International Standard for HBV DNA*).

SEROLOGY DURING ACUTE INFECTION *(FIG. 3)*

Acute infection is a phase of active viral replication and HBs Ag is the first viral replication marker found in serum. Synthesis peaks at the time of the acute phase of the hepatitis (marked elevation of transaminases). HBe Ag, which is closely linked to the viral nucleocapsid, is present at the time of full viral replication, when HBs Ag titres are high. Anti-HBc IgM Abs are present as soon as clinical signs develop.

Convalescence and resolution of the infection

Markers usually progress as follows:

– In the early convalescent phase, HBs antigenaemia falls and then disappears along with HBe/anti-HBe seroconversion as a result of arrest of viral replication

– After a variable period, anti-HBs Ab develop. Only anti-HBs Ab, anti-HBc Ab (IgG) and anti-Hbe Ab persist and then fall in people who have recovered. Their titres fall over the years.

Chronic progression

Chronic progression varies depending on the level of immunocompetence from 5 to 10% in adults to almost all children infected by their mother at birth. This progression follows the acute phase, which may be entirely clinically silent. Potential for progression is assessed from:
– Persistence of HBs Ag beyond 6 months

– Lack of HBe/anti-HBe seroconversion within 6 to 8 weeks following the acute phase (prolonged acute phase); persistence of HBe Ag indicates that infectious virions have not been removed by the immune system and the person remains highly infectious.

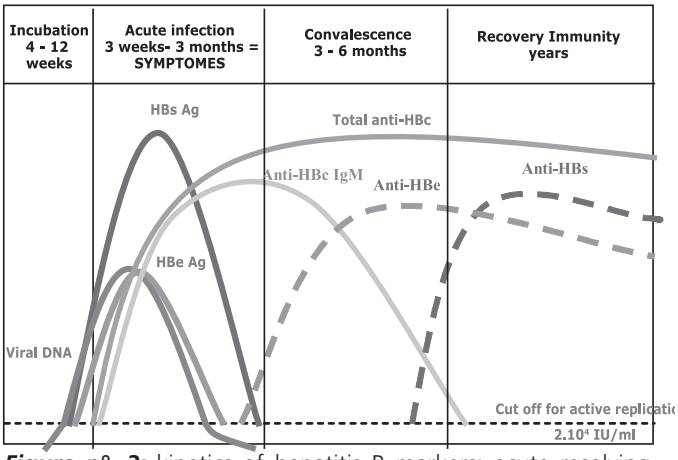


Figure n°. 3: kinetics of hepatitis B markers: acute resolving hepatitis.

SEROLOGY IN CHRONIC INFECTION *(FIG. 4)*

By conventional serology, indirect markers of viral replication (HBe Ag) and the person's immune response (anti-HBe) define two stages of infection representing either replication of viral genome or integration of the genome into the host genome, the second situation representing a lower level of infectivity.

HBe seroconversion in the natural history of chronic HBV infection is a spontaneous and constant finding. It is often preceded by exacerbation of the liver disease, which may raise diagnostic problems if the prior HBV marker status is unknown. This reduction in viral replication over time results in reducing prevalence of HBe Ag with age.

This "post-HBe-seroconversion" state is not irreversible: spontaneous re-activation may occur with recurrence of HBe Ag, generally within a year of HBe Ag disappearing (15% of chronic hepatitis at this stage have had Hbe seroconversion).

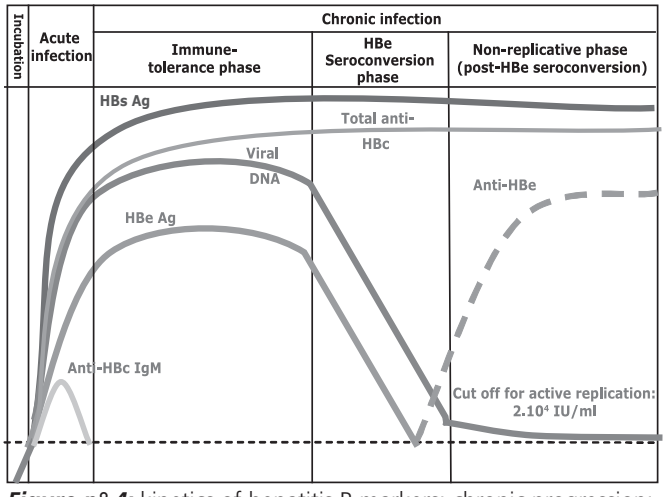


Figure n° 4: kinetics of hepatitis B markers: chronic progression: the 2 phases of chronic infection.

SPECIFIC SEROLOGICAL SITUATIONS

Isolated finding of total anti-HBc (no HBs Ag or anti-HBs Ab) may represent:

– The convalescence phase of acute hepatitis, the "serological window" between disappearance of HBs Ag and the appearance of anti-HBs Ab because complex formation with the antigen when it is first produced means that the antibody is detected later. In this case, anti-HBe antibodies are present and anti-HBc IgM are still detectable

– Recovered old infection in which anti-HBs Ab have become negative (in the elderly or immunosuppressed)

– A false positive: in low positivity areas some methods suffer from a lack of specificity, in this situation it is recommended that the parameter be tested using a different reagent

– More rarely, "occult" hepatitis B: defective quantitative production of HBs Ag despite a normal nucleotide sequence (low viral DNA in blood and liver)

– Far more rarely, an HBs Ag mutant which is not detected by the HBs Ag screening test.

Isolated positive anti-HBc IgM (with no HBs Ag or anti-HBs Ab). This may represent:

– A false positive (total anti-HBc itself may be falsely positive)

– Co-infection with hepatitis Delta virus: in 10% of cases this may switch off HBs Ag production.

Simultaneous finding of HBs Ag and anti-HBs Ab may represent:

– A false positive HBs Ag (to be confirmed with the neutralisation test); in this case anti-HBc Ab is negative

– Chronic hepatitis B (in this case the anti-HBs Ab titre is usually < 100 IU/l)

– Acute, particularly fulminant hepatitis.

Isolated finding of HBe Ag (without HBs Ag) may represent:

– A false positive HBe Ag: in this case anti-HBc Ab is negative

– More rarely, the presence of a HBs Ag mutant, which is not detected by the screening test: in this case anti-HBc Ab is positive.

USE OF QUANTIFICATION TESTS TO DIAGNOSE AND MONITOR PATIENTS

Recent acute hepatitis

HBV DNA can be detected before the hepatitic phase and development of HBs antigen. Detection and/or quantification of HBV viral DNA is of no use however in recent acute forms of the disease, when the diagnosis is made by the presence of HBs antigen and quantification of anti-HBc IgM.

Chronic hepatitis

This is defined by detection of HBs Ag in two samples, 6 months apart and the absence of Anti-HBc IgM. Determination of viraemia is then a key factor to assess the level of replication and risk of progression to decide on treatment, measure response to treatment or enable early detection of resistance to anti-retroviral agents.

Chronic hepatitis in the "immune tolerance" phase

In the immune tolerance phase viral replication is intense, as viral loads of over 2.10^8 IU/ml are found. This highlights the extremely infectious nature of patients' serum during the active HBV replication phase in both acute and chronic infection. During this initial phase, which may last for 1 to 15 years, aminotransferase activity is not particularly deranged.

Chronic hepatitis "immunoactive phase"

The development of an immune response leads to viral clearance, characterised by a fall in viraemia. The immune reaction against infected hepatocytes leads to a rise in aminotransferase activity (ALT, AST) and development of intra-hepatic inflammatory lesions.

Chronic hepatitis "non- or pauci-replicative phase or inactive viral carrier status"

A person is deemed to be a pauci-replicative chronic hepatitis carrier if the viraemia is less than 2.10^4 IU/ml (or 10^5 copies/ml). The HBe antigen is no longer detected in these people and anti-HBe antibodies develop ("e" seroconversion). The liver disease is not progressive, does not require treatment and aminotransferase activity returns to normal.

The following are seen in the liver:

– Either integration of the HBV viral genome into the hepatocyte genome, responsible for liver carcinogenesis

– Or episomic maintenance of the viral genome as super-role circular (DNAccc) responsible for the viral reactivation process.

Chronic hepatitis and viral reactivation

In chronic hepatitis B the DNAccc (closed covalent circular) of the HBV is not removed and anti-HBc antibodies persist throughout life. Secondary reservoirs of the virus exist (hepatocytes, lymphocytes, etc). Because of this, viral reactivation may occur in chronic carriers in the pauci-replicative stage and in patients whose infection has resolved: HBe Ag may re-emerge in people who have become HBe Ag negative (HBe seroreversion), together with HBs Ag in patients who are anti-HBs Ab positive (HBs seroreversion). Viral replication restarts with a rise in ALT and anti HBc IgM become positive again. Viral reactivation may be spontaneous or, more often iatrogenic, occurring after cytotoxic or immunosuppressant therapy (corticosteroids, rituximab, infliximab…). HBV reactivation is defined as an increase in viral HBV load of at least 1 log unit compared to its nadir (basal level) or a rise to high levels (> 2.105 IU/ml), after excluding recurrent systemic infection and infection with hepatitis A, C, D or E viruses. All patients at risk of reactivation, i.e. those with a past history of HBV infection who need to receive immunosuppressant or cytotoxic therapy, must now be managed preventatively. The compounds currently used prophylactically are tenofovir and entecavir.

HBV GENOTYPING

The variability of circulating HBV strains is due to variability in the epitopes of the surface antigen (HBs Ag). Recognition of different antigenic determinants carried by the HBs antigen by different immune sera, has led to HBV strains being divided into different serotypes. One major "a" epitope is shared by all types, whereas the d/y and w/r determinants are exclusive to one or other and are specific to the sub-types (sub-types, adw, adr, ayw, ayr).

A final level of variability exists due to the w determinant (w1, w2, w3 and w4). This antigenic variability is due to variability of the viral genome. Analysis of the nucleotide sequences of different strains has identified 7 genotypes (AG): these genotypes represent divergence between the nucleotide sequences of 8% or more. A relationship has been established between sub-type and genotype (table 1). Genotype A predominates in Western Europe, B and C in Asia and D and E genotypes in the Mediterranean basin.

Genotypes	A	B	C	D	E	F	G
Associated serotypes	adw2 (ayw1)	adw2 ayw1	adr adrq- ayr	ayw2 ayw3 ayw4	ayw4 (adw2)	adw4q-	adw2

Table 1: ***Classification of HBV genotypes and associated serotypes.***

Different methods are used to characterise the HBV genotypes. These are all based on PCR amplification of sequences located in the pre-S and S regions. The amplification products obtained are analysed either by restriction endonuclease treatment (***PCR-RFLP***, *PCR-Restriction fragment length polymorphism*), or by direct sequencing and then comparison to reference sequences or by reverse hybridisation of the products amplified with main types of specific oligonucleotide probes (LiPA method or *Line Probe Assay*) fixed to a nitrocellulose strip. Genotype characterisation of epidemiological interest (eg: more common association of preC mutants with non-A mutants from the Mediterranean basin, particularly the D genotype).

Genotype determination is also of prognostic use. The disease is more severe in Asia with the C genotype compared to the B genotype. Response to interferon alpha is better with the A genotypes (in Europe) compared to the D genotypes (Europe, America). Response is also better with the B genotypes (Asia) compared to the C genotypes (Asia).

CHARACTERISATION OF HBV MUTANTS

Whilst the existence of different circulating genotypes represents one level of variability, a second level of variability is the development of mutant viruses. Replication of the HBV viral DNA, which contains a reverse transcription stage, explains the frequent development of mutations during its replication phase. Some mutations are able to escape the host immunological surveillance mechanisms *(escape mutants)*. Other mutations are associated with development of a more severe form of the disease (mutations in the pre-core region, the promoter region and the capsid), resistance to the anti-viral agents (DNA polymerise mutations) or hepatocellular carcinogenesis (X mutants).

S Mutants

S mutants are characterised by the development of point mutations or insertions of nucleic acid sequences within the region coding for the HBs antigen. As the immunological response is ultimately directed against epitopes carried by the HBs antigen, the development of mutants in the S region allows mutants to escape the immune system surveillance. S mutants, however, are rare and are found more commonly in Asian regions. The emergence of S mutants is provoked by preventive treatments against HBV, in vaccination campaigns and in preventive treatments with serotherapy in liver transplant patients and in pregnant women carrying the virus (serotherapy is passive immunotherapy with specific anti-HBs Ig). Occasionally these mutants can develop in a chronic carrier of the virus. Mutations located in the S region produce false negative results in some HBs Ag screening tests, which are a risk to patients who are not identified and to the safety of the blood transfusion system. Carriers of these mutants are identified by detecting the anti-HBc antibody, anti-HBe antigen and testing for viral DNA. Confirmation, however, is provided by direct sequencing of the S region and comparison with reference sequences.

Pre-C mutants or HBe Ag (-) variants

Pre-C mutants represent mutations, nucleotide deletions or insertions located in the pre-C region responsible for reduction or failure of HBe Ag synthesis and persistent viral replication despite the presence of anti-HBe in these patients. The 1896G > A mutation (replacement of a guanine with an adenine in position 1 896 of the pre-C region) is the most common. This mutation causes premature development of a stop codon (TAG codon), leading to failure of HBe Ag synthesis. Hepatitis B due to the pre-C mutant is now widely found in populations in the Mediterranean basin, Africa, Asia and Europe (in France it is estimated that more than 50% of people infected with HBV carry a pre-C mutant). Patients with pre-C mutants are characterised by negative HBe Ag antigenaemia. Viral replication fluctuates at low level (10^3 to 10^6 copies/ml or 2.10^2 to 2.10^5 IU/ml), as does aminotransferase activity. The other mutations described in the pre-C region responsible for reduced synthesis of HBe antigen include the 1762A > T and 1764G > A mutations. This form of hepatitis is more severe and more difficult to treat that infection due to the wild type virus.

DHA polymerase mutants

Mutations in the region coding for DNA polymerase (mutations located in the catalytic domain of the enzyme), develop after approximately 6 months on long-term treatment with nucleotide analogues. In the case of lamivudine, two mutations, Met552Ile or Met552Val, affect the preserved Tyr-Met-Asp-Asp (YMDD) region, which is part of the active site (C domain) of the DNA polymerase. Famciclovir, another nucleotide analogue, can induce mutations within the DNA polymerase B domain (mutations Val521Leu and Leu528Met). By reducing the affinity of nucleotide analogues for viral polymerase DNA (DANP), these mutations raise the possibility of escape from treatment. Viral replication increases but does not reach levels documented before treatment was started. Because replication of the mutant virus is compromised in the vital region of DNAP activity, the wild type virus re-emerges at the end of drug therapy. Escape is characterised by a rise in serum transaminases associated with re-emergence of DNA or even HBe Ag.

Pre-C mutants and the region coding for DNA-polymerase (antiviral resistance) are characterised using molecular methods based on PCR amplification of target sequences located in the C or P regions. The amplification products obtained are analysed by sequencing or by reverse hybridisation of the PCR products with specific oligonucleotide probes for the main types (LiPA or *Line Probe Assay*) bound to a nitrocellulose strip.

Summary: main clinical-laboratory pictures.

"Resolved" hepatitis B	Inactive carrier status (post-HBe seroconversion stage)
Past history of acute or chronic hepatitis	HBs Ag (+) for more than 6 months
HBs Ag negative	HBe Ag (–) and anti-HBe Ab +
Anti HBc and anti-HBs Ab present ≥ 10 IU/l	HBV DNA < $2.10^{3/4}$ IU/ml
HBV DNA undetectable	Transaminases normal
Transaminases normal	
Chronic wild type hepatitis B (pre-HBe seroconversion) stage	**Chronic hepatitis B with pre-core mutant**
HBs Ag (+) for more than 6 months	HBs Ag (+) for more than 6 months
HBe Ag (+) and anti-HBe Ab (-)	HBe Ag (-) and anti-HBe Ab (+/-)
HBV DNA > 2.10^{5} IU/ml	HBV DNA ≥ $2.10^{3/4}$ IU/ml
Transaminases increased or normal	Transaminases raised or normal
	Histology: fibrosis ≥ F2
	PCR – pre-C sequencing: specific mutations

TREATMENT

PREVENTIVE TREATMENT: VACCINATION

Vaccination has been reintroduced into the French vaccine calendar for infants (3 injections at 2, 4 and 16 months) and is mandatory for health staff in whom a titre of more than 100 IU/ml is sought (hospital care staff, haemodialysis patients, transfusion centre staff, laboratory technicians), in newborn babies born to HBs Ag positive mothers and in populations travelling in highly endemic areas.

CURATIVE TREATMENT

Acute hepatitis: Acute hepatitis B virus infection does not require antiviral treatment.

Chronic hepatitis:

The treatments used are pegylated interferon alpha and nucleos(t)ide analogues: lamivudine, emtricitabine, adefovir, entecavir, tenofovir, clevudine, telbivudine.

The aims of treatment are to achieve a viral DNA of < 10-15 IU/ml, clinical, biochemical, and histological improvement and HBe seroconversion in patients who are initially HBe Ag +, with HBs Ag becoming negative (with possible anti-HBs seroconversion).

In HBe Ag positive patients (who usually have a high viral load), HBe seroconversion is usually obtained with pegylated IFN (30% of cases) although viral load becomes undetectable more often with analogues (entecavir, tenofovir: 67%, 74%). In HBe Ag negative patients (who often have a low viral load) the analogues are generally more effective (undetectable viral load obtained in 86 to 92% of cases with entecavir, telbivudine or tenofovir). Negative HBs Ag at 1 year is only obtained in a small number of cases (3% with IFN peg or tenofovir, 2% with entecavir, < 1% with the other analogues).

Who to treat? EASL recommendations (2009)

Patients with an HBV DNA > 2000 IU/ml and/or abnormal transaminases and hepatic fibrosis (as assessed by puncture liver biopsy or serum markers) ≥ A2 F2. Clinical factors must be taken into account (age, co-morbidities, etc.). In a specific case of people with cirrhosis: these people must be treated even if they have normal transaminases.

On the other hand treatment should be deferred if chronic hepatitis B is in the immune tolerance phase (with normal transaminases even with high viral load) and with ALT < twice normal and fibrosis/ Metavir score < A2 F2. These patients should be closely monitored.

Predictive factors for successful treatment

The pre-treatment predictive factors for virological response (HBe seroconversion or undetectable HBV DNA) are a viral load < 7 log IU/ml, ALT > 3N and Metavir score > A2. Other predictive markers for successful treatment appear promising in patients treated with IFNpeg, although are still being assessed: fall in HBe Ag and HBs Ag titres and A or B genotype appear to be good prognostic markers.

Evaluation and monitoring treatments: definitions

Virological assessment by HBV DNA

– Initial response: reduction in viral load (VL) > 1 log within 3 months

– Response to IFN: VL < 2000 IU/ml after treatment for 6 months

– Response to analogues: VL < 12 IU/ml at 1 year

– No primary response: fall in VL < 1 log within 3 months

– Virological response: undetectable viral load at one year (< 10 IU/ml)

– Partial response: reduced VL > 1 log unit but undetectable at 6 months

– Virological failure: increased VL > 1 log unit compared to the nadir, confirmed on two successive samples.

Biochemical and clinical evaluation and monitoring

– Biochemical failure: increasing ALT

– Histological failure: return or progression of fibrosis.

Serological evaluation and monitoring

– In HBe Ag negative patients: testing for HBs seroconversion

– In HBe Ag positive patients: HBe and HBs seroconversion

– The following serovaccination protocol is used to treat children born to mothers positive for HBs Ag: anti-HBs immunoglobulins, 100 IU/l within 12 to 24 hours after birth and 1st injection of vaccine against HBV, 2nd injection of vaccine 1 month after birth – 3rd injection of vaccine 2 months after birth– 4th injection of vaccine 3 months after birth or if the mother has replicative disease– 4th or 5th injection at 12 months.

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HEPATITIS C

DEFINITION

Hepatitis C virus (HCV: hepatitis C virus) is the main agent responsible for post-transfusion hepatitis, formerly called non-A non-B hepatitis. The virus is responsible for liver disease, which varies in severity between different people, which may progress slowly to cirrhosis, and then hepatic adenocarcinoma. Since 1989 when the genomic sequence and the hepatitis C virus was characterised, considerable advances have been made in our knowledge of this disease (structure of the virus, genetic variability, development of serological and molecular screening tests, changes in the disease over time, method of transmission, risk factors for infection and improvement in treatments). The infection, however, remains a public health problem with a worldwide prevalence of chronic hepatitis C of 3% (i.e. 170 million chronic carriers). HCV is mostly transmitted parenterally (by transfusions before 1990-1991 and by syringe sharing in drug addicts). Mother to child transmission is rare but is increased in mothers who are co-infected with HIV. Sexually transmitted infection appears to be limited if it even occurs.

30% of cases of infection, however, remain of unknown origin.

Synonyms: Family: *Flaviviridae*; Genus: Hepacivirus; Single chain positive polarity RNA virus, parenterally transmitted non-A, non-B viral hepatitis; HCV: Hepatitis C virus.

SYMPTOMS

HCV infection is characterised by an acute, usually asymptomatic, non-jaundiced phase progressing variably and slowly. A person infected with the hepatitis C virus develops acute hepatitis within the period of 1 to 6 months (and is asymptomatic in 65- 75% of cases). The severity of HCV infection is due to its progression to chronic infection (which occurs often, 50 to 80% depending on estimates) which itself may progress to cirrhosis with increased risk of hepatocellular carcinoma. The factors that predispose to more severe disease are the 1 genotype, male sex, the consumption of alcohol and HIV co-infection.

SEARCH INDICATIONS

– Testing for infection particularly in at risk groups (multiple transfusions, drug addicts): testing for anti-HCV antibodies

– Diagnosis of acute or chronic hepatitis C: testing for anti-HCV antibodies and qualitative detection of HCV RNA

– Diagnosis for transmission after a blood exposure accident (BEA): testing for anti-HCV antibodies and qualitative detection of HCV RNA followed by measurement of ALT and identification of seroconversion

– Diagnosis of mother-child transmission: in mothers infected with HCV (positive HCV RNA), testing for HCV RNA in the infant at between 6 and 12 months old and detection of anti-HCV antibodies after 18 months old.

INFORMATION

SAMPLE

Serum for anti-HCV antibodies.

Serum or EDTA plasma for molecular biology tests.

SAMPLE STORAGE AND TRANSPORT

Serology tests: store and transport at + 4°C.

Molecular biology tests tests (PCR, genotyping): once the sample has been taken it should be centrifuged promptly at 4,000 rpm for 20 minutes and the serum or plasma then collected into tubes which are frozen at -20°C (within 4 hours of sampling). It **MUST BE** stored and transported frozen at -20°C.

DIAGNOSIS

The indirect diagnosis is based on detecting specific HCV antibodies (serological tests), which are markers of recent or former contact with the virus. The direct diagnosis is based on tests that detect and/or quantify constituents of viral particles (genomic RNA and viral antigens).

SEROLOGICAL DIAGNOSIS

- **The screening tests currently used are third generation ELISAs** testing for circulating IgG antibodies by capturing them with recombinant peptides and/or proteins. There is no IgM test available. ***Fourth generation*** tests also exist which simultaneously detect IgG and the core antigen with better sensitivity than the third generation tests, although these cannot replace PCR for the diagnosis of primary infection as they are less sensitive.
- **Immunoblots** are almost no longer used.
- **Interpretation**

  – If anti-HCV serology is positive with an EIA test, it is recommended that the result be checked on a second sample using a different method to the first (an alternative EIA method). The HCV RNA may be tested on the repeat specimen using a qualitative test (PCR). The presence of HCV RNA confirms that the virus is present but does not distinguish acute from chronic infection

  – If anti-HCV serology is negative and acute hepatitis C is suspected, the HCV RNA may be tested for. If this is positive, the diagnosis of acute hepatitis C is confirmed on a later sample from anti-HCV seroconversion. In the immunosuppressed, if there are clinical, laboratory or epidemiological reasons to suspect HCV infection, patients must be tested using a qualitative test for HCV RNA regardless of the result of the anti-HCV Ab test

  – If anti-HCV serology is negative in haemodialysis renal failure patients and in renal transplant patients, annual monitoring of HCV serology must be performed because of the risk of nosocomial HCV transmission

  – Testing for HCV RNA must be performed in anti-HCV positive pregnant women to assess the risk of transmitting the HCV from mother to child. This risk ranges from 0 to 5% depending on the study in mothers found to be positive for HCV RNA, although this risk increases to 15 to 30% if the mother is co-infected with HIV.

VIROLOGICAL DIAGNOSIS

- **Qualitative detection of viral RNA:** HCV viral RNA detection methods are based on amplification of a target region of the viral genome.
- **Indications for measurement:**
  – After finding positive HCV serology
  – Suspected acute viral hepatitis C with negative serology
  – Suspected chronic hepatitis without detectable antibodies in haemodialysis patients or the immunosuppressed.
- **Interpretation:**
  – The presence of HCV viral RNA indicates that viral replication is occurring
  – Absence of RNA with positive HCV serology may suggest interference in serological tests or recovered old infection.
- **Quantification** of viral RNA (requested in the pre-treatment assessment and to monitor treatment). Viral RNA quantification methods or viral load are used to assess the extent of HCV replication in a patient.

  Molecular biology methods currently available on the market to detect and quantify the HCV genome are based on real-time amplification methods (real-time PCR) and have replaced the former end-point PCR methods. Real-time PCR methods are both sensitive (limit of detection 12 to 15 IU/ml depending on the kit) and have a very wide linear response range of at least 8 $\log_{10}$ units (from 12 or 43 IU/ml to 100 millions IU/ml). As they are carried out in a closed system, these real-time PCR methods prevent against any risk of contamination (producing a false positive result) and automation of the extraction and PCR/developing stages improves reproducibility over the entire working range of the method. Differences in analytical performance of the kits available on the market are found, however, particularly in terms of detecting and quantifying the 2 and 4 genotypes.

  The introduction of an international standard by the WHO *(WHO International Standard for HCV RNA)* now enables results to be harmonised in international units (IU/ml). A specific conversion factor for each commercial kit is used to convert unstandardised "copies/ml" units into IU/ml.
- **HCV genotyping:** the hepatitis C virus is a small RNA virus, the genome of which is extremely variable. Whilst genetic variability is seen throughout the entire genome, it occurs predominantly in the regions coding for envelope proteins. An international nomenclature has been established to classify the virus into genotypes and sub-types of virus. The genotypes are expressed in Arabic numbers (genotypes 1, 2, 3, etc.), and the sub-types by a small case letter (genotypes 1a, 1b, 2a, etc.). At the end of 2010, the classification listed 11 genotypes and more than 70 sub-types. The genotype is established by amplifying the target regions of the viral genome (5' non-coding region, the NS5B region). The amplification products obtained are analysed either by direct sequencing and then compared by aligning the sequence obtained with reference sequences, or by reverse hybridisation of the PCR products to specific oligonucleotide probes for the main types and sub-types (LiPA or *Line Probe Assay*) fixed onto a nitrocellulose strip. Some HCV genotypes are specifically linked to particular methods of transmission. The 1b genotype is seen more commonly in people infected from blood transfusions than the 1a and 3a types which are more often found in drug addicts. Whilst the genotype does not appear to influence the severity of the liver disease, it does influence response to treatment.

FIBROTEST® AND ACTITEST®

These two tests are proposed as an alternative to puncture-liver biopsy. The Fibrotest® uses 5 biochemical markers: alpha 2 macroglobulin, haptoglobin, bilirubin, apolipoprotein A1, Gamma GT (gamma glutamyl transferase), to calculate an age and sex related fibrosis index. By adding another marker, ALT, the Actitest® is used to calculate an index of inflammatory and necrotic activity.

TREATMENT

The reference treatment for chronic hepatitis C is a combination of pegylated interferon-α and ribavirin. This is not without its side-effects and must be considered taking account of age and underlying medical health. Only liver biopsy correlates with severity of disease.

In chronic hepatitis: treatment decisions are based on testing for viral RNA and determining the viral genotype. If viral replication is found (positive detection of viral RNA), treatment depends on the genotype and on viral load.

There are two types of treatment depending on viral genotype: treatment should last for 24 weeks in the absence of a major contraindication in people infected with genotype 2 or 3 HCV and carries a high likelihood of long-term virological response (70% of cases). In people infected with HCV genotype 1 (and also suggested for genotypes 4, 5 and 6), treatment should last for 48 weeks with a likelihood of long-term virological response in the region of 45%. The duration of treatment should take account of 4 factors, initial (pre-treatment) viral load, kinetics of the fall in viraemia, time required to obtain undetectable viraemia and extent of fibrosis.

Viral load must always be measured before treatment is started in patients infected with HCV genotypes 1, 4, 5 or 6, in order to assess the type of virological response. A viral load of less than 400,000 IU/ml is considered to be a low, and conversely, a viral load over 400,000 IU/ml represents a high pre-treatment viral load.

Viral load must be measured after 12 weeks of treatment in people infected with HCV genotypes 1 (4, 5, 6). If the viral load is undetectable, treatment should be continued until week 48. If it is still detectable after 12 weeks but has fallen by at least two log units compared to the pre-treatment viral load, treatment should be continued up to 72 weeks. Conversely, if viral load does not fall by at least 2 $\log_{10}$ units, treatment can be stopped (low likelihood of virological response).

The aim of treatment is to obtain an undetectable hepatitis C viral load at the end of treatment, confirmed 6 months after stopping treatment.

New decision-making algorithms for the virological treatment of hepatitis C based only on the speed of virological response, regardless of genotype may be introduced in the future. Failure to reduce viral load by at least 1 $\log_{10}$ unit after treatment for 4 weeks appears to define non-responders *(Null response)* in whom treatment can be stopped. An undetectable viral load after treatment for 4 weeks appears to identify patients with a rapid virological response (RVR: *Rapid Virological Response*). In

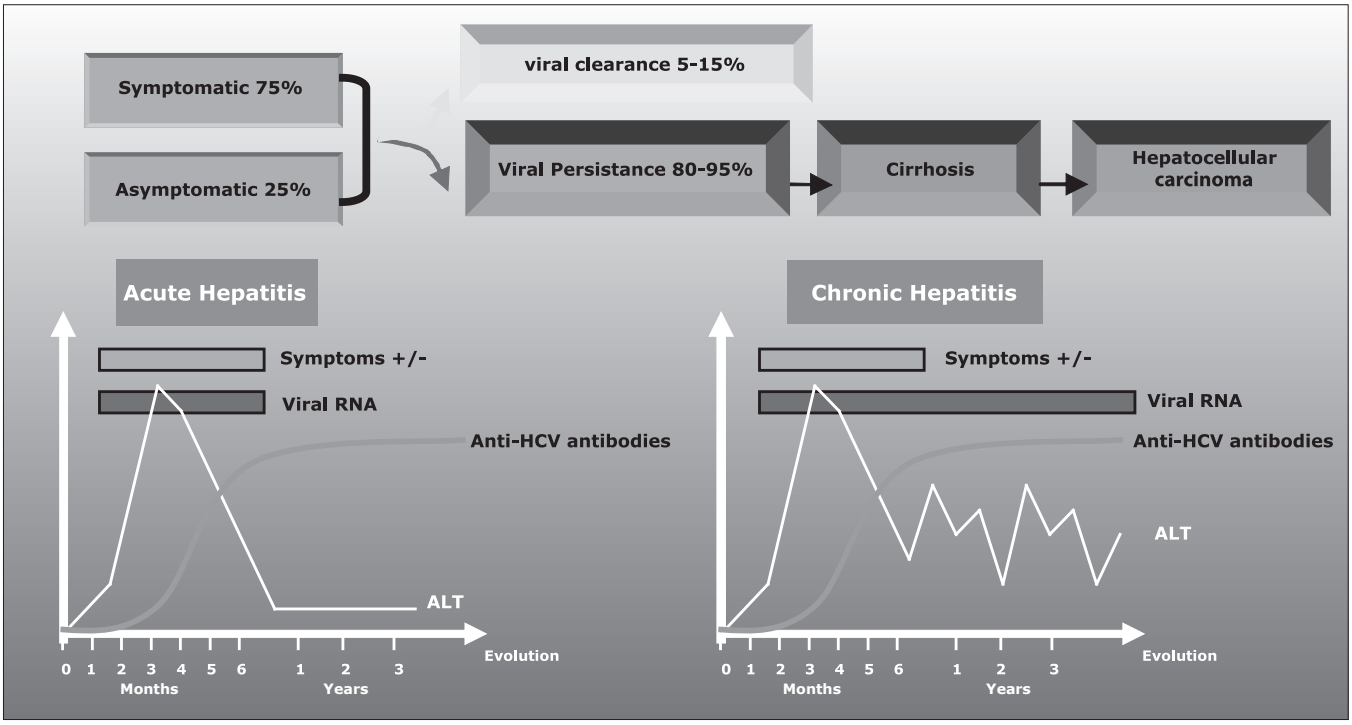

Figure 1: Serological and virological progression of hepatitis C

this case it may be possible to reduce the duration of treatment if the pre-treatment viral load is less than 400,000 IU/ml. Above 400,000 IU/ml, treatment should be continued up to 48 weeks. An undetectable viral load after treatment for 12 weeks appears to define patients with an early virological response *(EVR)*. Treatment can then be continued up to 48 weeks. A detectable viral load after treatment for 12 weeks but with a fall of at least 2 $\log_{10}$ units appears to define patients with a slow virological response *(SVR)* in which case treatment should be continued up to 72 weeks.

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HEPATITIS DELTA

DEFINITION

The D or delta virus is a defective satellite virus of hepatitis B (HBV). It can thus be acquired at the same time as HBV (co-infection) or as a complication of a chronic hepatitis B infection (superinfection).

This is the smallest virus (35-37 nm in diameter) known to infect humans. It is related to the viroids which infect plants and takes the form of a single-strand, circular RNA virus. The genome codes two proteins – the small and the large – associated with the genomic RNA in the virus particle, which play a part in regulating viral replication. They also carry the delta antigenicity. In certain regions, the delta virus genome presents considerable variability, which may explain the frequency of chronic forms and resistance to antiviral treatments. The envelope is constituted from HBs antigen taken from the co-infecting HBV.

Synonym: Delta Virus (HDV).

INTRODUCTION

EPIDEMIOLOGY

In spite of the dependency of delta virus with respect to HBV, there is not a perfect correlation between the prevalence levels of hepatitis B and Delta infections. Infection by HDV is common in geographical regions where there is a high prevalence of HBV, such as the Mediterranean, Eastern Europe and certain countries in Latin America and sub-Saharan Africa. In Western Europe, 1% of chronic carriers of HBV are carriers of the delta virus. In China and South-East Asia, on the other hand, where there is a high incidence of HBV infection, delta virus infections are far rarer in occurrence. In Western Europe and North America, chronic delta hepatitis mainly affects intravenous drug addicts and their sexual partners. In drug addicts, infection with HIV or HCV is often combined with HDV infection. Hepatitis delta can be transmitted from mother to child at the same time as hepatitis B.

SYMPTOMS

HDV causes acute hepatitis as a co-infection or superinfection, as well as chronic hepatitis.

– In co-infection acute hepatitis, infection by HDV is contemporaneous with that by HBV, and the hepatitis is serious more often than not (a larger proportion of fulminating hepatitis cases compared with infection by HBV alone).

– In superinfection acute hepatitis, infection by the delta virus occurs in patients who are chronic carriers of the HBs antigen.

– Chronic hepatitis D should be suspected in any subject at risk, presenting chronic hepatitis with a positive HBs antigen.

HDV causes a much more serious hepatic illness than that due to HBV alone: fulminating forms are 100 times more frequent, and the risk of evolution towards chronicity is greater. Paradoxically, B/delta infection often results in an inhibition of HBV replication, as well as an accelerated process of hepatic fibrosis and carcinogenesis.

SEARCH INDICATIONS

A possible diagnosis of infection by HDV is indicated in 3 circumstances:

– Acute hepatitis occurring in a known chronic carrier of the HBs antigen

– Severe acute hepatitis B occurring in a subject who belongs to a group at risk from hepatitis delta (drug addicts, haemophiliacs and patients receiving haemodialysis) or is from a region where hepatitis D is endemic (Southern Europe or the Near East)

– Severe relapse of acute hepatitis B.

INFORMATION

SAMPLE

For serological tests: serum

For molecular tests: freshly-separated serum or plasma (EDTA or citrate).

SAMPLE STORAGE AND TRANSPORT

For serological tests

If the analysis has to be deferred, the serum or plasma must be frozen rapidly and transported if necessary without interruption of the cold chain.

For molecular tests

Once the sample has been taken, centrifuge immediately at 4000 rpm for 20 minutes, and then collect the plasma in tubes to be frozen at -20° C within 4 hours for collection.

Samples should be stored and transported at –20 °C.

METHODS OF DIAGNOSIS AND INTERPRETATION

Indirect diagnosis

Only one marker should be requested in the first instance: total anti-HDV antibodies. If they are positive, a test for anti-HDV IgM's is required, of which the persistence is a marker of chronic delta virus infection (**NB:** They may be absent in certain African subjects).

Direct diagnosis

This is based on delta RNA, quantified by real-time PCR. The delta Antigen is fugacious and testing for it is of little practical interest.

DIAGNOSTIC ALGORITHM

If total anti-HDV antibodies are positive → anti-HDV IgM antibodies and HDV RNA (qualitative or quantitative RT-PCR)

1 - If IgM (-) and RNA (-): earlier and resolved infection.

2- If IgM (-) and RNA (+): acute or chronic infection.

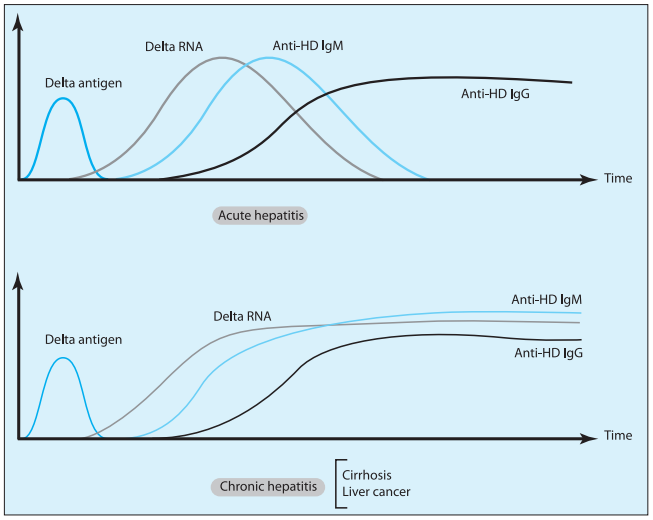
3- If IgM (+) and RNA (+): acute or chronic infection.

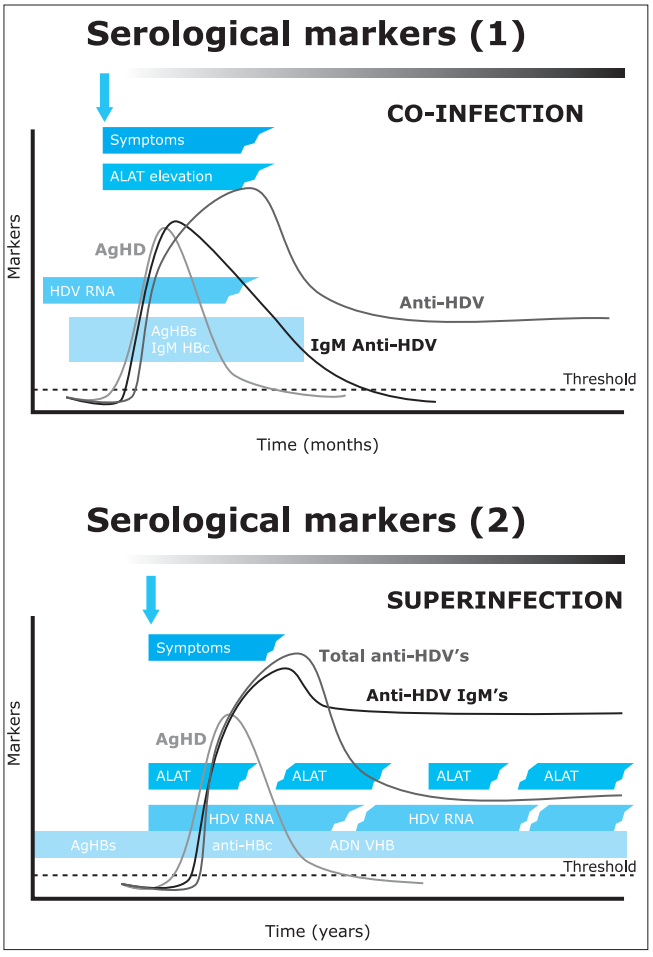
TREATMENT AND PREVENTION

Only alpha interferon is effective in chronic delta hepatitis.

Delta infection relapse after liver transplantation is less common and less serious than during HBV infection.

Prevention of hepatitis D coincides with that of hepatitis B in subjects at risk (drug addicts, haemophiliacs and patients receiving haemodialysis) and in new-born babies in endemic countries: systematic vaccination against hepatitis is effective against coinfection by HDV.





According to Dr E. Gordien, CHU Avicenne, Bobigny

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HEPATITIS E

DEFINITION

Hepatitis E virus (HEV), identified in 1983, is a small, non-enveloped single-strand RNA virus (a sphere 27 to 34 nm in diameter covered in spicules) classified in the *Hepevirus* genus of the *Hepeviridae* family. It is sensitive to high saline concentrations and to freezing-thawing cycles. There are four main genotypes, depending on geographical origin, but only one serotype.

INTRODUCTION

EPIDEMIOLOGY

Until the early 2000's, hepatitis E was an illness exclusively imported from hyperendemic regions such as India and, more recently, from Darfour and Uganda, with a few sporadic, travel-related cases in industrialised countries. HEV has now joined HBV and HCV as an aetiological agent of chronic hepatitis.

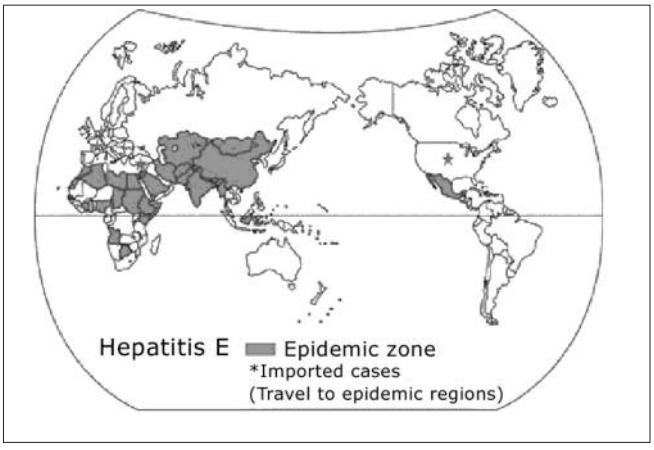

Figure 1: Geographical distribution of hepatitis E, according to E.M.C.

In the course of the early 2000's, the availability of serological tests made it possible to show that anti-HEV seroprevalence was high in India (20%), but also in France (3% to 20%). This makes it a cosmopolitan disease, rather than being solely liked to importation: cases of hepatitis E have been detected in France since 2004-2005 in subjects who have not travelled (approximately 150 cases at the national reference centre in 2008). This significant increase in the number of cases defined hepatitis E as an emerging infection in France (85% of detected cases in the south).

There are therefore two hepatitis E epidemiologies: one in the hyperendemic regions, with faecal-oral transmission and epidemics (genotypes 1 and 2), and the other in industrialised countries, for which the animal reservoir is essentially porcine and undoubtedly plays an important role (genotypes 3 and 4).

Modes of transmission

HEV is found in the blood and stools of infected subjects, as well as in water and molluscs. Its preferred mode of transmission is faecal-oral. Transmission from mother to child can occur, and transfusion-related cases have been described.

In addition, a great deal of data is now available which suggest transmission by pigs or wild boars (anthropozoonosis). Anti-HEV seroprevalence is indeed high in veterinarians, pig farmers and hunters (30% in the Toulouse region of France), while 50% of pigs have detectable levels of HEV in their blood, mostly of genotype 3. Contamination is in fact highly probable if poorly-cooked pig meat is eaten (HEV is inactivated at +60° C) or on contact with fresh porcine blood (hunters in particular). Most of the indigenous cases in France are believed to be linked to the consumption of pigs' liver sausage.

SYMPTOMS

After an incubation period of 15 to 60 days, which is comparable to that of hepatitis A, symptomatic hepatitis E (although the proportion of symptomatic cases is undoubtedly low) manifests itself in the acute stage by an influenza-like infectious syndrome with fever, jaundice (70% of cases) and significant asthenia. It is associated with a mortality rate of 0.2 to 5% in the general population, but this rises to 20 to 25% in pregnant women. It is the second cause of acute hepatitis in the world. It was originally said that there was no evolution to chronicity, but this is now known to be untrue. Cases have recently been observed in patients who are viraemic for longer than 6 months (which defines a chronic infection), with persistence of IgM's and/or IgG's for several months (up to 3 years in one renal transplant patient with disturbance of the hepatic balance). This means that hepatitis E can become chronic, particularly in immunosuppressed patients (organ transplantation) and develop into cirrhosis, sometimes in less than a year.

INDICATIONS FOR MEASUREMENT

Search for HEV infection in cases of reported acute (or fulminating) hepatitis, or unexplained hepatic cytolysis (ALAT more than 3 times normal). Carry out HEV serology (IgG + IgM) and HEV PCR in blood (+ sequencing if positive).

INFORMATION

SAMPLE

PCR: On EDTA plasma or stool sample in a coproculture container. Freeze to -20 °C or -80 °C.

For serology: Serum (dry tube, with or without phase separator) stable for several days at + 4 °C.

QUESTIONS FOR THE PATIENT

Have you travelled in the last 2 months? Eaten pig or wild boar meat? Profession (contacts with animals)?

DIAGNOSTIC METHODS

Diagnosis is direct and indirect.

The acute hepatitis is associated with an ALAT serum peak; the virus is present in the liver and stools; the RNA is detectable in the blood and stools and IgM's are detectable. One month later, the RNA is no longer detectable, the ALAT level returns to normal, IgM's disappear more often than not and IgG's, once they have appeared, persist for a variable period.

■ DIRECT DIAGNOSIS

Diagnosis by real-time PCR on EDTA-plasma or stool sample. HEV is detectable in stools and blood approximately 15 days after contamination and for a period of 4 to 5 weeks (up to 3 months in stools).

■ INDIRECT DIAGNOSIS

Serological diagnosis by the ELISA method.

Anti-HEV IgM's appear in the blood approximately 4 weeks after contamination, at the same time as transaminase levels rise, and disappear 2 to 3 months later. IgG's are detectable from 5 weeks and usually persist (**NB:** IgG's sometimes remain negative in immunosuppressed subjects). Rapid HEV IgM tests are now commercially available *(MP Biomedicals)*; from 20 to 50 µl of serum, they give a result in approximately 15 minutes, with satisfactory sensitivity and specificity.

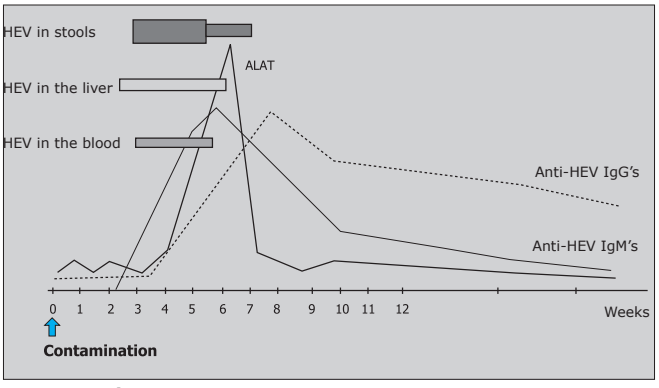

Figure 2 : Évolution des marqueurs du virus de l'hépatite E

TREATMENT AND PROPHYLAXIS

In the absence of an active principal against HEV, treatment in the acute phase remains symptomatic, taking care to avoid any medication which might induce additional hepatotoxicity. In the fulminating or subfulminating forms, liver transplantation, where the technique is available, has indications which can be considered similar to those present in the same circumstances for patients with hepatitis A.

Prophylaxis is based on the hygiene of water and effluents, and on cooking pork meat and sausages at high temperature. Vaccines are currently being developed.

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HEPATITIS G

DEFINITION

In 1967, an American surgeon aged 34, known only by his initials "GB", contracted a viral hepatitis of unknown aetiology. Injections of his serum into chimpanzees or tamarins produced an acute hepatitis. In 1995 and 1996, two groups independently used the techniques of molecular biology to characterise the viral agent responsible. A group from the Abbott Laboratory identified 3 viral types and named them GBV-A, GBV-B (serum from surgeon GB) and GBV-C (serum from different patients). Another group from Genelabs Technologies described the complete sequence of a virus which was named HGV. It was quickly seen, after analysing the viral genome sequences that GBV-C and VHG were identical, corresponding to 2 isolates of the same virus. We can therefore conclude the existence of 3 distinct but closely-related viruses i.e. GBV-A, GBV-B and GBV-C or HGV.

These are enveloped viruses of approximately 9400 nucleotides with single-strand RNA, possessing structural genes for the nucleocapsid (core) and envelope (E1-E2), non-structural genes NS1 to NS5, NS3 coding a helicase and a protease, NS5 a polymerase RNA and a replicase, and non-coding regions situated at the extremities 5′ and 3′. The capsid protein is defective for the HGV, GBV-B and GBV-C viruses and complete for GBV-A. These structural features enable the viruses concerned to be classified in the Flaviviridae family, and are distant cousins of the hepatitis C and yellow fever viruses.

Synonyms: Family: *Flaviviridae*; Genus: Hepacivirus; single-strand RNA viruses of positive polarity, viral hepatitis non-A, non-B and non-C transmissible by the parenteral route; HG-V, hepatitis G virus (HGV).

SYMPTOMS

The hepatitis G virus is essentially transmitted through blood. It is not found in patients who have never been transfused or are not at risk, such as intravenous drug abusers. Its transmission therefore seems very close to that of the hepatitis C virus, although other modes of transmission, not yet described, cannot be excluded at the present time. Note that possible mother-child transmission has been described in certain cases.

Studies of GBV-C/HGV prevalence conducted in blood donors with elevated transaminases show a prevalence of 1.5% to 4%, against 0.8% to 2% in blood donors with normal transaminase levels. In the drug addict population, prevalence reached 10% and is between 12% and 15% in polytransfused subjects. GBV-C/HGV is thought to represent 0.4% of acute hepatitis cases and 6% in the case of non-A-E hepatitis.

Acute GBV-C/HGV infection is generally benign. In biochemical terms, transaminase levels remain normal, although they may temporarily be moderately elevated. If GBV-C/HGV RNA persists for years after the subject becomes infected, no evolution towards chronic disease has been demonstrated.

SEARCH INDICATIONS

Diagnosis of *non-A*, *non-B* and *non-C* viral hepatitis.

INFORMATION

SAMPLE

Serum or plasma (EDTA) freshly separated from blood components.

SAMPLE STORAGE AND TRANSPORT

For serological tests

If the analysis has to be deferred, the serum or plasma must be frozen rapidly and transported if necessary without interruption of the cold chain.

For molecular tests

Once the sample has been taken, centrifuge immediately at 4000 rpm for 20 minutes, and then collect the plasma in tubes to be frozen at -20° C within 4 hours of collection. Store and transport the sample at –20 °C.

VIROLOGICAL DIAGNOSIS

Indirect virological diagnosis is based on the detection of specific antibodies directed against the viral envelope (anti-E2). Anti-E2 antibodies appear 3 to 6 months after the acute episode.

Direct virological diagnosis is based on molecular tests (RT-PCR) which detect the presence of the viral genome.

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HERPES SIMPLEX

DEFINITION

The *Herpes simplex* viruses (HSV) are part of the *Alphaherpesvirina* sub-family of the *Herpesviridae* family. Like the other herpes viruses, the virions are approximately 150 nm in size and enveloped. The genome is linear dual-strand DNA and viral replication is intranuclear. The two serotypes which exist (HSV-1 or HHV-1 and HSV-2 or HHV-2) are genetically homologous to more than 50%. After the primary infection, HSV's persist in a latent state, as do all the *Herpesviridae*. The latency site is located in the trigeminal ganglia in the case of HSV-1 and in the sacral ganglia for HSV-2. Reactivation occurs following deterioration in general health (fever, infectious disease or immunosuppression) or in response to certain stimuli (UV radiation, stress, etc.). The presence of antibodies does not prevent reactivation or infection by a different HSV serotype.

INTRODUCTION

EPIDEMIOLOGY

The virus reservoir is strictly human: HSV's are relatively delicate in the external environment. More than 80% of adults have antibodies against HSV-1. This virus usually causes cutaneomucosal lesions of the oropharyngeal region and the eyes, with transmission taking place by direct contact.

HSV-2 is classically responsible for genital herpes, a sexually transmitted infection whose seroprevalence varies between 10 and 50%, according to the populations considered (age, sexual behaviour and socio-economic level). The distinction between HSV-2 genital herpes and HSV-1 non-genital herpes should be kept in perspective, since 50% of genital herpes is due to HSV-1, particularly in adolescents and young women. Neonatal transmission is possible and occurs during birth if the mother is carrying HSV in her genital passages.

SYMPTOMS

Primary infection by HSV-1

In most cases, this is asymptomatic or non-specific. In children, however, it may produce gingivostomatitis, angina and ulcerated vesicopustular lesions, accompanied by fever and eating difficulties. A primary infection with ocular localisation oculaire is characterised by keratoconjunctivitis accompanied by pre-auricular adenopathy, in which case recovery is slow (1 month).

Primary infection by HSV-2

This is not apparent in two thirds of cases. The typical clinical expression is an acute genital infection, with localised or widespread vesicopustular lesions which may be accompanied by local pain, dysuria, fever and inguinal adenopathy. The symptoms persist for 2 to 3 weeks.

Herpes reactivation

Viral reactivation or recurrence is generally located at the same site as the primary infection. In the case of HSV-1, the outbreak usually takes the form of a "cold sore", which is annoying but benign. Ocular recurrence, because of its repetitive nature, may lead to irreversible corneal damage with a risk of blindness. Cutaneous herpes, located in a hand or finger, is less common and prefers individuals who are subject to allergies.

The clinical signs of a genital herpes recurrence are less striking and of shorter duration than during the primary infection, usually involving a discrete rash of genital vesicles or even a simple irritation.

It should be noted that while herpes recurrences are frequently not obvious, viral shedding is real and contributes to spreading the infection.

Herpetic meningoencephalitis is a very serious form of HSV-1 infection which mainly affects adults aged 50 and over. Although relatively rare, it is the leading cause of acute necrotising encephalitis in temperate latitudes. It results either from a reactivation or a recontamination by a viral strain which is antigenically different from the resident strain (exogenic reinfection). The onset may be sudden (fever, headache, confusion or epileptic attacks) or more gradual. Imaging shows unilateral or bilateral localised lesions in the temporal or frontal lobes. Hyperlymphocytosis of the cerebrospinal fluid is variable and not always obvious. In the absence of treatment, the necrosis caused by the virus and the intensity of the inflammatory reaction (cerebral oedema) may lead to death or induce severe neurological damage.

Mollaret's meningitis is a lymphocytic form which recurs at intervals of a few months or years and is always benign. This type of recurrence is particular to HSV-2.

Neonatal herpes

The risk is high if a primary genital herpes infection occurs during the last month of pregnancy, particularly at term or just beforehand, even in the absence of visible lesions. The baby is infected in the event of early membrane rupture and in particular during birth. The illness may present itself in the early days of life, in the form of a very serious septicaemia (fulminating hepatitis, necrotising meningoencephalitis and pneumopathy), which is often fatal or has grave consequences. If the infection occurs later, we essentially observe isolated encephalitis. In the best case, neonatal infection is localised in the skin, the oropharynx and/or the eye. There are usually numerous recurrences.

Neonatal herpes can be caused by HSV-1 or HSV-2. The serious forms are believed to be more often due to HSV-2.

Herpes infection in the immunosuppressed

The seriousness of the infection depends on the degree of immunosuppression. Recurrences are, in general, more frequent and more extensive. Oesophageal or pulmonary dissemination, or hepatitis, may be observed. Encephalitis is rare.

SEARCH INDICATIONS

Aetiological diagnosis of cutaneomucosal, genital or ocular lesions.

Aetiological diagnosis of a neurological syndrome (meningoencephalitis).

Exploration of vesicular angina.

Exploration of a pneumopathy or digestive symptoms in immunosuppressed subjects.

Monitoring of pregnant women.

Aetiological diagnosis of neonatal pathology (septicaemic form and localised form).

INFORMATION

SAMPLES

Depending on the symptoms, numerous samples may be taken e.g. cutaneomucosal samples (skin, nasopharynx, eye and genital region in men and women), bronchial secretions, bronchoalveolar irrigation, neonate whole blood collected on EDTA or citrate, urine, CSF, liver biopsy, etc.

Searching for antibodies is carried out on non-haemolysed serum.

QUESTIONS FOR THE PATIENT

Clinical signs, onset of symptoms, possibility of recurrence, immune status, possible HIV seropositive status, pregnancy, abnormalities on imaging?

SAMPLE STORAGE AND TRANSPORT

HSV's are relatively fragile in the external environment. Samples must be refrigerated and delivered rapidly to the laboratory (a few hours if possible, and less than 3 days). Swab samples (eye, throat or cervix) must be placed in a transport medium. Freezing is recommended for CSF to be tested for viral DNA, with serum being kept at +4° C if the laboratory is far from the sampling location.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

Histocytological examination

This involves staining to reveal large round cells presenting nuclear and cytoplasmic inclusions (Tzanck cytodiagnosis, Unna's bodies). This examination is not specific but may be useful for guidance.

Detection of viral antigens in sample cells

This involves the use of immunofluorescence, and immunoperoxydase if appropriate, e.g. on cell smears taken from suspect clinical lesions, or by biopsy. In most cases monoclonal antibodies are used to differentiate between HSV-1 and HSV-2.

Detection of the viral genome

This is usually performed by conventional or real-time PCR. Depending on the choice of primers and probes, it can either detect one of the viruses in the human herpes virus group (consensus PCR HSV-varicella-CMV-EBV-herpes virus 6), or HSV-1 and HSV-2, or differentiate directly between HSV-1 and HSV-2.

Viral isolation

HSV's are very easily cultured in numerous human and animal cell lines. The appearance of cell lesions is generally rapid (a few hours to a few days, depending on the virus concentration on the sample). The search can be accelerated by centrifuging the sample on the cellular monolayer, then after 36 to 48 hours revealing the presence of HSV by a marked monoclonal antibody (fluorescence or peroxydase).

INDIRECT DIAGNOSIS

Testing for IgG and IgM antibodies is currently carried out using ELISA. Seroconversion indicates the existence of a primary infection. The presence of antibodies at a generally stable level indicates an earlier contact with HSV, which implies as a corollary that the subject is carrying the virus in its latent state. Detection of IgM antibodies is an indicator of a recent primary infection or an active infection. One should always, however, be suspicious of a non-specific reactivity (e.g. infection by EBV or a varicella-zoster virus), particularly at low levels. Finding anti-herpes antibodies in the CSF and a calculation of the viral load can be helpful in retrospectively determining a meningoencephalitis.

In spite of the extensive antigenic commonality between the two HSV's, it is possible to distinguish antibodies due to HSV-2 using ELISA, immunoblot or immunochromatography. The antigen used is the gG2 surface glycoprotein, believed to be specific to HSV-2.

INDICATIONS AND INTERPRETATIONS

Diagnosis of a primary herpes infection

The absolute criterion is the detection of the virus or one of its components in a lesion, followed up by strain typing and the detection of seroconversion in two successive serum samples. The presence of IgM antibodies is a warning sign, although these may be detected during a secondary infection. Account should also be taken of the possibility of a non-specific positive result.

Diagnosis of an active infection

Serology has little to contribute in general. A search for the virus is always required, this being more sensitive and more specific than the detection of herpes antigens.

If neurological symptoms are present, testing for viral DNA in the CSF (or brain tissue) is the preferred technique, due to the fact that virus culture from CSF is often slow or fruitless.

Comparative serology in serum and CSF provides a retrospective argument. Note that a high level of alpha-interferon in CSF may suggest a neurological viral condition, without providing a specific aetiology.

In a woman with suspected genital herpes at the end of pregnancy, even if there are no clinically visible lesions, the detection of viral DNA enables the necessary precautions to be taken during childbirth. The baby will be subject to clinical and virological monitoring (oropharyngeal and ocular samples) for the first two weeks.

The simple detection of the virus or its components at an anatomical site or in a biological fluid should also be seen in perspective and discussed according to the clinical context and immune status. It may indeed be an "exit" infection which is the consequence and not the cause of the main pathology.

Determination of serological status with respect to HSV

The detection of antibodies against HSV-2 may be of interest inasmuch as the consequences of neonatal infection due to this serotype are considered to be more serious than those of HSV-1. It must however be specified that the absence of antibodies against HSV-2 does not exclude the existence of HSV-1 genital herpes. The determination of herpetic serological status is not currently recommended as a systematic step for pregnant women.

TREATMENT

CURATIVE

Anti-herpetic treatment is the first successful antiviral therapy. It has considerably improved the prognosis for meningoencephalitis and for infections in new-born babies and immunosuppressed subjects. This involves the use of Acyclovir or Valacyclovir, which has better bioavailability. Unfortunately, the emergence of genotypically resistant viral strains has been observed.

Antiviral treatment has little effect on herpes recurrences, genital or otherwise.

PREVENTIVE

In spite of a great deal of experimental work, there is not yet a vaccine against HSV.

During a clinically active herpes infection, the classical hygiene measures must be applied. Hospital, crèche or maternity personnel with herpes lesions of the fingers must not be in direct contact with children. If labial herpes is present, a mask must be worn.

If there is a confirmed risk of genital herpes, the use of a condom is strongly recommended.

If there is a primary genital infection in a pregnant woman, a caesarean section is necessary. Treatment with Acyclovir IV must be administered to mother and baby if it is feared that the mother may have herpetic hepatitis.

A recurrence of genital herpes in the week before term means that there must be no hesitation in performing a caesarean section, although the prescription of Acyclovir is the subject of debate.

In the most common case of a pregnant woman (or her partner) having a history of genital herpes, conventional childbirth is permissible. In both these cases, it is essential to test for asymptomatic viral excretion in the mother (major and minor labia and endocervix) and to disinfect the genital passages with Povidone-iodine before parturition, and to disinfect the skin of the neonate using an established method.

If there is no known genital herpes in the woman at the end of pregnancy, a careful gynaecological examination must be undertaken, testing for virus by culture or PCR if there is the slightest doubt.

FOR FURTHER INFORMATION

Prise en charge de l'herpès cutanéo-muqueux chez le sujet immunocompétent. Conférence de consensus, Société française de dermatologie; novembre 2001.

HIGH MOLECULAR WEIGHT KININOGEN

DEFINITION

High molecular weight kininogen (HMWK) is a glycoprotein, which acts as a coagulation co-factor. It is probably produced in the liver and its production is independent of vitamin K. Plasma concentrations are in the region of 70 mg/l (0.7 micromol/l).

Synonym: Fitzgerald factor, Flaujeac factor.

INTRODUCTION

HMWK is a cofactor for factor XIIa in surface-dependent activation of coagulation and is involved in fibrinolysis.

When the contact phase of coagulation is activated, initiated by binding to an electro-negative surface, factor XII auto-activates into factor XIIa. Factor XIIa then activates the conversion of prekallicrein bound to high molecular weight kininogen (HMWK) into kallicrein. Kallicrein then activates factor XII and hydrolyses HMWK into several fragments including bradykinin, which has hypotensive properties. Bradykinin also stimulates release of t-PA from the vascular endothelium. This is responsible for one of the pathways, which activates plasminogen into plasmin.

INDICATION FOR MEASUREMENT

HMWK deficiencies prolong the ACT (other coagulation tests, particularly the QT, are normal).

This contact factor is measured in isolated lengthening of the ACT corrected by adding control plasma in the absence of factor VIII, IX, XI or XII deficiency. The ACT is greatly increased if the reagent used contains silica or kaolin and less so if the activator used is ellagic acid. Lengthening of the ACT is only slightly corrected after incubating for 10 to 15 minutes compared to the direction obtained after incubating for 1 minute in HMWK deficiency whereas it is extensively corrected in prekallicrein deficiency. The diagnosis requires specific measurement of HMWK.

INFORMATION

■ SAMPLE

The sample is taken into 3.2% citrate (0.109 M) 1/10 (0.5 ml per 4.5 ml of blood). 3.8% citrated tubes (0.129 M) can be used.

A fasting sample is not necessary: a light fat free snack is permitted.

For further information, refer to the "General preanalytical conditions for haemostasis" section.

■ QUESTIONS FOR THE PATIENT

Current treatments, particularly unfractionated heparin, low molecular weight heparins, fondaparinux, hirudin and derivatives, rivaroxaban and dabigatran. These treatments may prolong the ACT *(cf "indications for measurement" section)*.

■ SAMPLE STORAGE AND TRANSPORT

Plasma can be stored for 2 to 4 hours at laboratory temperature, for 1 week at – 20°C, and at – 80°C beyond this time. It should be thawed quickly in a water bath at 37°C.

Transport: sample should be centrifuged, separated and frozen within 2 hours of sampling.

ASSAY METHODS

Chronometric assay (coagulation method).

REFERENCE VALUES

50 to 150%.

INTERPRETATION

HMWK deficiencies are transmitted as autosomal recessive conditions and do not cause any bleeding tendency even in profound deficiency.

However, reduced plasma HMWK concentrations may be associated with an increased risk of thrombosis. This has not however been demonstrated in clinical studies.

If HMWK deficiency is discovered a medical certificate must be produced and given to the patient to avoid further investigations if a prolonged ACT is subsequently discovered, and urgent surgery if necessary should not be delayed.

FOR FURTHER INFORMATION

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■ Abdelouahed M., Elalamy I., Samama M.M., *Physiologie de l'hémostase*, Encycl Méd Chir (Elsevier, Paris), Angéiologie, 19-0100,1997, 9 p.

HIPPURIC ACID - METHYLHIPPURIC ACIDS

DEFINITION

Hippuric acid is the main metabolite of toluene and the methylhippuric acids are xylene metabolites. Toluene and the xylenes (1,2-, 1,3- and 1,4-dimethylbenzenes) are aromatic hydrocarbons used as synthesis intermediates in the manufacture of many products, including solvents for paint and fuel additives. The utilisation of these toxic solvents is strictly regulated by French health and safety legislation (Threshold Limit Value = 150 ppm, i.e. 550 mg/m^3 for toluene, and 650 mg/m3 for the xylenes).

INTRODUCTION

After absorption, mostly via the lungs but also through the skin; toluene and xylenes rapidly spread in the blood and concentrate in lipid-rich tissues. Toluene is metabolised in the liver by cytochrome p450 monooxygenases to give benzoic acid which is in turn conjugated with glycine to yield hippuric acid. A very small fraction (<1 %) is converted into ortho-, meta- or para-cresol. Hippuric acid is excreted in the urine. Its clearance half-life is about 3 hours and all is eliminated from the body within 24 hours. Xylenes are metabolised in the liver to yield the corresponding methylbenzoic acids and then methylhippuric acids. About 80% of the xylenes absorbed are excreted in the urine as methylhippuric acids within 24 hours. Methylhippuric acid excretion is a two-phase process with respective half-lives of 3.6 hours and 30 hours.

INDICATIONS FOR MEASUREMENT

Biological Monitoring for exposure in at-risk populations:

– The hippuric acid concentration in the urine gives a measure, in a group of workers, of exposure to toluene the day of the sampling and the day before, if exposure was moderate or heavy (> 50 ppm). For low-level exposure and at the individual level, this marker is unreliable because of metabolic differences between different individuals, low specificity and interference; assaying ortho-cresol in the urine is more sensitive.

– Measuring methylhippuric acids in the urine at the end of the work shift gives a specific estimate of xylene exposure that day—correlating well with the degree of exposure. More of the meta-isomer is excreted in the urine than the other isomers.

– Sodium benzoate is used to treat urea cycle enzymopathies and, in this context, assaying hippuric acid can be used to monitor the efficacy of treatment.

INFORMATION

SAMPLE

Urine should be collected over the last four hours of the work shift, into an unused recipient made of polyethylene or polystyrene.

SAMPLE STORAGE AND TRANSPORT

– 15 days at +4 °C.

– Transport at room temperature if the transport time is short; otherwise, at +4 °C.

ASSAY METHODS

High performance liquid chromatography (HPLC) with UV detection.

UNITS AND REFERENCE VALUES

– Hippuric acid in the urine

< 1.5 g/g creatinine in the general population

< 2.5 g/g creatinine at the end of a shift (French guidelines)

< 1.6 g/g creatinine at the end of a shift (Biological Exposure Index, ACGIH BEI).

– Methylhippuric acids: French guidelines and Biological Exposure Index (ACGIH BEI) < 1.5 g/g creatinine at the end of a shift.

INTERPRETATION

INTERFERENCE FACTORS

Hippuric acid is also excreted in the urine after the breakdown of other compounds, including ethylbenzene, styrene, dietary benzoic acid (a common preservative) and certain amino acids. A number of factors can interfere with its metabolism, including enzyme-inducing medicinal products and enzymatic inhibitors such as styrene, benzene, acetone, ethanol, trichloroethylene and alcohol.

When interpreting the result of a methylhippuric acid assay, the factors to be taken into account are: possible exposure to interfering compounds, notably enzyme-inducing medicinal products, aspirin, ethylbenzene, toluene and trichloroethane which will all inhibit its metabolism; the possibility of absorption through the skin; and physical exertion.

FOR FURTHER INFORMATION

■ Institut National de Recherche et de Sécurité (INRS). www.inrs.fr

■ *Toluène*. Fiche toxicologique N° 74 de l'INRS (Institut National de Recherche et de Sécurité). www.inrs.fr

■ *Xylènes*. Fiche toxicologique N° 77 de l'INRS (Institut National de Recherche et de Sécurité). www.inrs.fr

HISTAMINE

DEFINITION

Histamine is a haptene and it is the main mediator of the early phase of immediate hypersensitivity.

INTRODUCTION

Histamine is produced in the body from the decarboxylation of histidine by histidine decarboxylase, and then stored in the metachromatic granules at concentrations which are 2 to 10 times higher in mastocytes than in basophils.

The circulating fraction is rapidly metabolised by the liver, filtered by the kidney and found in urine with its metabolites. This is the predominant pathway in healthy people and is replaced by the diamine oxidase (DAO) pathway when this is present in the placenta, intestine and kidney. Histamine is catabolised abnormally rapidly by DAO in the 2nd trimester of pregnancy and during heparin treatment (activation of DAO is proportional to heparin concentrations – NB: low molecular weight heparins have not been studied).

Contact with an allergen provokes mastocyte and basophil degranulation and massive histamine release, which acts on H1 and H2 receptors on the surface of cardiovascular, respiratory, gastro-intestinal and skin cells.

Histamine release is objective evidence of cell degranulation without indicating the mechanism involved. This can be immunological, with the production of specific IgE in anaphylactic reactions, or non-immunological caused by pharmacological agents or by complement activation in anaphylactoid reactions.

INDICATIONS FOR MEASUREMENT

– Anaphylactic reaction: Histamine is released rapidly depending on the severity of the reaction but not in all cases (only in 56% of patients). In order to improve its diagnostic sensitivity it is recommended that several measurements are made during the reaction and that these are combined with the measurement of tryptase. Plasma histamine appears to be a more sensitive marker than tryptase in mild and moderate reactions.

– Hospital-based provocation tests: Sequential samples during a provocation tests to an allergen (hymenoptera venom, foods and drugs) are used to construct a histamine release curve and assess the patient's response to the allergen tested. The histamine peak is seen very rapidly (from the first minute in venom reactions) and correlates with the severity of the reaction.

– Histamine release tests (HRT): A dose-response curve is constructed to study the in-vitro cell reaction to an allergen. This is not a first line diagnostic test but is useful if the measurement of circulating specific IgE cannot be performed in the absence of an allergen bound to a solid support. It is an aid to diagnosis in allergies, particularly hymenoptera venoms when the clinical picture is inconsistent with skin and/or laboratory tests. It forms part of the SFAR- French national society of anaesthesia and intensive care recommendations, in choosing subsequent anaesthesia in a patient who has previously developed an anaphylactic reaction.

INFORMATION

SAMPLE

Histamine: Blood collected into EDTA.

Histamine release test: Blood, which must be collected into heparin.

Histamine adsorbs onto glass. All glass materials must therefore be avoided.

Because of its short half-life in healthy people (102 seconds) and increased half-life in severe reactions (5 to 30 minutes), the sample may be taken as soon as clinical signs develop in relatively non-severe reactions (5 to 30 minutes) and can be delayed by 30 minutes to 2 hours in severe reactions.

Plasma is separated after centrifugation at + 4°C at 1000 g for 15 minutes and gently aspirated, avoiding the 2 cm above the clot as these contain leukocytes and platelets.

Haemolysis should be avoided as overestimation may occur. A linear reaction is seen between histamine concentration and haemoglobin.

STORAGE AND TRANSPORT

Histamine is stable in the presence of EDTA in blood overnight at + 4°C or for 2 hours at 20-25°C.

Plasma histamine concentrations remain stable at + 4°C for 48 hours and for several years at–20 °C. If the sample is to be sent, freeze within 4 hours of sampling if the transport time is > 48 h.

If the blood is intended for HRT it must be kept at 20-25°C for a maximum of 24 hours.

ESSENTIAL INFORMATION

Sampling time after the onset of symptoms.

Drugs administered.

ASSAY METHODS

Many non-immunological assay methods have been developed which meet the various essential requirements depending on context (routine, expert assessment or research) and fields of application (allergy, medicine, toxicology or neurobiology); biological, chemical, radioenzymatic, chromatographic and fluorimetric, with many adaptations to make these more specific, together with gas chromatography coupled to mass spectrometry.

Radioimmunological and immunoenzymatic methods are used routinely.

REFERENCE VALUES

NORMAL EXPECTED VALUES

Plasma: 10 nmol/l or less.

Whole blood: 200 to 2000 nmol/l.

PATHOLOGICAL VALUES

Plasma histamine > 10 nmol/l.

FOR FURTHER INFORMATION

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HIV

DEFINITION

The two viruses (HIV-1 and HIV-2) responsible for chronic viral infection, which in the long-term causes the Acquired ImmunoDeficiency Syndrome (AIDS) were discovered in 1983 and 1986 respectively in France and were initially called LAV *(lymphadenopathy associated virus)* by Montagnier and HTLV III *(human T cell leukaemia/lymphoma virus)* by Gallo. The name HIV *(Human Immunodeficiency Virus)* was given definitively in 1986. The AIDS epidemic probably started in Africa in the 1970s and has spread to all continents, mostly affecting the developing countries.

■ VIROLOGICAL FEATURES

HIV-1 and HIV-2 belong to the *Retroviridae* family and *Lentivirus* genus, which cause slowly progressive diseases. The genome of the viruses consists of two identical, single stranded RNA molecules, contained in a cylindrical capsid, which also contains structural proteins and enzymes required for viral replication. The nucleocapsid is surrounded by a lipid bilayer or envelope carrying viral and cellular proteins on its surface, including the glycoproteins gp120 and gp41 for HIV-1 and gp105 and gp36 for HIV-2, obtained by cleaving a precursor, gp160. The viral gene contains three structural genes, *gag, pol* and *env*, and regulatory genes *(vif, vpr, tat, rev, nef, vpu* for HIV-1 and *vpx* for HIV-2) for expressing viral proteins. HIV replicates by transcribing the RNA into DNA using a viral enzyme reverse transcriptase (RT) and incorporating this DNA into the cell genome. HIV has tropism for cells expressing the CD4 molecule on their surface: *helper* T lymphocytes (CD4 + lymphocytes) and antigen presenting cells, such as monocytes, macrophages, microglial cells and Langerhans cells (dendritic cells). Infection of these cells results in the major immune dysfunction, causing the human immunodeficiency syndrome (AIDS).

The strains of viruses can be schematically classified by their preferential tropism for antigen presenting cells (viruses with macrophage tropism) or T lymphocytes (viruses with T lymphocyte tropism):

- viruses with macrophage tropism do not replicate to a large extent and are not particularly cytopathogenic. They enter the cells using the CCR5 co-receptor and as a result are called R5. These are found mostly in asymptomatic people

- viruses with T lymphocyte tropism replicate extensively in activated CD4 + T lymphocytes producing a cytopathogenic effect. They use the co-receptor CXCR4 and are called X4. These are found mostly in symptomatic people.

■ EPIDEMIOLOGY

■ Transmission routes:

The main routes of HIV transmission are:

Sexual transmission, which represents almost 90% of worldwide infection with increased risk in homosexual intercourse. Heterosexual intercourse can also infect, with a higher risk in the direction man → woman.

Parenteral transmission:

– Intravenous drug addiction: the risk of transmission from syringe sharing is estimated to be 0.67%

– Occupational accidents with contaminated materials, which are estimated to involve 0.32% of such accidents. The risk of transmission is influenced by the size of the inoculum, the depth of the needlestick injury, the type of penetrating object involved (hollow needle), whether wearing gloves and viral load

– Blood transfusion: the residual risk from blood donations in France is now 1 per 2.5 million donations, since the introduction of viral genome screening combined with serology.

Materno-foetal transmission (MFT): the risk of spontaneous transmission (i.e. without treatment) is estimated to be 15-20%. Breast-feeding carries an additional risk of approximately 10%. HIV seropositive mothers in industrialised countries are strongly advised therefore not to breast-feed their babies . Materno-foetal transmission occurs *in-utero* in a third of cases (up to 95% in the last 4 weeks), and in two thirds of cases on the day of birth. The risk of HIV transmission has been considerably reduced thanks to administration of AZT from the 14th week of pregnancy. Combining a planned caesarean section (and therefore without initial rupture of membranes) with AZT can reduce the risk of viral transmission to 1-2%. Sexual transmissibility of HIV-2 appears to be five times less than HIV-1 and materno-foetal transmission to be in the region of 0.6%.

The expert group recommends that triple therapy with an association of two Reverse Transcriptase Nucleoside Inhibitors (RTNI) and a Protease Inhibitor (PI) be used to prevent MFT and that the anti-retroviral treatment is continued in women treated before their pregnancy if it has been effective and well tolerated (observing the contra-indications).

■ Geographical distribution

HIV infection affects most of the planet and is due principally to HIV-1. HIV-2 is still localised, mostly in West Africa. The majority of people infected with HIV (95%) live in developing countries (Africa, Asia, Latin America and the Caribbean). One of the key features of the HIV genome is its wide variability. HIV-1 isolates have been classified into two main groups: M (for *major*) and a minor group O (for *outlier*). The M group is divided into nine subtypes or clades (A, B, C, D, F, G, H, J and K), the E and I subtypes, which were initially described actually being recombinant viruses. Because of patients becoming co-infected with different subtypes of HIV-1, recombinant viruses have emerged and are called *circulating recombinant forms* or CRF.
HIV-2 is also divided into several subtypes (A to E). This genetic variability is also seen in each subtype in different people and in the same person with many related strains of virus.

SYMPTOMS

The clinical progression of HIV infection involves three successive phases:

1) Primary HIV infection is symptomatic in 50 to 70% of cases. Symptoms develop between 1 and 6 weeks after infection. The usual clinical picture is one of a non-specific

mononucleosis syndrome, which may not be noticed. This is accompanied by intense viral replication, which is then controlled by the immune system

2) The latent clinical phase which lasts for an average of eight years. Persistent viral replication and progressive deterioration in the immune system occurs during this phase. Patients are asymptomatic or pauci-symptomatic

3) The symptomatic stage. At this advanced stage, immunosuppression predisposes to infectious and/or neoplastic complications.

Three clinical categories have been defined since 1993: A, B and C, stratified according to CD4 count. The clinical features of stage C define AIDS. These are major opportunistic infections, HIV encephalopathy, HIV related cachexia and neoplasia (Kaposi sarcoma, non-Hodgkin's lymphoma and invasive cervical carcinoma).

SEARCH INDICATIONS

– In the presence of clinical signs suggestive of primary HIV infection

– In possible occupational or non-occupational HIV exposure, such as after unprotected sexual intercourse

– Recommended at the start of pregnancy and before surgery.

Doctors must inform the person that the test is being requested and the person must give their agreement in France. Patients must also be informed of the significance of the test and counselled in advance about the result.

INFORMATION

SAMPLE

– Serology (Antibody, p24 Ag): serum or plasma (EDTA or citrate)

– PCR: whole blood withdrawn into EDTA, CSF (1 ml)

– Proviral DNA: EDTA whole blood

– Genotyping: antiviral resistance profile: EDTA plasma (2 x 2 ml).

SAMPLE STORAGE AND TRANSPORT

For serological tests: separated serum can be stored for several days at + 4°C. Transport at + 4°C.

For molecular tests: the sample (EDTA whole blood) must be centrifuged at 4000 rpm for 20 minutes within 4 hours of sampling and the plasma then collected into tubes which are frozen at -20°C. CSF must be frozen within an hour of sampling. Store and transport the samples frozen at – 20°C.

Proviral DNA: store and transport at + 4°C.

Genotyping: freeze plasma separated within an hour of sampling. If the sample is to be transported, state the last known viral load. CD4/CD8 count and different antiviral treatments being taken.

LABORATORY MARKERS OF HIV INFECTION

The laboratory markers widely used from a blood sample are:

– anti-HIV 1+2 antibodies (Ab) using screening and confirmatory serological methods

– p24 antigen (p24 Ag) using immuno-enzymatic methods (ELISA)

– HIV RNA using molecular biology techniques

– Testing for pro-viral DNA and isolating the virus by culture are only performed in specialist laboratories.

KINETICS OF DEVELOPMENT OF THE DIFFERENT MARKERS OF HIV INFECTION

The viral replication is intense in primary infection and the HIV-RNA is the first marker to appear, between the 7th and 14th day after infection (average 10 days), rapidly reaching a peak, which seems to vary between individuals (10^2 to 10^7 copies/ml of plasma). Without treatment replication decreases as a result of the immune response and the viral load (viral RNA) stabilises from 4 – 6 months during the clinically silent years.

p24 antigenaemia can be detected between the 10th and 26th day of infection (average 14th day) and becomes negative over 3 to 4 weeks. When it recurs, often years after primary infection, it indicates intense viral replication.

Finally, serum antibodies can be detected with the most sensitive ELISA tests between 15 and 45 days after infection and remain at high titres throughout the infection.

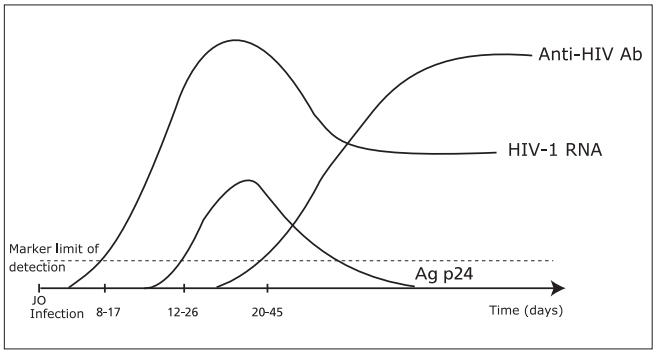


Figure 1. Diagrammatic kinetics of virology markers during the early phase of HIV-1 infection.

SEROLOGICAL SCREENING TESTS

Testing for anti-HIV Ab is the method of choice to diagnose HIV infection in someone over 18 months old. Since the development of the first screening test (in June 1986) considerable advances have been made and the sensitivity and specificity of the reagents have improved year by year through changes in methods from viral proteins (1st generation) to recombinant peptides and proteins (2nd generation), indirect ELISA *(Enzyme Linked ImmunoSorbent Assay)* to direct or sandwich ELISA (3rd generation) and detection of anti-HIV 1/2 antibodies to combined screening for these antibodies and the p24 antigen (4th generation).

All of the kits available have now become very reliable in detecting patients infected with groups M and O HIV-1 and HIV-2. Anti-HIV Ab screening uses either an ELISA method or a "rapid one-off" method with immunochromatography on various types of support (nylon, plastic membrane, etc.). The recommendations are that a combined HIV Elisa test (Anti-HIV Ab and p24 Ag) be used with a p24 Ag limit of detection, no greater than the minimum threshold required by the European Regulations of 2 IU/ml (WHO NIBSC 90/636 panel). If this test is positive, a confirmatory test (Western blot or immunoblot) should be triggered by the laboratory specialist, on the same

sample, in order to establish the specificity of the anti-HIV Ab present in the initial serum. If the confirmatory test is equivocal or negative, p24 Ag or viral load may be measured at the initiative of the laboratory specialist.

If the confirmatory test is positive, the presence of HIV1/HIV2 Ab or p24 Ag is only confirmed in a person after a screening test has been performed on a second sample, using an identical or different reagent to the one used in the initial test. HIV infection is only confirmed if the result is positive on a second sample. *(Fig 2 cf HIV infection diagnostic algorithm)*.

■ CONFIRMATORY TESTS

Western blot (WB) is used to visualise antibodies against the gene proteins env, gag and pol. The other available confirmatory test, immunoblot (IB) uses recombinant proteins and/or synthetic peptides.

Criteria for interpreting Western-blot:

Unequivocal seropositivity: A minimum of 2 bands in the envelope antigens (generally gp120 and gp160) and one band in the antigens from the *gag* or *pol* gene.

Probable seropositivity: either one p24 band and one gp160 band or 2 *env* bands present.

Profiles to retest: isolated gp160, isolated p24 or isolated p34. In these latter two cases, HIV-2 Western blot must be performed. It is essential that a second sample is taken 15 days later to demonstrate seroconversion, as other bands will appear with seroconversion and seropositivity criteria will be present. Conversely, a stable profile in both serum samples taken apart, without p24 antigenaemia suggests a cross-reaction with some viral proteins, without the person actually being infected by HIV. An incomplete profile with poor reactivity against gp120 and gp41, or a p24 associated with p31 in isolation, suggest HIV-1 O infection.

Whilst the confirmatory tests (WB or IB) offer excellent specificity they are less sensitive than the screening tests and very recent seroconversion detected by ELISA may produce no signal in the confirmatory test.

■ P24 Ag

The p24 antigen (p24 Ag) can be detected in serum or plasma using immunocapture ELISA techniques. The limit of detection of the method used must be no greater than the minimum level required by the European regulations of 2 IU/ml (WHO NIBSC 90/636 panel). All positive reactions must be confirmed by a neutralisation test using a specific monoclonal antibody.

■ VIRAL LOAD OR QUANTIFICATION OF PLASMA HIV-RNA

The plasma HIV-RNA quantification method widely called measurement of viral load is performed using commercially available standardised molecular biology methods

The molecular biology methods used at present to measure plasma viral load in HIV-infected patients mostly involve real time PCR. .

Results are expressed as the number of copies/ml and log10 of the number of copies/ml. Only differences of more than 0.7 log units between two successive samples are significant. Inter-current infections or vaccination can produce a transient rise in viral load. Differences are seen between results obtained with the different kits. As a result, it is recommended that the same test be used to monitor plasma HIV RNA concentrations sequentially in the same patient.

DIAGNOSIS OF HIV INFECTION

■ DIAGNOSIS OF PRIMARY INFECTION

Primary HIV infection cannot be diagnosed by testing for antibodies, which are absent in this phase. It is therefore recommended that testing for anti-HIV Ab be combined with investigation for plasma p24 Ag or HIV-RNA. Plasma HIV-RNA methods however produce false positives for values close to the limit of detection and some viruses are not detected (HIV-2, HIV-1 variants). The diagnosis of primary HIV infection cannot therefore be made on the basis of any one of these methods. The first anti-HIV antibodies to be detected during sero-conversion are against envelope proteins (gp160 for HIV-1) and the capsid protein (p24 for HIV-1). The WB is equivocal at this stage and becomes positive a few days later with the progressive development of other antibodies. The kinetics with which the Anti-HIV Ab develop may be delayed when anti-retroviral treatment is started early with a positive result (plasma HIV-RNA or p24 Ag). If a primary HIV infection is suspected, the patient can be deemed "not to be infected" if antibodies have not developed after a period of 3 months.

■ DIAGNOSIS OF INFECTION IN THE NEWBORN

An accurate early diagnosis is required in children born to a seropositive mother because of the treatments available. Because maternal antibodies are present up to the age of 15-18 months old, no serological diagnosis can be made until this time. Beyond the age of 18 months the diagnosis is made in the same way as in adults (ELISA and Western blot).

Early diagnosis is based therefore on finding the virus on culture or identifying the pro-viral DNA by PCR or from plasma HIV-1-RNA. Three samples should ideally be taken from newborn babies: during the first week after birth (do not use cord blood), and then at 1 month and 3 months. For a child to be deemed to be infected, two consecutive samples must be positive by PCR or culture. p24 antigenaemia tested at birth is not sensitive in making the diagnosis, although if positive it is a poor prognostic indicator, reflecting very high viral replication.

■ DIAGNOSIS OF INFECTION IN PEOPLE OVER 18 MONTHS OLD

Most requests for HIV serology are not made in cases of suspected primary infection. The diagnostic approach is based on testing for anti-HIV Ab combined with p24 Ag and a WB in positive cases.

Figure 2 shows an algorithm for interpreting HIV serology tests, which takes account of the problems inherent to serology (labelling and separation errors) and interpretative difficulties and attempts to make the result as reliable as possible.

■ VIROLOGICAL DIAGNOSIS FOLLOWING POTENTIAL EXPOSURE TO HIV

In accidental blood exposure (ABE), whether or not occupational, or accidental sexual exposure, the risk of infection must be assessed as quickly as possible in the

patient by establishing the HIV status of the potential source. A rapid test can be used in this emergency situation (on the sample from the potential source) which, if positive is a strong argument in favour of offering immediate anti-retroviral prophylaxis to the exposed person. This test needs to be confirmed by a classical ELISA. If the potential source is HIV negative the exposed person does not need further laboratory monitoring for HIV unless ongoing seroconversion is suspected in the potential source.

VIROLOGICAL MONITORING OF INFECTED PATIENTS

Laboratory monitoring of HIV-infected patients is based on the CD4 lymphocyte count, measurement of viral load, measurement of anti-retroviral agents and determining the anti-viral resistance genotype. Tests to measure plasma viral load and the emergence of new anti-retroviral compounds have considerably changed the therapeutic management of HIV-infected people since 1996.

■ MONITORING UNTREATED PATIENTS

Regular close interval monitoring of clinical, immunological and virological indices is recommended for the first year in patients with a diagnosis of primary infection who are not treated from the outset: every month for the first three months then every three months for the first year to assess initial progression and to estimate the immunovirological steady state reached between three to six months after infection.

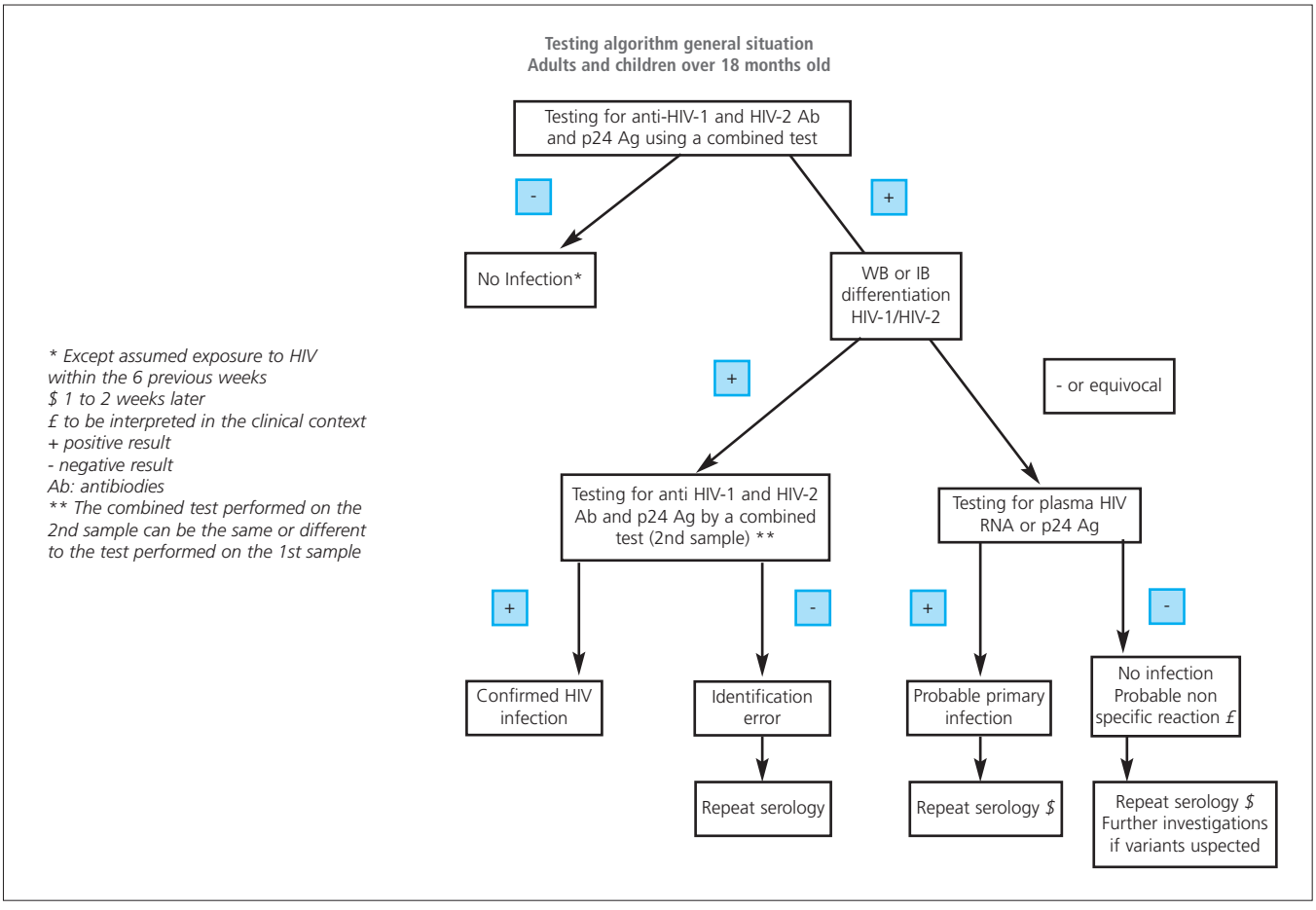
■ MONITORING TREATED PATIENTS

The recommendations state that patients with severe symptoms, particularly neurological and those with a CD4 count of less than 500/mm^3 at diagnosis should be treated early.

The aim of initial antiretroviral treatment is to reduce the plasma viral load to undetectable levels at 6 months using current methods with limits of detection ranging from 20 to 50 copies/ml. In order to achieve this and reduce the risk of HIV antiretroviral resistance, several different antiretroviral drugs need to be used in combination. The anti-retrovirals used must be chosen in a context of long-term treatment and alternatives must be considered if they are poorly tolerated or ineffective. This is the responsibility of the clinician with the agreement of the patient, taking account of the clinical and psychological context, potential side effects, adherence problems, past history, a concern to spare drugs for later treatment phases, potential interactions with other treatments and concomitant diseases.

After treatment is started it is recommended that a first measurement be performed at one month (viral load must fall by at least two log10 unit copies/ml) and then at the third month (viral load must be less than 400 copies/ml). If the viral

Fig. 2 Diagnostic algorithm for HIV infection



load rebounds on treatment, a change in treatment should only be considered after retesting the viral load. In patients whose treatment fails measurement of concentrations of nucleoside and non-nucleoside reverse transcriptase inhibitors or protein inhibitors should be measured in order to adjust doses if necessary in order to optimise treatment.

HIV ANTIRETROVIRAL RESISTANCE GENOTYPE TESTS

Routine methods use Sanger sequencing after RT-PCR amplification for genes coding for antiretroviral target proteins: reverse transcriptase, the protease or gp41. Commercial kits are available, the results of which correlate well with those obtained from classical sequencing. Algorithms to interpret the genotype must be consulted (e.g. the Stanford algorithm: http://hivdb.stanford.edu/). These are being continually updated depending on new published findings and the most recent clinical and virological studies.

The current recommendations state that a resistance genotype test should be performed along with viral sub-type determination in the initial assessment of any HIV infection and when treatment is started, in cases of possible re-exposure and in treatment escape. In this latter situation the choice of new antiretroviral combinations will be guided by the results of genotype testing, the patient's therapeutic history and measurement of anti-retrovirals. In these situations it is useful to determine the genotype for CCR5 tropism if prescription of a CCR5 co-receptor antagonist is being considered.

PHENOTYPE TESTS

These are not performed routinely, although they remain useful to assess new compounds and in multi-treated patients.

PROVIRAL DNA

Testing for proviral DNA is currently used to detect infection of newborn babies to seropositive mothers.

PLASMA ANTIRETROVIRAL MEASUREMENTS

The reasons for treatment failure can be explained at least in part by low trough protease inhibitor (PI) or NNRTI concentrations. This may be due to temporary lack of adherence (forgetting, stopping, travel, etc.) or due to adverse therapeutic interactions, including interactions between the anti-retrovirals. The blood sample should be taken pre-dose in the morning, observing the usual times between two doses. When treatment is started the samples must be taken at steady state between D15 and one month after starting for the PI and efavirenz and from the first month for nevirapine. The doses of antiretroviral drugs, time and date of last dose and time of date of the sample must be provided along with the sample for optimal interpretation. The methods used currently are chromatographic (high performance liquid chromatography, HPLC or liquid chromatography linked to tandem mass spectrometry, LC-MSMS).

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HLA CLASS I ANTIGENS

DEFINITION

The HLA *(Human Leukocyte Antigens)* system or major histocompatibility complex (MHC) is a group of transmembrane glycoproteins present on the cell surface. These proteins are the products of a group of genes located on chromosome 6. The system is divided into 6 classes of molecules, class I molecules coded for by genes from loci A, B and C, class II molecules coded for by genes from loci DP, DR and DQ and class III molecules (complement C2, C4 fractions, cytokines, etc). HLA system genes exhibit extensive polymorphisms, each locus having many different alleles. They are transmitted *en masse* by a codominant mendelial model and each allele for the different loci present on each chromosome is expressed.

INTRODUCTION

The HLA class I molecules (HLA-A, HLA-B and HLA-Cw) are transmembrane glycoproteins present on most nucleated cells. They are formed from an α chain, the extracellular region of which itself is subdivided into 3 domains: α1, α2 and α3. The sites of polymorphic variations are located in the α1 and α2 domains. The α chain is bound non-covalently to β2 microglobulin, a protein coded for by a gene located on chromosome 15.

Class I HLA molecules take part in initiation of the immune response, their role being to bind and present endogenous peptides (of viral or tumour origin) to "killer" cells in order to suppress these "infected" cells.

HLA molecules take part in selecting T clones and in forming the functional T repertoire. A number of autoreactive T clones are selected although have low affinity for autoantigens. Some situations in life (viral infections) cause breakage of the tolerance to "self" antigens which may explain the appearance and development of some autoimmune diseases. The pathophysiological mechanisms of autoimmune diseases are poorly understood and the association between HLA molecules and diseases is known but poorly characterised.

SEARCH INDICATIONS

– Testing for compatibility between donor and recipient in organ or bone marrow transplantation

– Paternity exclusion

– Testing for susceptibility to some diseases.

INFORMATION

SAMPLE

HLA class I: 5 ml of EDTA whole blood or ACD whole blood (citric acid, sodium citrate, dextrose: capped tube at room temperature).

HLA B27: 5 ml of EDTA whole blood at room temperature. A strict fasting sample is not essential.

SAMPLE STORAGE AND TRANSPORT

Serological analysis of HLA specificities using the microlymphocytotoxicity method and testing for HLA B27 antigen expression by flow cytometry is performed on "fresh cells". The maximum storage time is 48 hours.

The sample should be transported to the laboratory as soon as possible and is not to be frozen.

The B*27 allele can be tested for by using molecular biology techniques, in which case the storage time is 1 month.

QUESTIONS FOR THE PATIENT

The laboratory must always ensure that the request is not being made in the context of organ or bone marrow transplantation or testing for paternity exclusion. Sample taking and investigation of HLA molecules in these two situations are regulated in France and reserved for accredited centres (EFS, (French Blood Establishment), medico-legal haematology laboratory).

HLA and disease associations:

– Disease suspected by the doctor

– Past family history of autoimmune disease

– Immunosuppressants being taken (cause of non-reaction).

ASSAY METHODS

HLA class I

The reference method is microlymphocytotoxicity on a Terasaki plate. This involves isolation of T CD8+ lymphocytes followed by a lysis reaction on lymphocytes expressing the antigen recognised by specific immune sera, in the presence of rabbit complement. Addition of a living cell stage visualises the lysed lymphocytes and intact unlysed cells remain refringent. The proportion of living cells is assessed by reading the microtitre plates on the reverse phase microscope.

Molecular biology techniques, which have already been used to type HLA class II molecules, are developing to characterise nucleotide sequences of HLA class I genes. This involves a PCR amplification of a genomic DNA fragment and revealing on a strip using specific oligonucleotide probes (PCR-SSO *reverse* or *dot blot reverse*).

Specific testing for HLA B27 antigen

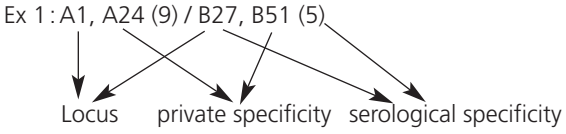
The most widely used method is flow cytometry. After lysing and removing red blood cells the lymphocytes are incubated with anti-HLA-B27 antibodies labelled with a fluorochrome (fluorescein). The labelled HLA-B27 lymphocytes are excited with a light source (laser) and the light fraction re-emitted at a specific wavelength is used to visualise and quantify them. If the response is equivocal, microlymphocytotoxicity or molecular biology (amplification of specific B*27 allele sequences) confirmation may be performed.

RESULTS AND INTERPRETATION

RESULTS

HLA class I: Serological nomenclature

The letter, A, B and possibly Cw, defines the locus. Serological specificity is indicated by a figure or number (HLA-A1, HLA-B27, etc.). Some serological specificities are subdivided into private specificities or sub-specificities (HLA-B51 (5) and HLA-B52 (5)).



Case n°. 1: The patient expresses antigens A1, A24, B27 and B51 on the cell surface (serological specificity 5).

Ex 2: A2, A3/B7,

Case nº. 2: The patient expresses antigens A2, A3, and either a double dose of B7 or B7 and another unidentified B antigen on the cell surface.

Ex 3: A1, A28/B8, B35

Case nº. 3: The patient expresses antigens A1, A68 (28) or A69 (28), B8 and B35 on the cell surface. Antigen A28 is subdivided into 2 private specificities: A68 and A69.

Ex 4: A2, A36 or -/B18, B44 (12)

Case nº. 4: The patient expresses antigens A2, A36 or A2 (and therefore a double dose) or another unidentified A antigen, B18 and B44 on the cell surface.

Specific testing for HLA-B27 antigen

The result is reported as presence (or positive) or absence (or negative) of HLA-B27 antigen expression.

INTERPRETATION

The association between HLA antigen and disease is measured in terms of relative risk (RR). The relative risk reflects the risk of developing a given disease in an exposed group (in this case expression of a given HLA antigen) compared to an unexposed group (non-expression of the same HLA antigen).

	Disease present	Disease absent
Expression of a given HLA antigen	A	B
Non expression of a given HLA antigen	C	D

RR = (A/A+B)/ (C/C+D)

When RR > 1: the risk of developing the disease is increased

When RR < 1: the risk of developing the disease is reduced (protective antigen against the disease)

Disease	HLA Antigens	Relative Risk	Other laboratory markers
Reactive arthritis	HLA-B27	**38**	Past history of Chlamydia urethritis or shigellosis, salmonellosis or yersiniosis
21-hydroxylase deficiency, early form	HLA-B47	**16**	17 Hydroxyprogesterone
21-hydroxylase deficiency, late form	HLA-B14	**40**	21 deoxycortisol, after Synacthène® stimulation 17 Hydroxyprogesterone
Haemochromatosis	Associations: HLA-A3/HLA-B7 or B14 HLA-A11/HLA-B5 or B35	**8**	Testing for Cys 282Y and H63D mutations Trasnferrin saturation coefficient
Behcet's disease	HLA-B51	**6**	-
Myasthenia	HLA-B8, HLA-A1, HLA-DR3	**3**	Acetylcholine anti-receptor antibodies + test to anticholinesterase (10 mg IV of Prostigmine)
Birdshot retinopathy	HLA-A29	**100**	-
Ankylosing spondylitis	HLA-B27	**88**	-
Reiter's syndrome	HLA-B27	**37**	Past history of Chlamydia urethritis or shigellosis, salmonellosis, yersiniosis
de Quervain Thyroiditis	HLA-B35	**14**	TSH, free T4, anti-thyroglobulin and anti-thyroid peroxidase antibodies
Anterior uveitis	HLA-B27	**10**	-

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HLA CLASS II ANTIGENS

DEFINITION

The HLA system was discovered in 1958 by Jean Dausset, George Snell and Baruj Benacerraf from the ability of an antigen (called Mac and then HLA-A2) to cause leuko-agglutination in the presence of antibodies from multiply transfused patients. This discovery won them the Nobel Prize in physiology in 1980. Genetically, the HLA (Human leukocyte antigen) system or major histocompatibility complex (MHC) consists of a group of genes (more than 200 identified) located on the short arm of chromosome 6 (6p21.3). Based on structural and functional differences the HLA system is subdivided into 3 genetic regions, one region formed from genes coding for molecules or HLA class I antigen, one region containing genes coding for HLA class II molecules. These two regions surround the HLA class III regions, which are formed from genes coding for molecules unrelated to the HLA molecules, but which are involved in the immune response.

Synonyms: HLA class II; Human leukocyte antigens class II ; MHC class II; Major histocompatibility complex class II; HLA class II gene; MHC class II Genes; Gene, Ir.

INTRODUCTION

HLA class II (or MHC II) molecules are coded for by a group of genes located in the peri-centromere position on the short arm of chromosome 6 *(figure 1)*. The class II region contains many genes and pseudogenes. The genes for the DP, DQ and DR *loci* are of practical clinical interest.

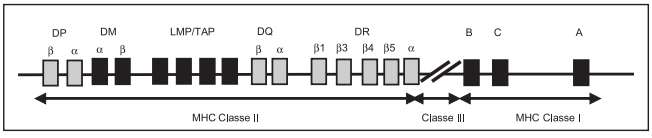


Fig. 1. Diagrammatic representation of the organisation of the MHC.

HLA class II molecules (DP, DQ, DR) are heterodimer transmembrane glycoproteins expressed on cell surfaces. The HLA class II molecules consist of an α light chain combined with a β heavy chain. The DP, DQ and DR loci contain a gene coding for the α chain (DRA, DPA1, DQA1 gene) and a gene coding for the β chain (DRB1, DPB1, DQB1) gene. The extracellular domain of the HLA class II molecules is formed from 2 loop domains (domains α1, α2 for the α chain and domains β1, β2 for the β chain). The α1 and β1 domains form a depression or furrow in which antigenic peptides bind.

The HLA molecules play a crucial role in the initiation and development of immunodependent T and B cell responses. HLA molecules act as the "presenter" of peptides to T CD4+ and T CD8+ lymphocytes. They are also the molecules recognised by Natural Killer cell receptors.

SEARCH INDICATIONS

Typing the HLA molecules has the following purposes:

- **In organ and bone marrow transplantation**

  HLA typing is essential for organ and bone marrow transplantations. For transplants in France, HLA system typing is a procedure reserved for the "Etablissements Français du sang (EFS)", (French Blood Establishments). As close a match as possible is sought for the A, B, C, DR, DQ and DP loci for bone marrow transplantations. Close compatibility is also desirable for kidney transplants. It is not always possible to obtain full compatibility between donor and recipient in other transplants because of the urgency of the transplant.

- **In disease associations** (risk of susceptibility to autoimmune and infectious diseases)

  The HLA system is used in autoimmune diseases as a marker of susceptibility to some diseases. Various studies have shown that certain HLA class I and class II specificities have protective or susceptibility factors for some infectious diseases.

- **To exclude paternity (genetic fingerprinting)**

  Because of its extensive polymorphism, the HLA system is a very informative marker used in population genetic studies or in legal medicine to test family relationships. Studying the HLA system to produce genetic fingerprint (paternity exclusion test) is a procedure reserved for accredited organisations in France. HLA markers are now giving way to the use of highly polymorphic markers, the STR (Short Tandem Repeats) Simultaneous amplification of several STR sites by PCR (PCR-multiplex) allows to produce a highly discriminatory genetic profile.

- **In anti-tumour immunity**

  Studying the HLA system may become a prerequisite for future anti-tumour therapies based on injecting peptides derived from tumour antigens.

INFORMATION

SAMPLE

5 ml of whole blood, drawn into an EDTA tube.

QUESTIONS FOR THE PATIENT

– Legal requirements: In France, a test order from the requesting physician is accompanied by a genetic consultation certification (or failing this the requesting physician's test order and the patient's informed consent) is essential in order to take the sample and enable the analysis to be performed (in accordance with article R.11315 of decree no. 2008-321 of 4 April 2008). The result and, depending on the analysis performed, a report, are only sent to the requesting physician

– Request form: Information about the reason for the investigation, i.e. clinical information and the locus to be investigated.

SAMPLE STORAGE AND TRANSPORT

Room temperature or + 4°C (if transport > 24 h).

DIAGNOSTIC METHODS

HLA II molecules were first studied using mixed lymphocyte culture techniques (MLR - Mixed Lymphocyte Reaction and MLC

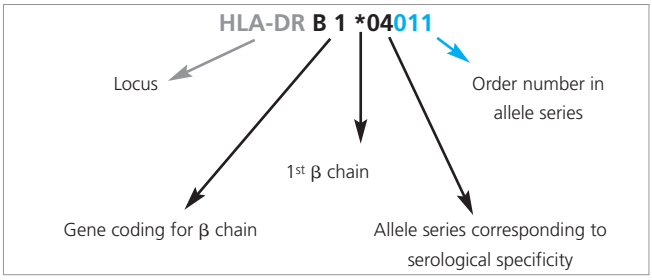
- Mixed Lymphocyte Culture) and then characterised with allosera. The routine methods now used to study HLA II molecules involve molecular biology. Molecular methods characterise gene nucleotide sequences coding for the α or β chains of the HLA class II molecules. Currently, genotyping methods for HLA class II alleles use PCR (polymerase chain reaction). This involves gene amplification with allele specific primers (PCRSSP: PCR-sequence-specific primers) and revealing the PCR products with allele specific oligonucleotide probes (PCR-SSO: specific oligonucleotide probes) or sequencing the PCR products.

NOMENCLATURE AND INTERPRETATION

Serological nomenclature of the HLA class II

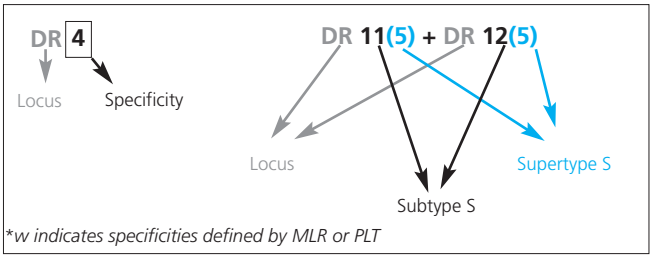
For HLA class II molecules, two letters indicate the locus studied (DP, DQ, and DR). Serological specificity is designated by one or two figures (HLA-DR4,-DR15). Some serological specificities (super-type specificities) are subdivided into private specificities (DR5 includes the private or subtype specificities DR11 and DR12).

Finally, DR serological specificity is occasionally designated Dw (Dw1 corresponding to DR1, Dw3 corresponding to DR3). The "w" indicates the specificities defined by MLR (Mixed Lymphocyte Culture) or PLT (Primed lymphocyte Test or secondary MLR) cell methods.



Genotypic nomenclature HLA class II

For the alleles coding for HLA class II molecules, 2 letters indicate the locus studied (DP, DQ, and DR). The letters "A" or "B" indicate the gene studied coding for the α chain or β chain respectively. Co-existence of functional genes and pseudogenes is indicated by a figure (A1*, A2*, B1*, B2*). Finally, the different alleles are defined by 4 or 5 figures. The first two figures refer to the serological specificity and the subsequent figures are the allele order number.



Example of interpretation of a result

Subject A	HLA DRB1*0805 and DRB1*0101 HLA DQB1*0201 and DQB1*0402 (or HLA-DR8,-DR1 and HLA-DQ2,-DQ4)
Subject B	HLA DRB1*0402 and DRB1*1301 HLA DQB1*03011 and DQB1*0501

Subject A has alleles DRB1*0805 and DRB1*0101 corresponding to the serological specificities DR8 and DR1. Subject A carries the alleles DQB1*0201 and DQB1*0402 corresponding to the serological specificities DQ2 and DQ4.

Subject B has alleles DRB1*0402 and DRB1*1301 corresponding to the serological specificities DR4 and DR13 (6). Subject B carries the alleles DQB1*03 011 and DQB1*0501 corresponding to the serological specificities DQ7 (3) and DQ5 (1).

HLA CLASS II SYSTEM AND DISEASE ASSOCIATIONS

The association of HLA markers with a disease (risk measurement) is quantified as relative risk (RR). Relative risk compares the prevalence of an allele or HLA haplotype between healthy and affected subjects (RR = ad/bc, for RR > 1, the risk is increased for RR < 1, there is no risk). In this case, one or more HLA antigens or alleles are present in the affected subjects at a significantly higher prevalence than the prevalence seen in the general population. HLA molecules are only susceptibility factors involved in the onset and development of some diseases, particularly diseases with autoimmune involvement. This susceptibility is probably dependent on environmental factors and other non HLA genes (genetic basis – multifactorial origin). The suspected associations between HLA class II and diseases include rheumatoid arthritis, type-1 diabetes, coeliac disease and narcolepsy *(table 1)*.

Relative risk	Disease	HLA concerned
6 - 10	***Rheumatoid arthritis***	DR4: alleles DRB1*0401, 0404, in 60% of cases DR1: allele DRB1*0101, in 35% of cases Poor prognosis for heterozygote carriers DRB1*0401/ DRB1*0404
40 - 45	***Type-1 diabetes***	2 DR-DQ predisposing haplotypes DR3 (DRB1*0301 - DQA1*0501-DQB1*0201) DR4 (DRB1*0401 - DQA1*0301-DQB1*0302) Synergism DR3/DR4
60	***Coeliac disease***	More than 90% of subjects express the DQ2 molecule. Haplotype: DQA1*0501/DQB1*0201 cis in DR3 subjects and trans in DR5/DR7 subjects
135	***Narcolepsy (Gélineau's Disease)***	Haplotype : DQA1*0102/DQB1*0602 Haplotype : DR2 (15)/DQ1 (DQ1: DQ5 and DQ6) Haplotype : DRB1*1 501 and DQB1*0602

Table 1. HLA Class II association with diseases.

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HOMOCYSTEINE & THE MTHFR GENE MUTATION

DEFINITION

Homocysteine (Hcy) is a sulphur-containing amino acid produced from methionine metabolism (an essential amino acid). It circulates in plasma bound to plasma proteins (70 to 80% of Hcy) and in a free form, a reduced free form (less than 1% of total Hcy) and oxidised free forms (20 to 30%), mostly homocystine (Hcy-Hcy disulphide) and a mixed Hcy-cysteine disulphide. Total Hcy measurement includes all free and bound forms.

Homocysteine sits at the crossroads of two metabolic pathways; remethylation and trans-sulphidation. Remethylation maintains a cell methionine pool: methionine synthase, which is vitamin B12-dependent, catalyses the conversion of homocysteine into methionine in the presence of a methyl group donor substrate, N-5-methyltetrahydrofolate. 5-methyltetrahydrofolate is obtained from reduction of 5, 10-methylene-tetrahydrofolate by methylene-tetra-hydrofolate-reductase (MTHFR). Remethylation occurs to a lesser extent by betaine and betaine-homocysteine-methyltransferase.

The trans-sulphidation pathway converts homocysteine into cysteine, the precursor of glutathione. This involves methionine adenosyl transferase, cystathionine synthase (CBS), cystathionase and vitamin B6 as a co-factor.

INTRODUCTION

The toxic vascular effects of hyperhomocysteinaemia, has been recognised from congenital homocystinuria due to CBS deficiency. This is an autosomal recessive disease in which severe hyperhomocysteinaemia occurs (generally > 100 µmol/l), and is characterised by early atherosclerosis associated with arterial and venous thrombo-embolism.

Moderate hyperhomocysteinaemia (< 50 µmol/l) is usually secondary to deficiency of vitamins involved in the metabolism of homocysteine (vitamins B6, B12 and folic acid) or to a mutation in the gene coding for MTHFR (heat labile C677T variant).

Moderate hyperhomocysteinaemia is an independent risk factor for arterial and venous thrombosis and repeated foetal loss. Vascular risk raises progressively in parallel to the increase in plasma homocysteine concentrations (a 5 µmol/l of Hcy may increase cardiovascular risk by as much as a 0.5 mmol/l rise in cholesterol). Screening for hyperhomocysteinaemia is important, as it can be corrected by a folate rich diet and/or vitamin treatment with folic acid, either alone (0.5 to 5 mg/day) or in association with vitamin B12 (0.02 to 1 mg/day) and vitamin B6 (2 to 50 mg/day) which reduces or normalises plasma homocysteine concentrations. These treatments may represent a relatively non-toxic and inexpensive way of preventing many vascular diseases.

The MTHFR heat labile variant is an enzyme which has reduced activity compared to the wild strain enzyme, and is due to the C677T mutation in the MTHFR gene. Unlike plasma homocysteine concentrations, this mutation is not associated with increased risk of thrombosis. Homozygotes with the mutation have markedly higher concentrations of Hcy than those who do not have the mutation and have higher folate requirements (testing for the C677T mutation may be indicated secondarily to a finding of hyperhomocysteinaemia). The involvement of moderate hyperhomocysteinaemia in arterial and venous thromboses of these cases has been examined in recent studies, some of which have produced contradictory results.

The methionine loading test is believed to increase the sensitivity of the test in people with hyperhomocysteinaemia (fasting measurement followed by measurement after 100 mg/kg of oral L-methionine). There is no consensus however, on the post-loading sampling time (2 hours, 3 hours, 4 hours or 6 hours) or on how to interpret results.

INDICATIONS FOR MEASUREMENT

Homocysteine measurement measurement is indicated in any aetiological assessment of venous or arterial thrombosis, particularly in a young person. It is recommended that vitamin B12 and folate be measured simultaneously in order to identify (or exclude) hyperhomocysteinaemia due to vitamin deficiency.

Homocysteine measurement is also proposed during a methionine loading test *(cf above)*.

Some authors have suggested that moderate hyperhomocysteinaemia may be a marker for osteoporosis and an independent risk factor for fractures in the elderly. Further studies are needed to confirm its use in this situation.

Testing for the MTHFR gene C677T mutation is a second line test in hyperhomocysteinaemia.

INFORMATION

SAMPLE

Recommendations for Hcy measurement are:

– Take the sample in the morning with the person fasting, into EDTA or lithium heparinate (EDTA is the reference anticoagulant)

– Immediately after sampling, keep the tube in melting ice; centrifuge and separate within an hour of sampling, and if the analysis is to be performed later, freeze within 4 hours of sampling. Failure to observe these precautions results in homocysteine release from red blood cells into plasma and overestimation of the plasma homocysteine concentration

– ***MTHFR gene C677T mutation:*** EDTA whole blood at + 4°C.

QUESTIONS FOR THE PATIENT

Sex and age, smoker or non-smoker? Renal or hepatic insufficiency?

Do you have a past personal or family history of venous or arterial thrombosis?

Are you taking any of the following drugs?

Drugs which increase plasma homocysteine concentrations:

– Anti-folate agents, such as phenytoin, carbamazepine, methotrexate, trimethroprim, pyrimethamine, triamterene, sulfasalazine, nitrogen monoxide and pancreatic extracts

– Drugs which induce vitamin B6 or pyridoxal 5-phosphate deficiency, such as theophylline, oral contraceptives containing oestrogens, isoniazid, gentamicin and procarbazine

– Anti-B12 drugs: nitrous oxide

– Others: cholestyramine and fibrates.

Drugs which reduce plasma homocysteine concentrations:

– Folic acid, L-thyroxine, insulin and penicillamine.

■ SAMPLE STORAGE AND TRANSPORT

Plasma can be stored at – 20°C for several months.

Transport frozen at – 20°C.

ASSAY METHODS

A large number of methods are now available to measure plasma total homocysteine. The reference method is HPLC (high performance liquid chromatography) coupled with fluorescence detection. Immunoassay kits are commercially available and have the advantage of enabling the assay to be automated. The results obtained are similar and correlate well with HPLC measurements. These are the ABBOTT kit on the IMX auto-analyser (fluorescence polarisation immunoassay (FPIA) and the DPC kit on the Immulite 2000 auto-analyser (competitive immunochemoluminescence assay). In both of these methods the bound, oxidised forms are reduced by dithiothreitol (DTT) treatment and total homocysteine is measured after conversion to S-adenosyl-L-homocysteine (SAH) by SAH hydrolase with excess adenosine.

There is no international standard for homocysteine measurement and the results and reference values may therefore vary between assay methods.

NORMAL EXPECTED VALUES

Published physiological plasma homocysteine levels are between 5 and 15 µmol/l. There is current controversy between experts about the threshold value to use (12, 13 or 15 µmol/l). Homocysteine measurement in a person should be interpreted according to the clinical context, past cardiovascular history and assay method used.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Plasma homocysteine concentrations are sex and age-dependent. They increase physiologically with age and are approximately 20% higher in men than in women ("normal" values in women rise to reach those in men after the menopause).

Smoking (smokers and ex-smokers), coffee and alcohol cause an increase in plasma homocysteine concentrations (approximately 12% increase in heavy smoking men and 23% increase in heavy smoking women).

■ PATHOLOGICAL VARIATIONS

NB: Reduced blood homocysteine has no clinical consequences.

All situations of hyperhomocysteinaemia regardless of cause represent a risk factor for vascular disease independent of other known risk factors and increase the risk of vascular accidents and death. Many studies have examined this, and according to a meta-analysis which combined 27 case control studies on the risk of arterial thrombosis, and one which combined 9 case control studies on the risk of venous disease, the risks associated with hyperhomocysteinaemia were:

– Coronary artery disease: Odds-ratio 1.7 (95% CI: 1.5 to 1.9)

– Cerebrovascular accidents: Odds-ratio 12.5 (95% CI: 2 to 3)

– Peripheral arterial disease: Odds-ratio 6.8 (95% CI: 2.9 to 15.8)

– Venous thrombosis: Odds-ratio 2.6 (95% CI: 1.8 to 3.8).

Recent studies have examined the existence of a relationship between moderate hyperhomocysteinaemia and venous thrombo-embolism.

Hyperhomocysteinaemia has also been reported to cause gynaecological and neonatal problems (repeated miscarriages, obstetric complications, neural tube closure abnormalities, neonatal cardiac malformations, etc.), and in addition, psychiatric and cognitive disorders.

In addition, hyperhomocysteinaemia has been associated with an increased risk of osteoporotic fractures in the elderly (men and women).

Main causes of hyperhomocysteinaemia:

■ Severe hyperhomocysteinaemia (> 100 µmol/l)

– Classical homocystinuria: Homozygous cystathionine beta synthase (CBS) deficiency, which has two clinical forms, one of which is B6 sensitive, the other of which is B6 resistant

– Homocystinuria due to homozygous deficiency of one of the enzymes involved in the remethylation of homocysteine into methionine, particularly MTHFR (methylene tetrahydrofolate reductase) or methionine synthase (MS). In this type of homocystinuria, plasma methionine is reduced whereas it is raised in classical homocystinuria

– Cobalamine deficiencies.

■ Intermediary (31 to 100 µmol/l) or moderate (16 to 30 µmol/l) hyperhomocysteinaemia

– Heterozygous deficiency of the enzyme CBS

– MTHFR C677T heat labile variant associated with reduced folate concentrations. (NB: When plasma folate concentrations are normal the plasma homocysteine is not raised.) The estimated prevalence of heterozygotes in the general Caucasian population is in the region of 50%, compared to 10% for homozygotes. Patients who are homozygous for this polymorphism have higher plasma homocysteine concentrations than those who do not carry the gene and their folate requirements are higher

– Deficiency of vitamin B6, B12 or folate intake or absorption defects; particularly alcoholism, Biermer's disease (pernicious anaemia), some cases of severe B12 deficiency; the hyperhomocysteinaemia may be very severe (> 100 µmol/l)

– Renal insufficiency: Plasma homocysteine concentrations rise in proportion to the severity of renal insufficiency

– Solid tumours and leukaemia: All cell proliferation produces large amounts of homocysteine

– Hypothyroidism: The mechanism of this has not been completely established

– Coeliac disease: Due to defective folate absorption

– Others: Hepatic insufficiency, hypertension, hypothyroidism, psoriasis and in some pathological pregnancies.

■ **Drug induced causes**

Cf. above.

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HOMOVANILLIC ACID

DEFINITION

Dopamine (DA) is a biogenic amine with a catechol core synthesised by chromaffin cells and catecholaminergic neurones, especially in the brain, lung, liver and gut. In the brain, dopamine is found at high concentrations in the pars compacta region of the substantia nigra which controls involuntary motility and muscle tone. The degeneration in this region underlies Parkinson's disease. Dopamine is broken down by two enzymes working in tandem, namely catechol-O-methyltransferase (COMT) and monoaminoxidase (MAO), to yield homovanillic acid (HVA) which is excreted in the urine. The amount of HVA in the urine gives a measure of overall dopamine production.

Synonym: HVA.

INTRODUCTION

Two cell-types secrete dopamine. These are the adrenal medulla cells and cells in sympathetic nerve fibres. A neuroblastoma is a malignant tumour of relatively undifferentiated cells in sympathetic nervous tissue. It is among the commonest of solid tumours in children. The symptoms—production of dopamine and its derivatives in inappropriate tissues— afford little help with diagnosis. The classic biochemical picture is an elevation of dopamine, HVA and vanylmandelic acid (VMA) and assaying these three parameters in parallel makes diagnosis possible in 95% of cases. Predominant HVA secretion gives a poor prognosis and the prognosis is better if HVA secretion is normal and VMA secretion is high.

A pheochromocytoma is a tumour of the chromaffin cells of the adrenal medulla. These cells are mature and 90% of these tumours are benign. Symptoms of hypertension, sweating, pallor and headache are of more help with diagnosis than those of neuroblastoma. A HVA assay is of limited relevance in pheochromocytoma in which the cells predominantly secrete noradrenaline and adrenaline, although it can be useful in the investigation of atypical pheochromocytomas in which VMA is not excreted in the urine.

INDICATIONS FOR MEASUREMENT

Most HVA assays are ordered for the diagnosis and monitoring of tumours:

– For diagnosis and establishing prognosis in neuroblastoma in children

– As a supplementary marker for confirmation of a diagnosis of pheochromocytoma in adults.

INFORMATION

SAMPLE

A 24-hour urine collection should be acidified at the laboratory using 6 N HCl (1 ml per 100 ml) to adjust the pH of the urine to between 2 and 3. If the pH is too low (< 1), the compounds to be assayed may not be efficiently extracted and the result may be underestimated.

In children, 24-hour urine collection is complicated and a simple urine sample can be assayed with expression of the HVA concentration with respect to the sample creatinine concentration.

QUESTIONS FOR THE PATIENT

Ask about how well the subject has complied with the dietary recommendations for the 48-hour period prior to the urine collection, i.e. reduced consumption of coffee, tea, vanilla, chocolate, tomatoes and citrus fruit; bananas should have been completely excluded from the diet.

In addition, dopaminergic medications (levodopa) and dopamine antagonists (clonidine, reserpine) should not have been taken recently.

SAMPLE STORAGE AND TRANSPORT

HVA is unstable (due to its phenolic catechol group) but, if the sample is acidified, it can be conserved at +4°C and transported. Urine collected on 10% disodium EDTA can be stored at –20 °C.

ASSAY METHODS

High performance liquid chromatography (HPLC) is the reference method. It is used with an electrochemical detection system.

NORMAL EXPECTED VALUES

For reference:< 8 mg per 24 hours, i.e. < 43 µmol/24 hours.

The mean concentration varies with age *(see Table)*.

Age	mg/24 h	µmol/24h
0 – 5	1 - 3	5.5 - 16.5
5 – 10	1 - 4	5.5 - 22.0
10 – 15	2 - 5	11.0 - 27.5
Over 15	3 - 8	16.5 - 43.0

INTERPRETATION

In 80% of childhood neuroblastomas, the urine HVA concentration is elevated and this test is useful in both diagnosis and establishing prognosis. Elevated HVA is often associated with a rise in urine VMA. Assaying dopamine in the urine together with these phenolic acids (VMA and HVA) affords 95% diagnostic sensitivity with the other 5% corresponding to non-secretory forms. Certain rare neuroblastomas (ganglioneuroblastomas) secrete adrenaline and noradrenaline.

In adults, a HVA assay confirms pheochromocytoma if VMA is normal, in which case HVA is elevated. However, a direct assay of catecholamine in the urine is more informative.

Minor elevations in HVA have been observed with certain tumours, namely medullar thyroid cancer, tumours of the small intestine and retinoblastoma.

In Parkinson's disease, reduced HVA excretion in the urine is seen after the destruction of dopaminergic neurones.

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HTLV

DEFINITION

HTLV-I and II *(Human T-cell Leukemia/lymphoma virus)* viruses belong to the *Retroviridae* family, Oncovirus genus. The HTLV1 virus was the first retrovirus discovered by Gallo in the United States in 1980. It is associated firstly with adult T cell leukaemias and lymphomas *(ATL adult T-cell leukemia)*, secondly with a neuromyelopathy known as tropical spastic paraparesis (TSP or HAM – *HTLV-I associated myelopathy*). The HTLV-II virus is associated with an atypical form of tricholeukocytic leukaemia.

INTRODUCTION

The HTLV viruses have features which are common to the retroviruses. The genome consists of a single chain RNA. Each viral particle contains two identical RNA molecules associated with an enzyme, reverse transcriptase. The genetic material is contained in an icosahedric capsid which itself is surrounded by an envelope.

Their genetic organisation is similar to that of HIV. HTLV viruses have *gag*, *pol* and *env* genes which code for structural proteins but also have two additional genes, *tax* and *rex*, which code for non-structural proteins which regulate viral replication.

Unlike HIV (human immunodeficiency virus), little genetic variation is seen between different HTLV virus isolates. HTLV-I variability is estimated to be less than 3%.

The HTLV viruses are lymphotropic viruses, although they are also able to infect a large number of different cell types. It is likely, therefore, that the receptor for these viruses is a ubiquitous molecule.

After the virus binds to the cell receptor, membrane fusion occurs which enables the viral capsids to enter the target cells and the genetic material is then released. Viral DNA which migrates to the cell nucleus is then synthesised from the genomic RNA through the action of the reverse transcriptase. This viral DNA then randomly incorporates into the host cell genome. The incorporated virus is called the provirus. Proviral DNA is then transcribed into genomic RNA and into viral messenger RNA, enabling viral proteins which are essential for the production of new viral particles in the cytoplasm to be synthesised. The envelope glycoproteins coded for by the env gene, accumulate on the cell membrane surface which forms the viral envelope after nuclear capsid budding. It should be noted that the incorporated provirus is transmitted to daughter cells like a cell gene.

The HTLV viruses are also transforming viruses, i.e. they cause malignant transformation of infected cells..

EPIDEMIOLOGY

HTLV viruses are transmitted through blood, via blood transfusions or from intravenous drug abusers using dirty needles, by sexual contact or from mother to child (mostly through breastfeeding).

HTLV-I (15 to 25 million carriers throughout the world) is endemic in Japan, in tropical Africa, in several regions of Central and South America, in the Caribbean (sero-positivity rates in blood donors in Martinique and Guadeloupe, are 2.2 and 0.6% respectively) and in some regions of the Middle East, such as North East Iran. An increase in seroprevalence with age, particularly in women over 30-40 years old, is a characteristic feature.

The prevalence of HTLV-I and HTLV-II infection in Europe and the United States is increasing in intravenous drug abusers.

SYMPTOMS

HTLV-I is associated with:

- **Adult T cell leukaemia** (ATL). The risk of developing ATL is 1% after incubation for 20 to 30 years.

  Several forms of these can be distinguished:

  – The pre-ATL form: This is a lymphocytosis which often resolves in asymptomatic people

  – Chronic leukaemia during which skin signs are seen without systemic features

  – Acute forms with raised lymphocytosis and characteristic T lymphoblasts. This form has a high mortality rate.

- **Tropical spastic paraparesis** is a neurological disease progressing to a four limb pyramidal syndrome in a third of cases, ending with patients being bedbound. The risk of developing tropical spastic paraparesis in HTLV-I positive patients is estimated to be 2% in Martinique and one in a thousand in Japan.

INFORMATION

SAMPLE

Serum or plasma (EDTA or citrate).

EDTA whole blood.

SAMPLE STORAGE AND TRANSPORT

For serological tests: If the analysis has to be deferred, the serum or plasma should be frozen promptly and ideally transported without breaking the cold chain.

For molecular tests: 5 ml of whole blood into EDTA, stored at + 4°C.

DIAGNOSTIC METHODS

Diagnostic methods for HTLV virus are mostly indirect, based on detection methods for anti-HTLV I and II antibodies in serum. This involves 2 stages: screening, following by confirmation and differentiation of the two serotypes.

Serology screening is performed using the ELISA technique or sensitised gelatin particle agglutination, followed if positive, by a confirmatory test on a second sample, possibly using Western blot. The positivity criteria for the Western blot test adopted by the French Society of Blood Transfusion "Retrovirus" working group are the presence of at least three positive reactions: rgp21 (common glycoprotein to the 2 viruses coded for by the env gene), p19 and p24 (coded for by the gag gene). Seropositivity is suspected if any of these three antibodies is absent, although further tests must be

performed. If an isolated anti-rgp21 is found the serology profile must be rechecked and in the absence of a change after two months it is concluded that HTLV virus infection is absent. An isolated finding of anti-p24 or anti-p19 in most cases represents a false positive reaction. A differentiation test between HTLV-1 and HTLV-2 is then performed. Information is gained from the intensity of the p19 and the p24 bands, where if the p19 band is equally intense or more intense than the p24, then HTLV-I is probably present; if the reverse is found, HTLV-II is probably present. MTA-1 or K55 proteins on the Bioptim® Western blot directly differentiate the two serotypes. PCR methods are required if indeterminate profiles are obtained.

Direct diagnosis based on cell culture isolation or PCR testing for proviral DNA in circulating leukocytes, is not widely used outside of trials and is only performed by specialist laboratories.

"Retrovirus" group interpretation criteria

Rgp21	p19	p24	Interpretation
+	+		Confirms positive HTLV. Differentiate according to p19, p24 and MTA-1 signals.
+	+	-	Suspected positive, confirm with PCR.
+	-	+	Suspected positive, confirm with PCR.
+	-	-	Recheck in 2 months. No change: person not infected.
-	-	+	Probable false positive. Monitor in a month.
-	+	-	Probable false positive. Monitor in a month.
-	+	+	Monitor in a month.

TREATMENT

There is no specific antiviral treatment HTLV virus infection.

The only means at present to prevent HTLV infection are:

– Screening for anti-HTLV antibodies in blood donors

– Non-use of shared syringes in IV drug abusers

– Use of condoms when the partner is seropositive

– Artificial milk for children born to seropositive mothers.

FOR FURTHER INFORMATION

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HÜHNER POST-COITAL TEST

DEFINITION

This test involves examining spermatozoa in cervical mucus after sexual intercourse. It is used to assess spermatozoa behaviour and their ability to penetrate the mucus in the pre-ovulatory period, to confirm the quality of the cervical mucus and to ensure that sexual intercourse has actually occurred.

SAMPLE

WHEN TO TAKE THE SAMPLE

- **For women:** during the pre-ovulatory period (one or two days before the projected date of ovulation) as the mucoprotein mesh structure tightens from ovulation onwards in response to increased progesterone. As a result, monitoring the menstrual temperature curve is a valuable aid to retrospective interpretation. Any stimulatory treatment (25 - 50 µg/d of ethinyl-oestradiol for around ten days from the fifth day of the cycle) may improve mucus production if this is found to be inadequate in a previous test.
- **In men:** sexual abstinence is recommended for 3 to 5 days before the intercourse preceding the test.

  Sexual intercourse must take place between 1 and 12 hours before the test and be followed by a 30 minutes rest period for the woman. No vaginal toilet must be performed before collecting the mucus.

MATERIALS

2 mucus aspirators.

1 disposable plastic speculum.

OPERATING PROCEDURE

After inserting the speculum (never use lubricant) and exposing the cervix (record its appearance: healthy, inflamed, bleeding and extent of opening), clean around the cervix with sterile swabs in order to avoid any contamination by vaginal secretions. The mucus is then aspirated from the internal cervical orifice (approximately 1 cm beyond the exocervix).

EXAMINATION OF THE SAMPLE

The sample is examined microscopically immediately after being taken (magnification x 40). Examine several fields (a minimum of 3) and calculate the mean value.

The mucus threadiness is assessed by grasping the mucus between the jaws of a set of forceps and gradually stretching it; threadiness is expressed in cm and is excellent if the mucus stretches 8-10 cm before breaking.

Crystallisation is studied by placing a drop on a slide and leaving it to dry at room temperature. Microscopic examination after an hour will reveal the classically described "fern leaf" crystallisation.

SAMPLE STORAGE

The mucus may be stored for up to 4 days in a refrigerator at 4°C. Freezing is not recommended.

RESULT REPORTING

The result should report the day of the cycle, the time since coitus, extent of cervical dilatation, amount of threadiness and transparency of mucus; progressive, non-progressive (with details of whether these are oscillating) and immobile spermatozoa density together with pH. Acid pH does not promote spermatozoa penetration (advise women to drink bicarbonate- rich water).

Mucus quality is scored using the Insler score

This score is obtained by adding the points attributed to each "characteristic" of the mucus (cervical opening, amount, threadiness, crystallisation).

	1 point	2 points	3 points
Cervix opening	Point (+)	Patent (++)	Gaping (+++)
Abundance	Minimal	Drop	Cascade
Threadiness	1 to 4 cm	5 to 10 cm	> 10 cm
Crystallisation	Linear	Partial	Total

INTERPRETATION OF RESULTS

The test is satisfactory if:

– The mucus is of good quality (correct oestrogenisation) with an Insler score > 10 (ovulatory mucus)

– On microscopic examination (x 40) the mucus contains more than 10 mobile spermatozoa per field. Interpret in the context of the sperm profile (report is positive if oligoasthenoteratospermia and ≥ 5 spermatozoa per field).

The test is negative if:

– The mucus is of poor quality with a low Insler score and/or

– Mobile live spermatozoa are occasional or absent on microscopy.

A negative Hühner score can be explained by a sample taken outside of the pre-ovulatory period, by infected mucus (numerous leucocytes will be present), by problems during coitus, azoospermia or anti-spermatozoa immunisation (which may be suspected from the "shaking" effect exhibited by the spermatozoa).

FOR FURTHER INFORMATION

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- www.aly-abbara.com

HUMAN HERPES VIRUS 6

DEFINITION

Human herpes virus 6 (HHV6) was first isolated from mononucleated cells of immunosuppressed patients (AIDS, lymphoma or leukaemia). It belongs to the *beta Herpes viridae* sub-family of *Herpes viridae*, like the closely-related cytomegalovirus. The β Herpes viruses (HHV5 or CMV, HHV 6 and 7) replicate *in-vitro* on a small number of cell lines; their replication cycle is long and they have little lytic effect on the cells which they infect.

HHV6 is an enveloped virus containing an icosahedral nucleocapsid, within which there is linear dual-stranded DNA. It has a preferential tropism for CD4+ T lymphocytes (which its other name, *Human B lymphotrope virus*, does not suggest), but also infects macrophages, monocytes, megakaryocytes, fibroblasts, salivary epithelial cells, glial cells and oligodendrocytes. Humans are the only reservoir of the virus. HHV6, like the other herpes viruses, is directly transmitted by biological fluids (saliva in particular in the case of HHV6) on close contact between individuals, such as maternity care, sexual relationships, blood transfusions (to a small degree for HHV6, due to low viraemia) and grafts of organs or tissues. Maternofoetal transmission in the course of pregnancy or during parturition is rare, although possible. HHV6 has not been found in maternal milk.

In immunosuppressed subjects, HHV6 is chiefly responsible for *exanthema subitum* or infantile roseola, also referred to as the 6th rash-causing disease of childhood.

Synonyms: Human B lymphotrope virus and Human Herpes Virus 6.

INTRODUCTION

EPIDEMIOLOGY - SYMPTOMS

HHV6 is widely distributed throughout the world. Approximately 90% of children have specific antibodies before the end of their second year of life. The seropositive rate then falls in the course of childhood and adolescence, before rising again to around 90% in adulthood. After primary infection, characterised by active multiplication of the virus and lymphocytic viraemia, the virus persists in a latent state, mainly in the salivary glands (with intermittent viral excretion in the saliva), monocytes, CSF and lungs. In immunosuppressed subjects, the forms of recurrence are usually asymptomatic or clinically non-specific (fever and asthenia).

Pathology in immunocompetent subjects

– *Exanthema subitum* in babies: The primary infection usually occurs suddenly between 6 months and 4 years of age. After an incubation period of 5 to 15 days, the illness appears with a rapid onset of fever, peaking at up to 40.5° C for 2 to 8 days (on average 4 days), with febrile seizures as a complication in some 10% of cases. When the fever subsides, a rubella-like rash appears on the trunk and neck (20% of cases), subsequently spreading to the limbs and face (confluent maculae). It lasts for 24 to 48 hours and is non-pruriginous. Angina is frequently associated, with the presence of cervical adenopathies.

– HHV6 meningitis: This rare condition is characterised by the presence of the HHV6 genome in the cerebrospinal fluid (CSF), combined with an increase in leucocytes and proteinorachia. Encephalitis is rare, but can be fatal.

– Other effects: Hepatitis (mainly in adults), diarrhoea (60 to 70 % of cases in children) and oedema of the eyelids (30% of children).

Pathology in immunosuppressed subjects

Generally speaking, HHV6 (after primary infection or viral reactivation) can be responsible following organ or tissue transplantation, for pneumopathy, medullary insufficiency, encephalitis, fulminating hepatitis or macrophage activation syndrome, which are potentially fatal. In paediatrics, HHV6 has been incriminated in the onset of interstitial pneumonia after bone marrow transplantation.

SEARCH INDICATIONS

– Search for the HHV6 genome by PCR in plasma: Aetiological diagnosis of fever; either isolated or associated with a rash or hepatitis; monitoring of a viral infection or reactivation in an immunosuppressed subject (after organ transplantation) and its treatment.

– Search for the HHV6 genome by PCR in CSF: Aetiological diagnosis of meningitis/encephalitis, particularly after bone marrow transplantation.

– HHV6 serology: Epidemiological interest.

INFORMATION

SAMPLE

Serology: Serum (dry tube). A fasting sample is not necessary.

PCR: Blood collected in ACD tube or CSF (sterile dry container).

SAMPLE STORAGE AND TRANSPORT

Blood collection for serology: Serum can be stored for 48 hours at +4° C or 1 year at -20° C.

For PCR: The sample can be stored for 48 hours at +4° C. For longer than this, freeze to -20° C

CSF: The sample can be stored for 1 hour at +4° C; for longer than this, freeze to -20° C.

DIAGNOSTIC METHODS

Serology (search for IgG and IgM): Indirect immunofluorescence on infected cells or EIA.

Classical or real-time PCR: On blood (peripheral blood lymphocytes), CSF, saliva, biopsies, etc.

INTERPRETATION OF RESULTS

– PCR on blood or CSF: A positive PCR suggests an HHV6 infection. Real-time PCR also provides quantification of viral load, which is useful in monitoring immunosuppressed patients under treatment.

– Serology: IgM's appear a few days after infection, reaching a peak after approximately 3 weeks and then disappearing in about two months. IgG's are detected a few days after IgM's, subsequently persisting at a low level throughout life. In the event of reactivation, a rise in IgG's is observed, sometimes associated with a reappearance of IgM's. HHV6 infections must be treated in the event of febrile seizures, encephalitis or severe post-transplantation infection. Treatment is preferably based on ganciclovir (10 mg/kg/day) or, if appropriate, on foscavir.

FOR FURTHER INFORMATION

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HUMAN HERPES VIRUS 8

DEFINITION

Isolated from a Kaposi's sarcoma skin lesion in an AIDS patient, human herpes virus 8 (HHV8) belongs to the *Herpes viridae* family, subfamily *gamma Herpes viridae* (genus rhadinovirus). It is related to Epstein-Barr virus, of which it possesses the oncogenic properties, particularly in immunosuppressed subjects. *Gamma Herpes* viruses replicate *in-vitro* on lymphoblastoid cells. Like the other herpes viruses, HHV8 is an enveloped virus containing an icosahedral nucleocapsid and its genome, DNA with a length of 140 kb comprising 81 genes, some of which present homologies with human cell genes. In human pathology, HHV8 is associated with Kaposi's sarcoma and certain lymphomatous processes.

Synonyms: HHV8, Kaposi's sarcoma-associated herpes virus (KSHV).

INTRODUCTION

CLINICAL EPIDEMIOLOGY

HHV8 is a non-ubiquitous virus. In countries with low endemic virus levels (Northern Europe and USA), the majority of individuals infected with HHV8 are homosexual men. In individuals who are seropositive for HIV, viral seroprevalence is as high as 70%. Transmission of HHV8 during sexual activity therefore seems to play a major role in the spread of the virus among homosexuals. The virus is present in saliva and semen (at low levels and in an irregular manner).

In countries with high endemic levels, (essentially East and Central Africa, and Mediterranean basin countries to a lesser degree), the seroprevalence of HHV8 is often already high in children. Virus transmission mostly seems to take place before puberty, which excludes sexual transmission. Transmission is though to occur mainly from mother to child and between children (close contact, particularly involving saliva, and sharing of domestic utensils).

Saliva seems in all cases to be a major virus reservoir.

HHV8 is though to be the agent responsible for 4 clinical forms of Kaposi's sarcoma:

– Classical form: Cutaneous lesions of the limbs and feet, mainly affecting elderly men originating from Central Europe or the Mediterranean basin.

– The endemic form in Eastern and Central Africa: Sometimes more severe, with infiltrating lesions, disseminated nodules and ganglionic or visceral impairment. It frequently affects elderly men, although children may also suffer from the ganglionic form.

– Iatrogenic or post-transplantation form: Kaposi's sarcoma occurring in a context of long-term immunosuppression. Lesions are mainly cutaneous and improvement is possible on completion of treatment.

– Epidemic form associated with immunosuppression linked to HIV infection: Frequent cutaneous, mucosal, ganglionic and visceral lesions.

HHV8 is also associated with primitive lymphoma of the serous membranes or *primary effusion lymphoma* (PEL), clonal B-lymphoproliferation, essentially affecting male homosexuals infected with HIV (pleural, pericardial or peritoneal localisations), and certain forms of multicentric Castleman's disease (polyclonal B-lymphoproliferation, characterised by fever, adenopathies and angiofollicular hyperplasia), occurring in HIV+ patients.

SEARCH INDICATIONS

Search for the HHV8 genome by qualitative or quantitative PCR in blood (or biopsies): Suspected Kaposi's sarcoma, PEL or multicentric Castleman's disease.

HHV8 serology: Epidemiological interest.

INFORMATION

SAMPLE

Serology: Serum (dry tube). A fasting sample is not required.

Qualitative or quantitative PCR: Whole blood EDTA, cutaneous lesions and biopsies. Fasting is not necessary.

SAMPLE STORAGE AND TRANSPORT

Blood collection for serology: Store serum for 48 hours at +4° C or 1 year at -20° C.

Blood collection for PCR: Store for 48 hours at +4° C. For longer than this, freeze to -20° C.

DIAGNOSTIC METHODS

Serology (search for IgG) by indirect immunofluorescence on chronically-infected cell lines, immunoblot, western blot or ELISA. IgM's can be tested for specifically.

Classical or nested PCR on blood (peripheral blood lymphocytes), saliva, biopsies, etc.

INTERPRETATION OF RESULTS

– Positive HHV8 PCR probably means infection with the virus. Detection of the HHV8 genome in tumour lesions is often associated with that of the HHV6 and/or EBV genome. PCR is not of great interest in epidemiological investigations; due to its low sensitivity in this context (it is in fact positive in 10 to 20% of healthy seropositive subjects).

– Serology: Results must be interpreted according to the tests used (test detecting antibodies directed against lytical or latent antigens). In countries with low endemic levels, HHV8 seroprevalence in adults is < 5%. In countries with high endemic levels, it varies between 10 and 80%.

In countries with low endemic levels, the prevalence of anti-HHV8 antibodies is approximately 80% in HIV-infected patients suffering from Kaposi's sarcoma, and close to 100% in patients suffering from Kaposi's sarcoma who are not infected with HIV. Antibody prevalence is approximately 30% in HIV+ homosexuals who do not have Kaposi's sarcoma. In blood donors or other groups at risk of HIV infection (haemophiliacs, drug addicts, etc.), it is much lower, ranging from 0 to 5%.

– Treatment of HHV8 infections uses ganciclovir or cidofovir. Evolution depends essentially on that of the HIV infection.

FOR FURTHER INFORMATION

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■ Plancoulaine S., Abel L., Gessain A., *Aspects épidémiologiques du virus herpès humain 8*, La Lettre de l'Infectiologue, 2002; XVII : 175-80.

HUMAN PLACENTAL LACTOGEN

DEFINITION

Human placental lactogen (hPL) or chorionic somato-mammotropic hormone (HCS) is a 190 amino acid single chain peptide (approximately 22000 Da) synthesised by syncytiotrophoblast tissue in pregnant women. It has considerable (85%) sequence homology with human pituitary growth hormone (hGH) and to a lesser extent (35%) with prolactin (hPRL).

It is mostly secreted in the maternal compartment and secretion closely parallels the change in placental mass.

It disappears rapidly after childbirth and has a plasma half-life (T1/2) in the region of 20 to 30 minutes.

INTRODUCTION

The action of hPL is still very poorly understood. Because of its direct "GH" lipolytic activity and its effect on insulin secretion, it is believed to play a metabolic role in regulating energy flow and maintaining glucose supplies to the foetus (insulin resistance). It may also stimulate the synthesis of somatomedins or *insulin growth like factors* (particularly IGF 1 and 2).

Functionally, it has lactogenic properties via prolactin (hPRL) receptors and effects on foetal growth via growth hormone (hGH) receptors.

Its secretion increases regularly during pregnancy rising to approximately 3 g/day in the 3rd trimester.

INDICATIONS FOR MEASUREMENT

As a marker of placental mass and function.

To investigate for foeto-placental distress, threatened mis-carriage or eclampsia.

INFORMATION

■ SAMPLE

Preferably serum (dry tube); EDTA plasma can be used for some methods. Avoid haemolysis.

A fasting sample is preferable.

■ QUESTIONS FOR THE PATIENT

Suspected diseases and clinical details; date of last period; other related investigations (urinary estriol, often combined); current treatment?

■ SAMPLE STORAGE

Serum: 5 days at + 4°C, beyond that freeze at –20°C.

Avoid freeze/thaw cycles.

ASSAY METHODS

Current methods are mostly immunometric: RIA (iodine 125), ELISA (direct sandwich) and CLIA (chemoluminescence).

As the antibodies are specific, cross reactions with prolactin and growth hormone do not occur.

NORMAL EXPECTED VALUES

Assays are calibrated against the WHO 73/545 standard.

hPL concentrations increase slowly from the 5th to 36th week of pregnancy and then fall very gradually until term.

Usual values in pregnancy (RIA):

Week of pregnancy	Mg/l (5th and 95th percentile)
5-12	0 - 0.9
13-16	0.3 - 2.0
17-20	1.0 - 2.8
21-24	1.5 - 4.5
25-28	1.5 - 6.2
29-32	3.5 - 7.7
33-36	5.0 - 10
37-40	4.5 - 9.5

Values are increased by approximately 60% in twin pregnancies.

PATHOLOGICAL VALUES

Reduced serum hPL values are seen in:

Toxaemia, choriocarcinoma, poor placental development, foeto-placental distress, threatened miscarriage, eclampsia and hydatiform moles (in which case it is associated with raised hCG).

High serum hPL values are seen in:

Multiple pregnancies, trophoblastic tumours, gestational diabetes and rhesus incompatibility.

Note:

Insulin-induced hypoglycaemia produces a large rise in hPL concentrations (by up to 25%).

FOR FURTHER INFORMATION

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■ Evain-Brion D., *Hormones placentaires humaines*, Nutrition physiological role et métabolisme, 2002 ; 16 : 206-209.

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HYALURONIC ACID

DEFINITION

Hyaluronic acid is a polysaccharide belonging to the glycosaminoglycan family which is produced by most cell-types, notably mesenchymatous cells. It is found in extracellular spaces throughout the body. It has a short half-life in plasma, of the order of a few minutes. It is mainly broken down by endothelial cells in the liver.

Synonyms: hyaluronate, HA test, hyaluran.

INTRODUCTION

Hyaluronic acid contributes to the elasticity and viscosity of the extracellular medium, and is involved in interactions between cells.

Malignant cell proliferation is often accompanied by a rise in hyaluronic acid production.

INDICATIONS FOR MEASUREMENT

■ SERUM/PLASMA

Hyaluronic acid is a marker for hepatic fibrosis and is used in hepatology to monitor chronic liver disease.

Hyaluronic acid is also sometimes used as a marker for synovial inflammation in rheumatology in patients with active rheumatoid arthritis and other forms of arthrosis.

Finally, it can be used to monitor the progress of HA-secreting pleural mesotheliomas and Wilms' tumours.

■ PLEURAL FLUID AND ASCITES

Hyaluronic acid is a tumour marker for mesothelioma, a primary, malignant tumour of the pleura; the aetiology is attributed to asbestos in 70% of cases. About 70% of mesotheliomas secrete hyaluronic acid into the pleural fluid and an increase in concentration is useful in diagnosis. However, such an increase hyaluronic acid in pleural fluid is not specific to mesothelioma because it also occurs in pleural inflammation and pulmonary neoplasia.

INFORMATION

■ SAMPLE

Serum or heparinised plasma: A fasting sample is preferred.

Pleural fluid or ascites, into a tube without anticoagulant.

■ QUESTIONS FOR THE PATIENT

Are you being treated with hyaluronic acid?

This medicinal product is administered by intra-articular injection for pain management in arthrosis. Nevertheless, unless the product enters the circulation, such treatment should not affect the serum concentration of hyaluronic acid.

Please ensure the age of the patient and the reason for requesting the test (at least the clinical details) is included on the request form.

■ SAMPLE STORAGE AND TRANSPORT

If the test is not going to be performed within 4 hours of drawing the sample, then please ensure it is rapidly separated and frozen at –20°C.

ASSAY METHODS

Radio-immunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), HPLC (assayed in aspiration biopsy fluids).

NORMAL EXPECTED VALUES

Serum: for reference:

Age	Expected Value
10 – 30	9 – 27 µg/l
30 – 40	7 – 47 µg/l
40 – 50	17 – 57 µg/l
50 – 60	18 – 80 µg/l
> 60	30 – 108 µg/l

Pleural fluid or ascites: < 80 mg/l.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Age-dependent: See *"Normal Values"*.

Eating tends to raise the serum concentration of hyaluronic acid.

■ PATHOLOGICAL VARIATIONS

■ **Serum/plasma:**

Liver disease

– The serum concentration of hyaluronic acid is higher in patients with chronic liver disease, notably cirrhosis. This test is the best non-invasive diagnostic marker for cirrhosis.

– Hyaluronic acid is an excellent marker for hepatic fibrosis and is used in patients with chronic viral hepatitis C. Regularly monitoring the hyaluronic acid concentration in the serum of these patients gives a measure of the extent of hepatic fibrosis without having to take liver biopsies every time.

– In full-blown cirrhosis, a rise in the serum concentration of hyaluronic acid is predictive of complications.

– A rise in the serum concentration of hyaluronic acid is also seen in alcohol-induced liver disease, notably cirrhosis. For the same degree of fibrosis, this rise tends to be greater in an alcoholic subject who is still drinking.

– Primary biliary cirrhosis: An increase in the serum concentration of hyaluronic acid correlates with the histological grade of the disease and is a poor prognostic indicator.

Rheumatology

– The hyaluronic acid level reflects the activity of the synovial membrane. The initial serum concentration of hyaluronic acid in patients with arthrosis of the knee is of predictive value,

therefore the higher it is at the time of diagnosis, the greater the risk of progressive gonarthrosis. The initial intensity of the synovitis is therefore a good indicator of arthritic activity.

– In patients with rheumatoid arthritis, an increase in the serum hyaluronic acid concentration (up to seven times greater than the normal value) reflects the activity of the disease.

Other situations in which the serum concentration of hyaluronic acid can rise:

Scleroderma and mesothelioma.

- **Pleural fluid or ascites:**

Hyaluronic acid is a tumour marker for mesothelioma. When its concentration is high, interpretation necessitates comparison with results from pleural biopsies because elevated hyaluronic acid values are also seen in benign forms of asbestosis. Only very marked increases are of use when it comes to the diagnosis of mesothelioma.

FOR FURTHER INFORMATION

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- Hillerdal G., Lindqvuist U. and Engstrom-Laurent A., *Hyaluronan in pleural effusions and in serum*, Cancer, 1991; 67: 2410-4.

HYDATID DISEASE

DEFINITION

Hydatid disease is an anthropozoonosis caused by the growth of *Echinococcus granulosus* larvae in human beings.

This is a cestode belonging to the Taenidae family which belongs to the *Cyclophyllidae* order *Cestodes* class and Platyhelminth branch.

It exists in 3 forms:

– ***The adult*** is a small taenia worm 4 to 6 mm long, the body of which consists of 3 to 5 segments and lives in the small intestine of the final host (particularly dog)

– ***The egg*** contains a hexacanth embryo with 6 hooks

– ***The hydatid cyst*** is a hollow vesicular sphere filled with fluid and contained by two membranes. The external cuticle, which surrounds the internal germ membrane which gives rise to the parts of the larva.

Synonyms: Hydatid echinococcosis, cystic echinococcosis and hydatid cyst.

INTRODUCTION

EPIDEMIOLOGY

Hydatid disease is a cosmopolitan disease but is seen particularly in sheep farming areas of Latin America, Oceania, China, North and East Africa (Kenya, Tanzania and Ethiopia) and central and southern Europe. Outbreaks are seen in France in Provence, Corsica and in the South West.

Human infestation occurs by eating food or drinking water contaminated by *Echinococcus granulosus* embryophores or by direct contact with a carrier dog.

LIFE CYCLE

- **The usual cycle is domestic,** involving the final host (dog) and the intermediary host (sheep).

  Adults live in the dog intestine. The embryonated eggs are excreted externally in dog faeces and are highly resistant to external conditions, being able to survive for several months. The sheep becomes infested by grazing on contaminated grass. The embryons enter the sheep gastro-intestinal wall, reaching the liver through the portal system and occasionally the lung or more rarely other sites. The embryon is converted into the hydatid larva in the sheep organs. The dog becomes infected by eating the organs (liver and lungs) of infested sheep.

- **The cycle in human beings:** Humans can accidentally enter the cycle, acting as the intermediary host, then forming a parasite blind alley. They are infested by eating eggs excreted externally in dog faeces, either directly or indirectly. The egg releases a hexacanth embryon which follows a similar pathway to that of the sheep in human beings. The embryon is transformed slowly into a larva or hydatid cyst. The structure of the cyst is identical in human beings and animals.

SYMPTOMS

The incubation and invasion phases are long and can last for several years.

Clinical features suggest tumour associated with the sites containing larvae.

- **The commonest form is hepatic** (60 to 80% of cases) and is often asymptomatic and discovered in a routine examination. This classically involves an association of hepatomegaly, jaundice and painless abdominal swelling. Complicated forms are due to cyst rupture in the biliary tract, biliary tract compression or a liver abscess.
- **The pulmonary form** makes up 20 to 30% of cases and is also often asymptomatic and found in a routine radiological investigation. Rupture of the cyst in the bronchi leads to vomiting associated with haemoptysis, cough and dyspnoea.
- **Other sites:** Hydatid cysts can develop more rarely in other organs such as bone, brain, kidney, spleen and heart.

INDICATIONS FOR MEASUREMENT

Diagnosis of hydatid disease in a person returning from an endemic region with suggestive clinical and/or radiological and/or biological signs.

Diagnosis of hydatid disease in a person in contact with dogs or particularly sheepdogs with suggestive clinical, laboratory or radiological signs.

Confirmatory diagnosis of hydatid disease after radiological discovery of cysts.

INFORMATION

SAMPLE

Blood:

– Taken into EDTA for a full blood count

– Taken into a dry tube (serum) for serological and immunological investigations.

Hydatid fluid in a pre-operative cyst sample.

QUESTIONS FOR THE PATIENT

Clinical signs?

History of direct contact with a sheepdog and/or return from an endemic region?

Current medical anti-helminth treatment?

SAMPLE STORAGE AND TRANSPORT

Serum can be stored at + 4°C for a week then frozen at– 30°C for a year.

DIAGNOSTIC METHODS

NON-SPECIFIC GUIDING DIAGNOSTIC FEATURES

- **Blood eosinophilia:** Usually normal but is raised in the invasive phase and if a cyst ruptures. Eosinophilia may be associated with allergic reactions.
- **Liver profile:** Disturbed if the biliary tract is compressed.
- **Total and specific IgE:** Raised in 60% of cases.

DIRECT SPECIFIC DIAGNOSIS

This has very limited use as it is only possible to test for the pathogen in pre-operative hydatid cyst samples. Cyst puncture is formally contraindicated as it carries a risk of dissemination with secondary echinoccocosis and anaphylactic shock.

INDIRECT DIAGNOSIS

Many serological reactions are available and use different qualities of antigens (soluble or cell based) resulting in variations in sensitivity and specificity.

SEROLOGICAL METHODS

SCREENING

- **Indirect immunofluorescence** uses cell-based antigens (*Echinoccocus granulosus* scolex sections obtained from a hydatid sand) and produces cross-reactions with *E. multilocularis* and cysticercosis.
- **The indirect haemaglutination reaction** uses sheep red blood cells sensitised by hydatid fluid, although produces cross-reactions with other helminths.
- **The ELISA reaction** uses a hydatid antigen purified from fertile cysts and offers good specificity. It is automatable and well suited for mass screening.
- **Electrosyneresis** is a precipitation reaction on a cellulose acetate medium or in agarose gel between the test serum and a soluble extract of the antigen obtained from a freeze-dried hydatid dust powder. This offers good specificity.

CONFIRMATION

- **Immunoelectrophoresis:** The presence of arc 5 specific to the *Echinococcus* genus indicates hydatid disease.
- **The immunoblotting method** is more sensitive and specific and easier to read. The profiles usually point the diagnosis towards infection with *E. granulosus* or *E. multilocularis*, or failing this, to the *Echinococcus* genus.

INTERPRETATION OF RESULTS

The diagnosis of certainty for hydatid disease is microscopic and histological examination of hydatid fluid from the surgical specimen.

Serology remains the essential stage in the pre-operative diagnosis

Serological methods enable the diagnosis to be made in 80 to 95% of hepatic hydatid disease and in 40 to 65% of pulmonary hydatid disease. Screening serology must be supplemented by a confirmatory method if positive. The results of serological reactions should be interpreted with caution; a positive result at a significant titre produces a positive diagnosis although a negative result does not exclude hydatid disease, particularly in cases of a calcified hydatid cyst or pulmonary disease.

Serology can also be used to monitor the effectiveness of treatment. Serum antibody concentrations rise within weeks of surgery and then fall, disappearing over 12 to 24 months. Persistent high antibody titres beyond this period, or a further rise after becoming negative, suggest treatment failure (recurrence or secondary echinococcosis).

TREATMENT

- **Surgery:** This is the treatment of choice, particularly in complicated disease. Precautions need to be taken during excision to avoid dissemination of the parasite due to cyst rupture.
- **Drugs:** Albendazole used as a complement to surgery or in inoperative hydatid disease.
- **Prophylaxis:**

General:

– Health measures: Monitor dogs food intake, exclude stray dogs from abattoirs, incinerate parasite contaminated offal, monitor slaughtering of butchery animals and regular worming of domestic dogs

– Farm sheep in enclosed pastures.

Individual:

– Avoid human-dog contact (licking, stroking, etc.), particularly in endemic areas

– Hand hygiene before preparing and eating meals, particularly after stroking a dog

– Preventative treatment of secondary echinococcosis with benzimidazoles.

FOR FURTHER INFORMATION

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- McManus D.P., Zhang W., Li J., Bartley P.B., *Echinococcosis*, Lancet 2003; 362: 1295-1304.

HYDROQUINIDINE

DEFINITION

Hydroquinidine is a "class Ia anti-arrhythmic" according to the Vaughan-Williams classification (membrane stabiliser class). It is marketed as delayed release capsules containing 30 mg of hydroquinidine hydrochloride and is indicated for use in prevention of recurrence of life-threatening ventricular tachycardias, documented symptomatic incapacitating ventricular tachycardias, in the absence of reduced left ventricular function, and documented supraventricular tachycardias in the absence of reduced left ventricular function.

Its anti-arrhythmic properties are due to a change in membrane permeability to sodium and potassium ions and an effect on myocardial cell depolarisation and repolarisation. The usual dose is 1 to 2 capsules daily at 12 hour intervals.

PHARMACOKINETICS

Bioavailability (oral)	Approximately 90%
Plasma peak	Tmax: 7 hours
Steady state achieved in	3 days
Protein binding	80%
Metabolism	Hepatic, mostly by hydroxylation. The main metabolite identified (18%) is 3-OH hydroquinidine, which is active.
Plasma half-life of elimination	7 to 9 hours
Elimination	Urinary, in form of metabolites and unchanged hydroquinidine (10 to 20%) Excretion is delayed by urinary alkalinisation and by reduced glomerular filtration.

INDICATION FOR MEASUREMENT

Monitoring the effectiveness of treatment (avoiding reaching toxic levels).

INFORMATION

■ SAMPLE

Serum or plasma taken into EDTA or heparin tubes. Avoid tubes with separator gel.

Take sample ***immediately before next dose*** (trough concentration), at steady stage (3 days after starting treatment).

■ QUESTIONS FOR THE PATIENT

Any request for drug measurement must include the reasons for requesting (investigation for efficacy or toxicity), sampling time, treatment start date and/or any change to dose, dosage information (amount administered, frequency, route of administration) and the age, height and weight of the person whenever possible.

Are you taking any other drug treatment? Association with oral magnesium causes an increase in blood hydroquinidine and a risk of overdose (from reduced urinary excretion).

In general, anti-arrhythmic treatment is with monotherapy (associations involve specialist advice). Hydroquinidine is contraindicated in association with other compounds which risk causing torsades de pointe: class 1 (disopyramide), class 3 (dofetilide, ibutilide, sotalol) anti-arrhythmics and others (cisapride, bepridil, sultopride, IV erythromycin, IV vincamine, etc.).

■ SAMPLE STORAGE AND TRANSPORT

Separated plasma/serum can be stored for up to 24 hours at room temperature, 1 week at + 4°C and at least 1 year at –20°C.

Transport: plasma should be separated and frozen at –20°C within 4 hours of sampling if the analysis is deferred.

ASSAY METHODS

High performance liquid chromatography with UV detection.

NORMAL EXPECTED VALUES

Therapeutic range for trough levels: 1 to 2.5 mg/l i.e. 3 to 7.5 µmol/l.

Plasma/serum hydroquinidine concentrations > 3 mg/l (> 9 µmol/l) are deemed to be toxic. Signs of overdose are seen after high doses (≥4g hydroquinidine base). These are gastro-intestinal, neurosensory (visual, auditory), respiratory (apnoea), agitation and hypotension. ECG signs range from bundle branch block, second degree block or widened QRS complexes to serious ventricular dysrhythmias. Specialist hospitalisation is required.

NB: There is no merit in adjusting doses in renal insufficiency although this is required in hepatic and cardiac insufficiency (reduced total clearance and longer half-life of elimination).

FOR FURTHER INFORMATION

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■ Delhotal B., Compagnon P., *Suivi thérapeutique de l'hydroquinidine*. In: Suivi thérapeutique pharmacologique pour l'adaptation de posologie des médicaments, Collection Option/Bio, Ed Elsevier, Paris, 2004: 337-42.

HYDROXY-CORTICOSTEROIDS (17-)

DEFINITION

The human adrenal cortex is formed from three cell layers which, from the periphery inwards, are the zona glomerulosa (which secretes aldosterone), the zona fasciculata (which secretes cortisol) and the zona reticulosa (which secretes androgens); these three types of steroid hormones secreted by the adrenal cortex have the same common sterol nucleus and the same synthetic pathway, as all are produced from cholesterol. 17-hydroxy corticosteroids (17-OHCS) are steroids containing a hydroxyl group on the 17 carbon of the sterol nucleus. The main 17-OHCS found in urine are cortisol, cortisone, 11-deoxycortisol and their di-, tetra-, and hexa-hydrogenated metabolites in the form of glucuronide conjugates.

INTRODUCTION

The adrenocortical hormones are inactivated in the liver and then removed by the kidneys. 17-OHCS measurement is used to investigate adrenal function and to assess the amount of cortisol excreted daily. This is often combined with measurement of 17-ketosteroids.

INDICATIONS FOR MEASUREMENT

As part of the investigation of hypo- or hypercorticism, but has largely been replaced by the measurement of plasma cortisol and free urinary cortisol. 17-OHCS measurement is subject to many artefacts. It is influenced by situations which change cortisol metabolism but in which production is not increased, and large overlaps are seen between values in patients with hypercorticism and the obese.

INFORMATION

■ SAMPLE

The 24 hour urine collection is collected into an appropriate bottle and transported promptly to the laboratory (urine should be stored in a refrigerator during the collection.

If a sample is sent to a referral laboratory, indicate the urine output, age and sex of the patient.

■ SAMPLE STORAGE

8 hours at room temperature

1 week at +4° C; stored at -20° C 1 month.

ASSAY METHODS

The method used is the Porter and Silber method which measures steroids containing the 17,21-diOH-20 keto group i.e. di- and tetra-hydrogenated cortisol, cortisone and 11 deoxycortisol derivatives. 17OHCS are measured on a hydrolysed sample of a complete 24 hour urine specimen (the glucuronide conjugates are hydrolysed enzymatically with β-glucuronidase); 17-OHCS produce a yellow colour in the presence of phenylhydrazine and sulphuric acid, the intensity of which is measured spectrophotometrically. Only 50% of cortisol metabolites, however, are measured by this method and it is important to be aware that some drugs (phenothiazine, spironolactone, meprobamate and isoniazid) interfere with the colorimetric method and enzyme inducers reduce urinary 17-OHCS.

NORMAL EXPECTED VALUES

Urinary 17-OHCS concentrations are expressed in µmol (or µg)/24 hours and vary by sex and age. Reference values are shown below:

Age (Years)	Women (µmol/24 hours)	Men (µmol/24 hours)
< 2	0.3 - 2.1	0.3 - 2.1
2 -4	1.4 - 5.5	1.4 - 5.5
5 - 9	2.8 - 9.7	2.8 - 9.7
10 - 14	5.5 - 16.5	8.3 - 16.5
15 - 19	5.5 - 16.5	7.0 - 22.5
20 - 44	5.5 - 16.5	9.0 - 23.0
45 - 59	6.9 - 16.5	9.0 - 23.0
60 - 74	5.5 - 16.5	8.9 - 22.0
> 74	4.0 - 14.0	7.0 - 15.0

PATHOLOGICAL VARIATIONS

– Urine 17-OHCS concentrations are raised in hypercorticism and reduced in adrenal insufficiency.

– Measurement of urinary 17-OHCS in the metyrapone test (using an agent which blocks 11β-hydroxylase and therefore inhibits cortisol synthesis, causing an increase in its precursor, 11 deoxycortisol) is used to assess the hypothalamic reserve of ACTH, secretion of which is increased following the fall in cortisol. Urine 17-OHCS concentrations should reach values 2 to 3 times higher than basal concentrations.

– Dexamethasone suppression tests (using a synthetic corticosteroid which inhibits pituitary release of ACTH and therefore adrenocortical secretion) have been standardised to various types (short, low dose and high dose suppression) and are useful for a positive aetiological diagnosis of hypercorticism. Urinary 17-OHCS concentrations should fall to at least 50% in a high dose suppression test to indicate the presence of Cushing's disease.

FOR FURTHER INFORMATION

■ bbBertagna X., Raux-Demay M.C., Guilhaume B., Girard F., Luton J-P. *Cushing's disease*. In: Melmed S. ed. The pituitary. Cambridge: Blackwell Science, 1995; 478-545.

5- HYDROXYINDOLACETIC ACID

DEFINITION

5-hydroxyindoleacetic acid (5-HIAA) is the main metabolite of serotonin (5-hydroxytryptamine) which is a biogenic amine generated by the hydroxylation and decarboxylation of tryptophan. It acts as a neurotransmitter in the central nervous system and, when secreted into the blood, as a neurohormone. 5-HIAA is usually assayed in urine or sometimes in plasma, together with an assay of serotonin in the blood and urine. This test is almost always ordered to screen for or monitor carcinoid tumours (most of which arise in the digestive tract).

Synonyms: 5-hydroxy-indolylacetic acid, 5-HIAA.

INTRODUCTION

Serotonin is produced by enterochromaffin cells which are mostly found in the digestive tract (the stomach, duodenum, jejunum, rectum and appendix) but also in the pancreas, lungs, bronchi, thyroid and ovaries. Serotonin has physiological effects in many tissues and organs:

– In the vascular system (inducing vascoconstriction)

– On platelets (promoting aggregation)

– On the kidneys (inhibiting diuresis)

– In muscle tissue (conferring tone on smooth muscle fibres, accelerating digestive peristalsis and stimulating transit through the intestine)

– In the nervous system; on the hypothalamopituitary system and on the immune system. In pathology, it is involved in many problems such as insomnia, migraine, certain psychiatric disorders (notably depression, anxiety and schizophrenia), hypertension and allergy.

INDICATIONS FOR MEASUREMENT

The 5-HIAA assay is used to screen for carcinoid tumours and monitor patients with such a tumour to assess the efficacy of treatment. It is also a predictor of the likelihood of recrudescence and metastasis.

INFORMATION

SAMPLE

– 24-hour urine collection: Store the sample at +4 °C during the collection period.

– At the laboratory, acidify the urine by adding 12 N hydrochloric acid (HCl) to adjust the pH to between 2 and 3 (5-20 ml of acid solution, depending on the volume of the urine sample and its initial pH).

– Record the volume of urine passed.

– For 48 hours preceding the collection, the patient should avoid consuming foodstuffs that are rich in tryptophan or serotonin, i.e. tomatoes, avocado, bananas, dried fruits, citrus fruits, pineapple, kiwi fruit, plums, chocolate and seafood (molluscs).

Plasma: In a heparinised tube. The same dietary restrictions apply for the urine test.

QUESTIONS FOR THE PATIENT

Have you closely followed the dietary instructions in the last 48 hours?

Tumour type and treatment details (modalities and dates)?

Are you taking any medication that affects serotonin metabolism, such as an imipramine antidepressant, a monoamine-oxidase inhibitor or a selective serotonin re-uptake inhibitor?

SAMPLE STORAGE AND TRANSPORT

– Urine: One week at +4 °C or several months frozen. Transport at +4 °C within one week or frozen.

– Plasma: Centrifuge, decant and freeze immediately after drawing. Transport frozen (-20°C).

ASSAY METHODS

Radio-immunoassay or high performance liquid chromatography (HPLC) with fluorometric detection.

NORMAL EXPECTED VALUES

For reference purposes, normal levels in the urine are between 5 and 45 µmol/24 hours (0.7-3.60 µmol/mmol creatinine).

PATHOLOGICAL VARIATIONS

The level of 5-HIAA rises in various pathological situations:

– Benign conditions: hypertension, certain types of migraine, coronary heart failure, various intestinal problems (coeliac disease, intestinal lipodystrophy) and certain bronchial diseases

– Malignancies: small-cell lung cancer, certain forms of cancer of the pancreas or gallbladder, ovarian cancer and carcinoid tumours.

5-HIAA and carcinoid tumours

Carcinoid tumours account for about 50% of tumours of the appendix, 20% of tumours of the small intestine, and 10% of rectal tumours. Half of these tumours are asymptomatic but they may cause carcinoid syndrome (especially with tumours in the small intestine) which is characterised by flushing of the skin of the face, neck and extremities, cardiovascular problems and diarrhoea. Most carcinoid tumours are characterised by the overproduction of serotonin; in some, serotonin is secreted exclusively into either the blood or the urine and in others only 5-HIAA is produced which is why all three parameters need to be assayed for the purposes of screening. In practice, any elevation in the concentration of any of these three parameters points to a carcinoid tumour.

For monitoring following surgery, a 5-HIAA test should be carried out once every three months for the first year, then every six months, and thereafter once a year. Any rise in its concentration points to relapse or metastasis.

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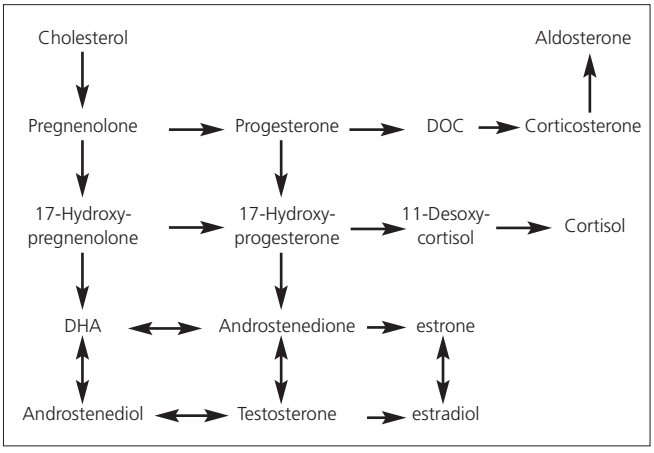
HYDROXYPREGNENOLONE (17-)

DEFINITION, SYNONYMS

17-hydroxypregnenolone (17-OH5P) is synthesised in the steroid-producing glands, the adrenocortical glands, testes and ovaries, where it is the obligatory intermediary in the biosynthesis pathways for glucocorticoids, androgens and oestrogens *(cf. figure)*. It should be noted, however, that in the adrenal cortex it is synthesised only in the zona fasciculata and zona reticulosa. It is one of the so-called "Δ5" steroids as it has a double bond between carbon atoms 5 and 6, unlike the so-called "Δ4" steroids (glucocorticoids, mineralocorticoids and sex steroids) where the double bond is located between carbon atoms 4 and 5. Interestingly, the steroid hormone cytostolic receptors do not bind "Δ5" steroids.



17-OH5P is secreted above all by the adrenal cortex and to a lesser extent by the testes, higher concentrations being found in spermatic veins than peripheral veins. This secretion follows a circadian cycle similar to that of cortisol, with a peak in the morning at around 06 00-08 00 hours and a trough around midnight.

17-OH5P can follow two metabolic pathways in steroid-producing glands. In the first it initially undergoes oxidative cleavage of the side chain, producing dehydroepiandrosterone (DHA), which is converted into androstenedione and testosterone. In the second pathway, 17-OH5P is converted into 17-hydroxyprogesterone, which can then either follow the androgen or cortisol metabolic pathways. The enzyme responsible for this conversion, 3β-hydroxysteroid dehydrogenase (3β-HSD), is expressed not only in the gonads, adrenals and placenta, but also in peripheral tissues (skin, adipose tissue, lung, brain, mammary glands and liver). It exists as two isoenzymes: 3β-HSD type 1 and 3β-HSD type 2. The gene coding for the type 1 enzyme is expressed above all in the skin, mammary glands and placenta, whereas the other gene is expressed almost exclusively in the adrenal glands, testes and ovaries.

The C20 ketone group of 17-OH5P is reduced in the liver producing pregnenetriol, which is then sulfo-conjugated before being removed in urine.

INDICATIONS FOR MEASUREMENT

17-OH5P measurement is indicated above all in the investigation of hirsutism and virilisation in girls and women. Measurement is also recommended in female pseudohermaphroditism and male pseudohermaphroditism to make a diagnosis of 3β-hydroxysteroid dehydrogenase deficiency.

INFORMATION

SAMPLE

17-OH5P can be measured both in plasma and in serum. The anticoagulant does not interfere in measurement. Lipaemic and haemolysed samples do not raise problems. The sample, however, must be taken in the morning before 10 00 hours in view of the circadian cycle variations in serum and plasma 17-OH5P concentrations and obviously, stressful conditions must be avoided.

ESSENTIAL INFORMATION

Because 17-OH5P is produced predominantly by the adrenal cortex, corticosteroid treatments (systemic, topical or intra-articular) must be reported. Similarly, the request should state whether measurement is part of an adrenocortical Synacthen® stimulation test.

SAMPLE STORAGE AND TRANSPORT

Separated plasma or serum is stable for several days at + 4°C and should be transported to the laboratory at this temperature.

Samples may be stored for 6 months to 1 year frozen at below -20°C.

ASSAY METHOD

It is assayed by radioimmunoassay with a pre-purification stage involving extraction followed by chromatography. This is required to obtain good specificity. The antisera available are not sufficiently specific to allow radioimmunoassay to be performed directly on the plasma or serum aliquot.

USUAL VALUES

17-OH5P concentrations are higher in men (0.60 to 3.40 ng/ml) than in women (0.10 to 2.40 ng/ml). Ng/ml are converted to nmol/ml by multiplying the ng/ml result by 3.008.

PHYSIOLOGICAL VARIATIONS

– As 17-OH5P comes mostly from the adrenal cortex, its concentrations vary during the day following a parallel circadian cycle to that seen for cortisol and dehydroepiandrosterone (DHA).

– Plasma 17-OH5P concentrations at birth are higher both in boys and girls and then fall regularly during the first year of life, reaching a plateau where they remain until the age of approximately 5 years old. Concentrations subsequently rise gradually until puberty reaching adult values.

– Functional investigations

The Synacthen® stimulation test is easy to perform and can reveal an enzyme deficiency in the adrenocortical steroid biosynthesis pathway which is not apparent in the basal state. This test is recommended particularly in women with signs of hyperandrogenism and involves injecting 0.25 mg of Synacthen® IV or IM and taking three blood samples, one before the injection and the other two after 30 and 60 minutes. The 17-OH5P response in women without hyperandrogenism varies between 2.10 and 9.50 ng/ml.

PATHOLOGICAL VARIATIONS

■ 3β-HYDROXYSTEROID DEHYDROGENASE DEFICIENCY

As 3β-HSD has two isoenzymes, which differ in tissue location, the features of the disease are very varied. The deficiency may be only adrenal and/or ovarian and/or hepatic. Like the other enzyme deficiencies, it is an autosomal recessive inherited disease.

■ Early presenting forms

Complete block of the 3β-HSD results in failure of cortisol, aldosterone, androgen and oestrogen biosynthesis. Clinically these forms result in:

– Adrenal insufficiency with salt loss and

– Pseudohermaphroditism:

- Ambiguous external genital organs in boys due to deficient testosterone and dihydrotestosterone during the pre-natal wave of testicular activity

- In girls, more or less pronounced masculinisation of the external genital organs generally attributed to excess testosterone arising from the conversion of Δ5 steroids into Δ4 steroids by hepatic or placental 3β-HSD.

■ Late presenting forms

These are characterised by hyperandrogenism, which is entirely similar to what is seen in 21-hydroxylase and 11β-hydroxylase deficiencies. The clinical picture may also resemble that of polycystic ovarian syndrome, which is secondary either to the effect of hyperandrogenism on the ovary or to 3β-HSD deficiency in the ovary.

The laboratory diagnosis is made by identifying the accumulation of Δ5 compounds (pregnenolone, 17OH5P, DHA) and reduced Δ4 compounds (progesterone, 17-hydroxyprogesterone, testosterone, androstenedione). It has therefore been recommended that the 17OH5P to 17-hydroxyprogesterone ratio should be calculated and that this should be less than 10.5, or alternatively the pregnenolone to progesterone ratio, which should be less than 12.5. Some Δ4 compounds can be raised because of the presence of a non-deficient hepatic 3β-HSD and as a result these ratios are not particularly reliable.

The immediate Synacthen® stimulation test is useful to diagnose late presenting forms. A very large rise in 5-P and 17-OH5P after Synacthen® reveals 3β-HSD deficiency. A 17-OH5P concentration of 15 ng/ml is generally accepted to support the diagnosis.

■ ADRENOCORTICAL ADENOMAS AND CARCINOMAS

Secretion of cortisol precursors, particularly the Δ5 compounds pregnenolone and 17-OH5P is often increased in adrenocortical adenomas and carcinomas. The respective urinary metabolites, pregnenediol and pregnenetriol, are also obviously greatly elevated.

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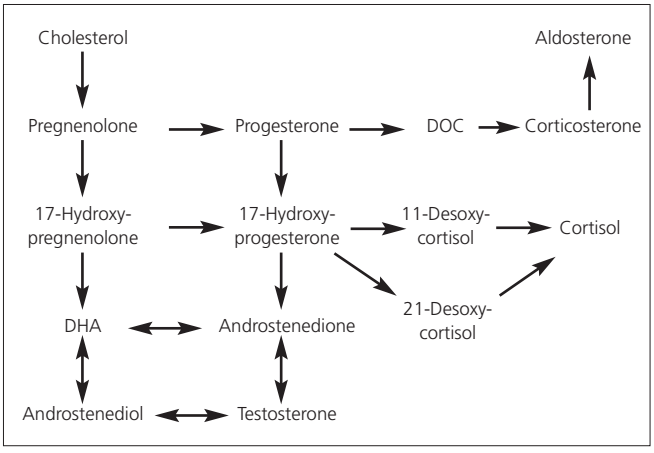
■ Orth D.N., Kovacs W.J., *The adrenal cortex*. Dans : Wilson J.D., Foster D.W., Kronenberg H.M., Larsen P.R., eds. Williams Textbook of Endocrinology, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, 1998:517-664.

HYDROXYPROGESTERONE (17-)

DEFINITION - INTRODUCTION

17-hydroxyprogesterone (17-OHP) is synthesised in all of the steroid-producing tissues, the ovaries, testes and adrenal glands, where it is an important stage in the biosynthesis of androgens, oestrogens and cortisol.



Circulating 17-OHP differs in origin depending on sex and age:

Subject	Main origin
Prepubertal child Woman (FP*) Postmenopausal woman	Adrenal
Woman (LP*)	Ovarian (Corpus luteum)
Man	Testes

**FP = follicular phase; LP = luteal phase*

17-OHP is obtained from the adrenal gland in prepubertal children and post-menopausal women. It is produced mostly from testicular secretion in men.

In women during the active ovarian phase of life, 17-OHP it is produced mostly by the adrenal cortex at the beginning of the follicular phase and almost entirely by the ovary in the pre-ovulatory and luteal phases.

During pregnancy, 17-OHP is produced by the gestational corpus luteum, which it is a better reflection of activity than progesterone because of the limited activity of placental 17-hydroxylase. At the end of pregnancy, 17-OHP comes from the foetal adrenal gland.

Regardless of origin, a large proportion of 17-OHP circulates in the blood bound to CBG *(Corticosteroid Binding Globulin)* and albumin and a very small proportion are unbound (approximately 2.5%).

17-OHP is metabolised in the liver by successive reductions. The main metabolite, 5β-pregnane-3α, 17α, 20α-triol, commonly called pregnanetriol, represents approximately 30% of the 17-OHP produced. It is conjugated to glucuronic acid and then removed in the urine as the glucuronide.

Synonyms: 17-Hydroxyprogesterone =17α-Hydroxy-progesterone = 17-OHP

INDICATIONS FOR MEASUREMENT

Measurement of 17-OHP is recommended in the investigation of hirsutism and virilisation in girls and women to identify 21-hydroxylase deficiency.

INFORMATION

■ SAMPLE

17-OHP is measured either in serum or in plasma. The anticoagulant does not interfere in the measurement. Similarly, lipaemic and haemolysed samples do not raise problems as the method used includes extraction. The sample, however, must be taken in the morning before 10 00 hours in view of the circadian variations in 17-OHP concentrations.

■ QUESTIONS FOR THE PATIENT

In addition to sex and age the request should state the phase of the menstrual cycle or term of pregnancy for a woman during the active ovarian phase of life. Similarly, contraceptive treatments and corticosteroids being taken should be reported. Synacthen® stimulation test or hCG must be reported as should patients suffering from 21-hydroxylase deficiency who are receiving corticosteroid treatment.

■ SAMPLE STORAGE AND TRANSPORT

Separated plasma or serum is stable for several days at + 4°C and can be transported to a specialist laboratory at this temperature.

It may be stored for 6 months to 1 year frozen at below –20°C.

ASSAY METHODS

The methods used are immunoassays with isotopic or non-isotopic labels. The antisera now available are sufficiently specific to enable the assay to be performed without chromatography although extraction prior to immunoassay is necessary in new-born babies and young children as 17-hydroxypregnenolone sulphate concentrations are very high in these situations and interfere with 17-OHP measurement, as they produce large cross-reactions with most antisera.

NORMAL EXPECTED VALUES – PHYSIOLOGICAL VARIATIONS

Results are expressed either in ng/ml or nmol/l. Ng/ml are converted to nmol/ml by multiplying the ng/ml result by 3.026.

17-OHP concentrations are high in cord blood, with no difference between boys and girls. Values are very high in both sexes at birth compared to adult men and adult women during the follicular phase. Concentrations then fall very quickly during the first 24 hours after birth and continue to fall during the first week both in girls and boys. Beyond this age the changes differ between males and females. In girls, concentrations continue to fall for the first year of life whereas in boys, 17-OHP begins to increase secondarily reaching a peak at between 30 and 60 days and then falling with a profile similar to that of testosterone.

From the end of the first year of life until pre-puberty, concentrations are low and similar in both sexes. The pubertal rise begins gradually from the age of 7-8 years old.

17-OHP remains at low levels in adult women during the first week of the menstrual cycle and then rise reaching a peak at pre-ovulation in parallel to that of LH. After ovulation, 17-OHP rises further, reaching concentrations, which are often higher than in the pre-ovulatory peak.

	FP (1st week)	FP (2nd week)	Pic Pre-ovulatory peak	LP
17-OHP (ng/ml)	0.3 – 0.8	0.3 – 1.5	1.4 – 3.1	1.7 – 5.2

FP = follicular phase; LP = luteal phase (D+4 to D+9 after the LH peak)

The changes in 17-OHP are very different to those of progesterone during pregnancy and are characterised by three phases. Concentrations at the beginning of pregnancy are above the mean for the luteal phase. The typical curve reaches a peak of around 7 ng/ml at the 5th week of pregnancy and then falls to a trough of approximately 3.5 ng/ml between 12 and 15 weeks. Concentrations then remain almost at a plateau until around the 30th week and then rise rapidly reaching the same levels as those seen at the 5th week.

Week of pregnancy	Concentration (ng/ml)
3 - 4	2.4 - 10.5
5 - 6	2.8 - 13.0
7 - 8	1.6 - 11.0
9 - 10	1.2 - 6.6
11 - 12	1.2 - 5.5
13 – 14	1.3 - 5.3
15 – 16	1.3 - 5.4

Plasma concentrations in adult men vary between 0.5 and 2.5 ng/ml in the morning.

PATHOLOGICAL VARIATIONS

■ DIAGNOSIS OF 21-HYDROXYLASE DEFICIENCY

17-OHP measurement is indicated particularly for the diagnosis of congenital adrenal hyperplasia due to 21-hydroxylase deficiency. Because of the enzyme block, the previous steroid in the pathway accumulates.

■ Basal state measurement

Two types of enzyme deficiency should be distinguished:

– Classical severe forms with salt loss or isolated virilisation in which the rise in plasma 17-OHP is diagnostic in itself

– Atypical forms, which have various names depending on the symptoms (latent, acquired, partial, attenuated, incomplete, adult, or late presenting forms) are characterised by early pseudo-puberty, acne, hirsutism or sterility. The enzyme block is less severe in these cases but obviously concentrations are lower than in the classical forms and vary greatly between individuals. They may therefore not be as straightforward to diagnosis.

In practical terms, providing that the sample has been taken under correct conditions, a 17-OHP concentration of more than 10 ng/ml provides a straightforward diagnosis. If the concentration is between 3 and 10 ng/ml, it is recommended that another sample be checked. Below values of 10 ng/ml, a Synacthen® test removes the doubt.

■ Measurement after stimulation

The most widely used Synacthen® test at present lasts for one hour and can be performed on an outpatient basis using the following protocol: one vial (0.25 mg) of Synacthen® is injected IM and samples taken for 17-OHP measurement before the injection (time 0) and 60 minutes later. Interpretation is usually based on the response peak.

An atypical deficiency can be diagnosed if the peak is more than 10-12 ng/ml. In this situation, the 17-OHP concentration is sufficient in itself to make a diagnosis of an atypical form of 21-hydroxylase deficiency. If the least doubt remains, then the diagnosis can be confirmed by measuring 21-deoxycortisol *(cf. 21-Deoxycortisol)*.

It should be noted that this test has been found to be rather difficult to use to diagnose heterozygotes, as there is a large overlap between the response of heterozygotes and normal controls. In this case the diagnosis is currently based on measurement of 21deoxycortisol for which there is clear separation between the response to Synacthen® by heterozygotes and normal controls *(cf. 21-Deoxycortisol)*.

■ Screening for atypical forms of deficiency

Screening has been proposed for atypical deficiencies at birth using blood spots on paper although is less justified than screening for congenital hypothyroidism.

■ DIAGNOSIS OF 17α-HYDROXYLASE DEFICIENCY

Very low 17-OHP concentrations associated with raised concentration of ACTH, progesterone and other non-17 hydroxylated steroids (corticosterone and DOC) provide a diagnosis in homozygotes, and should be considered in a patient with hypertension associated with hypergonadotrophic hypogonadism and delayed puberty in boys.

17-OHP progesterone and other steroid concentrations may be normal in heterozygotes, although the response to ACTH stimulation is poor or zero for 17-OHP and exaggerated for progesterone and the other non 17-hydroxylated steroids.

■ IDENTIFYING ANDROGEN DOPING

17-OHP measurement has recently been proposed to detect exogenous testosterone administration used for doping purposes in athletes. Androgen administration causes a fall in 17-OHP secretion because it is predominantly testicular in origin. Studying the testosterone/17-OHP ratio in serum or plasma therefore appears to be a good doping marker whereas the testosterone/epitestosterone ratio measured in urine, which is generally recommended may be falsely lowered by simultaneous administration of epitestosterone.

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HYDROXYPROLINE

DEFINITION

Hydroxyproline is an amino acid which is non-essential to human beings and is found in many proteins. It is formed by the hydroxylation of proline after its incorporation into proteins.

INTRODUCTION

Because of the rich hydroxyproline content of fibrillar collagens, urinary measurement of this amino acid has long been the most widely used marker of bone resorption. Free hydroxyproline and urine peptide hydroxyproline are produced from the metabolism of collagen and normal bone remodelling; 95% of hydroxyproline excreted in urine is bound to peptides.

Hydroxyproline is increased whenever increased bone resorption is present.

INDICATIONS FOR MEASUREMENT

This analyse has long been indicated for use in assessing any situation with increased bone modelling. Its use has declined greatly since the emergence of more specific markers of bone resorption such as cross laps (or CTx), NTx, and deoxypyridinoline.

INFORMATION

■ SAMPLE

In order to remove dietary interferences, patients must avoid eating foods rich in collagen for 48h before the sample is taken (meat, cooked pork meats, jelly, gelatine, ice creams and pastries).

Urine should be collected for 24 hours into a urine collection bottle without preservative. Keep the bottle at + 4°C between each passing of urine. Transport the sample promptly to the laboratory at the end of the collection.

■ SAMPLE STORAGE

The Sample can be stored for several months at -20°C.

■ QUESTIONS FOR THE PATIENT

Suspected disease and current treatment?

ASSAY METHODS

The reference method is ion exchange high performance liquid chromatography with electrochemical detection.

NORMAL EXPECTED VALUES

Adults: < 13 µmol/mmol of urine creatinine.

Urine hydroxyproline can vary from day to day in the same person and increases physiologically in pregnancy.

Values in children are 2 to 4 times higher because of the extent of bone remodelling during growth.

Conversion factor: µmol = mg x 7.63

mg = µmol x 0.131

PATHOLOGICAL VARIATIONS

Large increases

Paget's disease

Hyperparathyroidism

Hyperthyroidism

Acromegaly

Bone metastases.

Moderate increase

Osteomalacia

Marfan's syndrome

Klinefelter's syndrome

Extensive burns.

FOR FURTHER INFORMATION

■ Garnero P., Bianchi F., Carlier M.C., Genty V., Jacob N., Kamel S., Kindermans C., Plouvier E., Pressac M., Souberbielle J.-C., *Les marqueurs biologiques du remodelage osseux : variations pré-analytiques et recommandations pour leur utilisation*, Ann biol clin 2000; 58: 683-704.

HYPOXANTHINE

DEFINITION

Hypoxanthine or 6-hydroxypurine is a purine base. It is associated with nucleic acid metabolism and is produced from the degradation of inosine by nucleoside phosphorylase (PNP).

INTRODUCTION

Hypoxanthine is a purine base catabolite. These bases may be produced either from the diet or from *de novo* synthesis. The *de novo* synthesis is auto-controlled by hypoxanthine guanine phosphoribosyl transferase (HPRT), which catalyses guanine and hypoxanthine recovery by recycling them into nucleotides. Outside of this recovery process, hypoxanthine is rapidly catabolised into xanthine and then into uric acid, the end product of purine base catabolism.

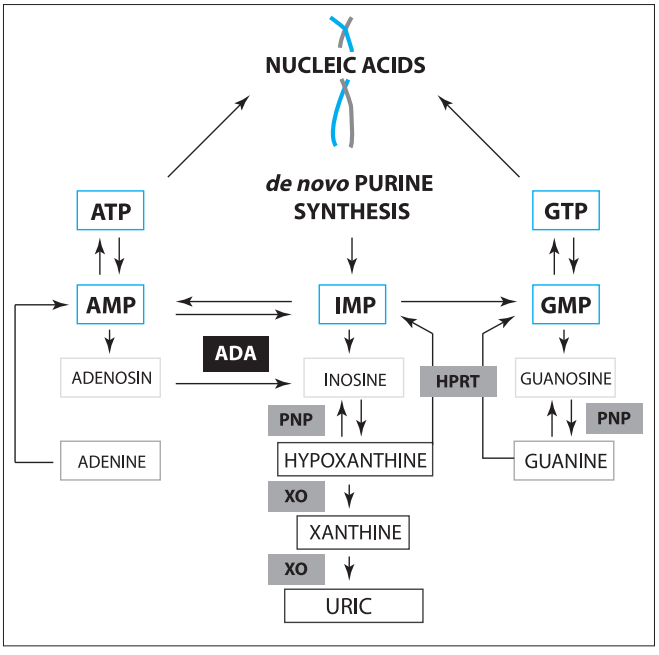

Figure 1: Purine base metabolism: metabolites: (ATP, AMP: Adenosine tri and mono phosphate; IMP: Inosine monophosphate; GTP, GMP: guanosine tri and mono phosphate) and enzymes (ADA: Adenosine desaminase, HPRT: Hypoxanthine guanine phosphoribosyl transferase, PNP: Nucleoside phosphorylase, XO: xanthine oxidase).

Serum and urine hypoxanthine concentrations are very low under normal physiological conditions. Excess hypoxanthine, which is soluble, is removed in urine. Hypoxanthine and uric acid metabolism are therefore closely linked.

Hypoxanthine measurements are used for:

– Aetiological investigation of disorders associated with excessive uric acid production:

– Haematological disorders with red cell lysis (haemolysis, blood dyscrasias and polycythaemia);

– Muscle disease in rhabdomyolysis or physical exercise;

– Problems associated with the diet, such as excessive intake of alcohol and/or purine rich foods (liver, offal, kidneys and anchovies) and obesity.

– Diagnosis of Lesch-Nyhan Syndrome:

This is an X-linked genetic disease characterised by deficiency of HPRT, the substrates for which are hypoxanthine and phosphoribosylpyrophosphate (PRPP). The confirmatory diagnosis of Lesch-Nyhan syndrome is made from the identification of deficiency of the enzyme HPRT or detection of an HPRT gene mutation.

Lesch-Nyhan syndrome presents from the first year of life with spasticity, choreoathetosis, mental retardation, compulsive self-harm and renal insufficiency due to nephrolithiasis. It is a rare disease with an incidence of 1 case per 300,000 births (there are around a hundred families in France). HPRT deficiency is complete in Lesch-Nyhan syndrome.

Different degrees of partial deficiencies exist characterised by nephrolithiasis, renal insufficiency, and gouty arthritis. This syndrome is referred to in the literature under the name Kelley-Seegmiller syndrome. Neuropsychiatric features do not occur in these cases and the diagnosis is made in patients around the age of 20 to 30 years. Increased urine and serum uric acid are responsible for renal stones.

Treatment with allopurinol (10 to 20 mg/kg), a xanthine oxidase inhibitor, inhibits uric acid formation and restriction of purine rich foods is also proposed.

INDICATIONS FOR MEASUREMENT

Suspected Lesch-Nyhan syndrome in a male infant suffering from self harm problems or uric acid crystalluria.

Aetiological investigation of attacks of gout or recurrent renal stones in adults.

INFORMATION

■ SAMPLE

24 hour urine collection. Patients should be recommended to avoid taking coffee, tea or chocolate for a day before and during the urine collection. The measurement should not be performed following a urinary tract infection.

■ QUESTIONS FOR THE PATIENT

Has the patient recently taken substances containing methylxanthine (coffee, chocolate, tea, etc.)?

Has the patient recently taken a urate-reducing agent such as allopurinol?

Does the patient suffer from attacks of gout or recurrent urinary stones? Has the patient a known family history of cases of Lesch-Nyhan syndrome?

■ SAMPLE STORAGE AND TRANSPORT

Urine can be stored 1 week at +4° C or must be frozen at –20°C if the measurement is to be performed later.

ASSAY METHODS

The assay method is enzymatic. Urine is treated with sodium hydroxide to remove the uric acid. Hypoxanthine is then converted into uric acid in the presence of xanthine oxidase. The conversion kinetics are read spectrophotometrically at 280 nm and 293 nm.

High performance liquid chromatography (HLPC) methods can be used to measure concentrations of all of the purine metabolites.

NORMAL EXPECTED VALUES

Urine hypoxanthine concentrations are less than 25 mg/24 hours, i.e. less than 0.20 mmol/24 hours.

FOR FURTHER INFORMATION

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IGF-1

DEFINITION – INTRODUCTION

Insulin-Like Growth Factor-1 (IGF-1) is a single chain, 70 amino acid, unglycosylated protein with a similar structure to that of proinsulin, and has a molecular mass of 7,649 daltons. IGF-1 gene expression is controlled primarily by growth hormone (or GH), although regulation involves other hormones; stimulation by estradiol, progesterone, androgens, insulin and thyroid hormones and in contrast, gene expression is reduced by cortisol. Regulation is also influenced by nutritional state. IGF-1 is produced almost ubiquitously, although the main producing tissues are the liver (responsible for 50% of plasma concentrations), muscle, lung, kidney and cartilage. IGF-1 is present in the circulation and in the extracellular spaces mostly as a form bound to high affinity proteins (5% of IGF-1 circulates free in the blood), the *Insulin-like Growth Factor Binding Proteins* (IGFBPs), 6 of which have been identified to date. This specific binding inactivates IGF-1 and its bioavailability is regulated by the IGFBPs. Only 5% of IGF-1 is free in circulating blood. IGF1 is removed mostly by the kidneys after enzymatic degradation.

IGF-1 plays an essential role in growth and a great majority of the effects of GH on growth are mediated by IGF-1 through both endocrine, paracrine and autocrine mechanisms. IGF-1 also exerts negative feedback control on pituitary GH production.

Synonyms: Insulin-like growth factor-1 and somatomedin C.

INDICATIONS FOR MEASUREMENT

In view of its role as the effector for GH activity, measurement of serum IGF-1 concentration is used to diagnose growth disorders: GH deficiency or acromegaly. IGF-1 secretion is considered to mostly reflect GH secretion, assuming no nutritional disorders (anorexia, or alternatively excess calorie intake) or chronic disease (renal or hepatic insufficiency, gastrointestinal diseases, etc.) are present. Its major advantage compared to GH, which is secreted in a pulsatile manner, is the absence of a 24 hour cycle of IGF-1 secretion. Interpretation of IGF-1 measurement, however, is limited in children under 5 years old (when IGF-1 concentrations are low).

It has recently been recommended that the biological effects of GH treatment should be monitored using regular IGF-1 measurements with the aim of maintaining levels within but not above the upper half of age-related physiological values, in view of the risk of, particularly gastro-intestinal, tumour induction with excessive GH exposure which has been demonstrated in acromegalic patients.

INFORMATION

SAMPLE

Serum or plasma collected into EDTA or heparin.

No circadian rhythm.

QUESTIONS FOR THE PATIENT

State the patient's age and include clinical details (growth disorders, nutritional disorders, symptoms of acromegaly, surgery, treatment, etc.). Ask if the patient is taking pegvisomant which lowers IGF1 (although increases GH).

SAMPLE STORAGE AND TRANSPORT

Centrifuge promptly. Serum or plasma is stable for 48 h at + 4°C. If the sample is to be transported, it should preferably be centrifuged and the serum or plasma frozen within 4 hours of the sample being taken. If the sample is already frozen, then transport at -20°C.

ASSAY METHODS

IGF-1 is assayed using isotopic or non-isotopic immunometric methods (so-called sandwich methods). Direct methods produce unreliable results because of the variable equilibrium between free and protein bound forms, particularly those bound to low affinity proteins from which IGF-1 may be easily detached.

Total IGF-1 is measured either after displacing IGF-1 from its protein binding with excess IGF-2 added to the sample or after breaking the bonds between IGF-1 and its transport proteins, notably IGFBP-1 and IGFBP-3. The reference method is exclusion chromatography on an acid gel. This is replaced routinely by an easy-to-use method which combines acid extraction of IGF-1 from its protein complexes and protein precipitation with ethanol.

Since 2008, assay kits have been calibrated against an international standard IS 87/518, although this is less than perfect and a new standard is currently being developed.

NORMAL EXPECTED VALUES

Physiological concentrations vary greatly with age and pubertal stage. Serum IGF-1 concentrations are very low at birth and increase greatly, reaching a peak at puberty and then fall thereafter throughout life.

Reference values may vary slightly between assay kits.

For reference:

µg/l → nmol/l conversion factor = x 0.1307

Adults (men – women)
18 to 20 years old: 220 to 580 µg/l
20 to 30 years old: 232 to 385 µg/l
30 to 40 years old: 177 to 382 µg/l
40 to 50 years old: 124 to 310 µg/l
50 to 60 years old: 71 to 263 µg/l
60 to 70 years old: 94 to 269 µg/l
70 to 80 years old: 76 to 160 µg/l

Boys
0 to 3 years old: 72 to 258 µg/l or 9.4 to 33.5 nmol/l
3 to 6 years old: 124 to 484 µg/l or 16.1 to 62.9 nmol/l
6 to 11 years old: 175 to 460 µg/l or 22.7 to 59.8 nmol/l
11 to 13 years old: 210 to 720 µg/l or 27.3 to 93.6 nmol/l
13 to 15 years old: 190 to 790 µg/l or 24.7 to 102.7 nmol/l
15 to 18 years old: 220 to 790 µg/l or 28.6 to 102.7 nmol/l

Girls
0 to 3 years old: 82 to 166 µg/l or 10.6 to 21.6 nmol/l
3 to 6 years old: 76 to 250 µg/l or 9.9 to 32.5 nmol/l
6 to 11 years old: 224 to 660 µg/l or 29.1 to 85.6 nmol/l
11 to 13 years old: 310 to 832 µg/l or 40.3 to 108.2 nmol/l
13 to 15 years old: 200 to 830 µg/l or 26.0 to 108.0 nmol/l
15 to 18 years old: 220 to 850 µg/l or 28.6 to 110.5 nmol/l

PATHOPHYSIOLOGICAL VARIATIONS

OUTSIDE OF THE GROWTH PERIOD

Corticosteroids

Corticosteroids suppress the somatotropic axis by a mixed central and peripheral mechanism.

Nutritional state and anorexia nervosa

The somatotropic axis is influenced by nutritional state and conversely, some somatotropic axis indices can be used to monitor a person's nutritional state. A raised GH /low IGF-1 (and low IGFBP-3) couple illustrates the GH resistance, which is always found in anorexia nervosa. In chronic malnutrition between 9 and 30 days are needed for IGF-1 values to return to normal after weight has been regained.

Osteoporosis

IGF-1 plays a very important role in the bone remodelling mechanism, stimulating osteoblast proliferation and type I collagen synthesis. It is also an important local mediator of the anabolic action of some hormones such as PTH on bone. Both bone mass and serum IGF-1 and IGFBP 3 concentrations have been clearly shown to fall with age.

Sport

The acute effects of exercise are mostly to lower free IGF-1 by raising carrier proteins, particularly IGFBP-1. Excessively intense physical activity causes a prolonged fall in IGF-1 due to a mismatch between nutritional intake and requirements.

Conversely, regular non-excessive exercise produces a chronic rise in plasma IGF1.

INVESTIGATION OF GROWTH DISORDERS

Investigation of somotropic function relies on measurement of GH (stimulation tests or investigation of physiological nocturnal secretion) combined with measurement of circulating IGF-1 and IGFBP-3.

RAISED IGF-1

The biological features of acromegaly include raised IGF-1 and oversecretion of GH which is not suppressed by a glucose load.

LOW IGF-1

– GH deficiency: Low IGF-1 and IGFBP-3. No response to GH stimulation tests. No physiological nocturnal GH secretion.

– Peripheral GH resistance: Normal or raised GH values associated with low IGF1 concentrations in a child with growth retardation suggest peripheral GH resistance, which may be either partial or complete (Laron dwarfism) associated with a mutation of the GH receptor gene. In equivocal cases the IGF1 stimulation test using an injection of GH can provide evidence supporting or against the diagnosis of partial resistance.

FOR FURTHER INFORMATION

- Chevenne D., Porquet D., *Hormone de croissance (GH) et insulin-like growth factor 1 (IGF-1) dans les états nutritionnels*, Ann Biol Clin, 1995; 53: 527-38
- Porquet D., *IGF-1 : métabolisme et action physiologique*, Nutr Clin Metabol, 1996; 10: 253-64
- Lahlou N., Roger M., *Mesures de l'hormone de croissance, des IGFs et de leurs protéines porteuses*, Med Therap. 1996, hors série n° 2 (Maladies de la croissance): 54-65.

IGFBP-3

DEFINITION

IGFBP-3 *(Insulin-like Growth Factor Binding Protein-3)* is the main IGF-1 *(Insulin-like Growth Factor-1)* binding protein. The IGFBPs are a group of proteins of homologous structure which specifically bind IGF-1 (somatomedin C) and IGF-2 with variable affinities. To date, six IGFBPs, coded for by different genes, have been isolated and cloned; these are classified as IGFBP-1 to IGFBP-6 and like IGF-1 are produced ubiquitously and are expressed variably depending on the tissue, stage of development and hormonal and nutritional environment. All of these proteins have been demonstrated in the extracellular spaces and in circulating blood. The liver is a major site of synthesis and the main source of circulating forms of the protein.

One of the main recognised functions of the IGFBPs is the transport of IGFs, particularly IGF1, in the circulation. IGFBP-3 plays an essential role as a circulating transporter of IGF-1 and is the factor controlling IGF-1 bioavailability to its receptor. The affinity of IGF-1 to IGFBP-3 is approximately 10 times greater than its affinity for the other IGFBPs and for its own receptor. 75 to 85% of IGF-1 circulates in a "large complex" form (140 to 150 kDa) which is one molecule of IGF-1 bound to one molecule of IGFBP-3 and a labile acid subunit (the "small complex" is formed mostly from binary IGF-1 and IGFBP-1, -2 and -4 complexes).

INDICATIONS FOR MEASUREMENT

Together with IGFBP-1, IGFBP-3 is the main IGF-1 transport protein which is clinically useful. IGFBP-3 secretion is partially GH dependent and this has been demonstrated by low IGFBP-3 concentrations in children with GH deficiency or genetic resistance (Laron dwarfism). IGFBP-3 measurement appears to provide similar information to that of IGF-1 in the investigation of GH deficiency. In a normal growth curve, plasma concentrations rise with age, although less so than IGF-1. Conversely, IGFBP-3 measurement in very young children does not pose sensitivity problems at low values unlike IGF-1.

INFORMATION

SAMPLE

Measurements should preferably be performed on serum, although heparinised plasma can be used.

Very lipaemic, haemolysed or jaundiced samples, or those with high protein concentrations should not be used.

No circadian variations appear to exist.

A fasting sample is not necessary.

ESSENTIAL INFORMATION

State the patient's age and include clinical details (growth disorders, nutritional disorders, clinical signs of acromegaly, surgery, treatment, etc.).

SAMPLE STORAGE AND TRANSPORT

Centrifuge and freeze the serum or plasma within 4 hours of the sampling to avoid IGFBP-3 degradation by serum proteases. Transport frozen at – 20°C.

ASSAY METHODS

Radioimmunometric or chemoluminescence assay.

NORMAL EXPECTED VALUES

These may vary depending on the method used.

Concentrations depend on age and pubertal stage, with a peak at puberty and then a progressive fall in adults.

IGFBP-3 values depending on age and pubertal status

(From HASEGAWA Y et al. Endocrine Journal 1993; 40: 185-190).

Age	IGFBP-3 in mg/l (mean +/-2 standard deviations)	
	Males	*Females*
1 month to 2 years	1.02 to 2.58	
2 to 4 years	1.32 to 3.12	
4 to 6 years	1.80 to 3.52	
6 to 8 years	1.61 to 3.73	2,00 to 4,00
8 to 10 years	2.00 to 3.96	2,18 to 4,02
10 to 12 years	2.22 to 4.62	
12 to 14 years	2.70 to 5.06	
14 to 18 years	2.57 to 4.53	
18 to 40 years	2.27 to 4.23	

Pubertal stage	IGFBP-3 in mg/l (mean +/- 2 standard deviations)
Tanner I	1.99 to 3.95
Tanner II	2.42 to 4.78
Tanner III	2.65 to 5.17
Tanner IV	2.93 to 4.73
Tanner V	2.81 to 4.77

PATHOLOGICAL VARIATIONS

Variations not specific to the somatotropic axis

– IGFBP-3 is useful in assessing nutritional status as it falls with protein and calorie restriction, although to a lesser extent than IGF-1.

– IGFBP-3 is increased in renal insufficiency because of defective removal.

– Other hormones interfere with IGFBP-3 synthesis: IGFBP-3 concentrations are low in pre-pubertal boys and increase in the presence of testosterone. IGFBP-3 falls by 40% after the menopause and can rise in women treated with oestrogen therapy. Concentrations are low in patients suffering from hypothyroidism and increase after thyroxine treatment. Poorly controlled diabetes can also cause growth retardation producing a fall in IGFBP-3.

– IGFBP-3 is also regulated by the action of a protease, which itself is altered in the following pathophysiological situations: Pregnancy (raise protease activity), GH deficiency, chronic renal insufficiency, diabetes, breast, ovarian and prostate cancer and recent surgery.

Pathological variations involving the somatotropic axis

– Childhood GH deficiency: Low IGFBP-3 and IGF-1, no response to the GH stimulation test and no physiological

nocturnal secretion. IGFBP-3 concentrations rise in these children after GH administration.

– Acromegaly: Raised IGFBP-3 and IGF-1 concentrations with increased GH secretion, not suppressed by an OGTT.

FOR FURTHER INFORMATION

- Chevenne D., Porquet D., *Hormone de croissance (GH) et insulin-like growth factor 1 (IGF-1) dans les états nutritionnels*, Ann Biol Clin 1995; 53: 527-38
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IMMUNOFIXATION

DEFINITION

Immunofixation is a sensitive identification method for a monoclonal immunoglobulin (mc Ig). It forms part of a global testing strategy for mc Ig involving serum protein electrophoresis, measurement of serum immunoglobulins and possibly testing for cryoglobulins.

It is a gel immunoprecipitation method. Samples, diluted to a greater or lesser extent, are loaded onto gels with predetermined electrophoretic migration lanes. After the serum constituents have been separated electrophoretically, the different lanes are incubated in the presence of specific antisera. When Ig is present it immunoprecipitates in the gel. The reaction is visualised by applying a protein stain after washing. Results must be accompanied by a comment from the laboratory specialist.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

Immunoglobulins are glycoproteins present in serum, extravascular fluids and secretions and have antibody activity. They are extremely heterogeneous despite having a common basic structure.

mc Ig represent a homogeneous population of immunoglobulins produced by a single plasmocyte clone. They are characterised by a single heavy chain class and a single light chain isotype and have the same allotype and same idiotype. They are often of normal structure but are produced in very large amounts. Their antibody activity has rarely been demonstrated.

A monoclonal Ig may be discovered fortuitously from an additional fraction on protein electrophoresis or may form part of the diagnostic investigation of lymphoid or lymphoplasmocyte proliferation. Immunofixation will occasionally reveal a monoclonal Ig which has not been seen on electrophoresis, particularly when present in small amounts (less than 1 g/l).This always enables the monoclonal Ig to be characterised.

In addition to its diagnostic use, immunofixation is used to monitor patients who have been shown to have a monoclonal Ig.

INFORMATION

SAMPLE

Dry tube (serum). Plasma must not be used.

Lipaemic or haemolysed sera must not be used.

ESSENTIAL INFORMATION

Clinical and laboratory context:

It is essential that clinical information is given particularly in the diagnostic situation (suspected lymphoplasmocyte proliferation, bone pain, etc; peripheral neuropathy), together with results of other laboratory (FBC, ESR, proteinuria, etc.) and radiological investigations.

SAMPLE STORAGE AND TRANSPORT

Samples can be stored for 4 days at + 4°C and for 3 months at – 20°C.

Transport at +4°C or at – 20°C if already frozen.

INTERPRETATION

– A relatively wide band indicates the presence of polyclonal Ig.

– A narrow band migrating at the same level as an anti-heavy chain serum (anti-gamma, alpha or mu) and an anti-light chain serum (anti-kappa or anti-lambda) indicates the presence of a monoclonal Ig. This narrow band is also seen on the first electrophoresis control lane.

– A narrow band found either in isolation with an anti-light chain serum without a narrow band at the level of a classical anti-heavy chain serum, requires further investigation. A second immunofixation must be performed, testing with anti-delta and anti-epsilon anti-serum is required before concluding that a monoclonal band is an Ig D or Ig E or when no precipitation occurs with these anti-sera, that a Bence-Jones protein is present (this migrates differently to the mc Ig with which it is occasionally associated as it has a different molecular weight).

– A narrow band revealed with an anti-heavy chain antiserum without a narrow band with an anti-light chain antiserum suggests heavy chain disease (depending on the clinical context).

– When a whole monoclonal Ig and/or Bence-Jones protein (BJP) is found in blood and/or urine by immunofixation, investigations must be continued for a possible malignant blood dyscrasia.

– For high concentrations of monoclonal IgG or IgA, the first line investigations are for myeloma, which has four immunochemical types. *(cf. table)*.

	Serum	Urine
1-whole mc Ig *	Ig +++	+/-
2-mc Ig + BJP	BJP +/-	+++
3-isolated BJP**	BJP +/-	+++
4-non-secretory myeloma	Ig/BJP = 0	0

** IgG in 50% of cases, IgA in 25% of cases, never IgM*

*** 24% of cases*

– The finding of IgM in the context of a blood disease points towards Waldenström's macroglobulinaemia (a monoclonal IgM is found in 100% of cases of this disease).

– A mc Ig is present in approximately 15% of cases of chronic lymphoid leukaemia, at low concentrations (< 5 g/l). This is usually IgG or IgM and rarely IgA.

– Benign monoclonal gammopathy (monoclonal gammopathy of unknown significance) is a diagnosis of exclusion. An isolated "benign" monoclonal Ig is found in 7.6% of people over 75 years old and in 10% of people over 80 years old. The Ig concentration remains stable and is always less than 30 g/l (usually < 15 g/l). No BJP, bone lesions, anaemia, hypercalcaemia or renal insufficiency are seen. It is recommended that clinical and laboratory investigations are monitored every 6 months in these patients combined with annual radiological investigation.

– "Benign" monoclonal Ig may also be associated with other disorders, such as primary or secondary immunodeficiencies, squamous cell cancers, some skin diseases, auto-immune diseases and viral or bacterial infections. A monoclonal Ig is often found in hepatitis C and HIV infections, usually at concentrations of approximately 5 g/l and normally disappears after a few months. A "benign" mc Ig has also been reported in approximately 10% of cases of primary hyperparathyroidism (particularly common in post-menopausal women).

INTERPRETATION DIFFICULTIES AND SOURCES OF ERROR

– Those applying to all antigen-antibody reactions

Antigen excess (zone effect) produces a band with a clear centre reflecting redissolution of the mc Ig. Conversely, antibody excess may occur when the serum is excessively diluted. Antisera can also vary in sensitivity (some reagents do not reveal small amounts of mc Ig which may however be demonstrated with other antisera). Analytical conditions must therefore be carefully followed and in particular, dilutions adjusted depending on the electrophoresis results.

– Type of serum

Staining in the loading zone on all lanes, regardless of the specificity of the antiserum used indicates proteins which have not migrated and have remained trapped in the gel. This may be a polymerised Ig (IgM or IgA) and/or cryoglobulin.

A reducing agent such as mercaptoethanol can be used to depolymerise the Ig. In the case of significant concentrations of cryoglobulin, the immunofixation should be repeated, heating the serum and placing both it and the buffer and gel at an appropriate temperature, depending on the temperature at which the cryoglobulin precipitates.

A narrow band seen on protein electrophoresis between the β and gamma globulins which is not revealed by an antiserum on immunofixation probably represents fibrinogen.

- Importance of clinical and laboratory information

It is important or indeed essential to have the electrophoresis results and clinical details:

Peripheral neuropathy, for example, is often accompanied by low concentrations of mc IgM (1 to 4 g/l) which may not be seen if co-existent hypergammaglobulinaemia is present. If this information is available, immunofixation can be organised, adjusting the serum dilutions.

It is also important to be aware of hypogammaglobulinaemia which can "mask" bone marrow invasion (the mc Ig may not be seen on protein electrophoresis). If a high clinical index of suspicion is present, all available methods should be used to investigate for a mc Ig.

FOR FURTHER INFORMATION

■ Pham B.N., Intrator L., *Immunoglobulines monoclonales : recherche et identification*. Le Cahier de formation, Biologie médicale n° 28, Bioforma, Paris, 2003: 44-65.

IMMUNOGLOBULINS G, A, M

DEFINITION

Immunoglobulins are glycoproteins present in serum, extravascular fluids and secretions, which have an antibody activity. They are extremely heterogeneous despite having a common basic structure.

They consist of monomeric structural units containing two identical halves, each consisting of a light chain (kappa or lambda) and heavy chain, connected together by one or more disulphide bridges. The heavy chains define the class and subclass specificity of the Ig:

– Gamma heavy chain: IgG class; subclasses IgG1, 2, 3, 4 (gamma 1, 2, 3 and 4 heavy chains respectively)

– Mu heavy chain (IgM)

– Alpha heavy chain (IgA class) containing two subclasses IgA1 (alpha1 heavy chain) and IgA2 (alpha 2 heavy chain)

– Delta heavy chain (IgD)

– Epsilon heavy chain (IgD).

The monomers can then be assembled into dimers, trimers or pentamers.

INTRODUCTION

Generally, immunoglobulins are involved in the humoral mediated immune response. Each class, however, has specific functions. Schematically, IgM forms the antigen receptor on the surface of B lymphocytes. They are the first to appear in serum after antigen stimulation and are the most effective in activating complement. IgG is mostly involved in the antigen removal process (by phagocytosis, antibody dependent cell mediated cytolysis or complement activation). IgA is involved in removal of bacterial or food allergens which have breached the intestinal wall. Secretory IgA (in saliva, tears, respiratory and gastrointestinal secretions and breast milk) is involved locally in protecting against an external agent entering through the mucosa. Finally, IgE is involved in allergy (triggering mastocyte and basophil degranulation).

INDICATIONS FOR MEASUREMENT

Immunoglobulin (Ig) measurements are common tests used in the investigation of humoral immunity. The electrophoretic gamma globulin protein fraction mostly reflects IgG and provides limited information about IgA and IgM.

Measurements are indicated in suspected immunodeficiency, particularly in certain infections (AIDS and shingles) or repeated infections with extracellular organisms (*Staphylococci, Streptococci, Pseudomonas, Haemophilus, Enterobacteriaciae*, etc.).

They are also used to monitor various diseases involving an immunological component, such as some malignant blood dyscrasias (myeloma, Waldenström's macroglobulinaemia and chronic lymphoid leukaemia) and alcoholic cirrhosis.

Monoclonal Ig can only be measured by immunoprecipitation (this specific immunoglobulin reacts different with immune sera and has a problem with antigen excess) in lymphoid proliferation with monoclonal Ig production. On the other hand, measurement of "residual" immunoglobulins is important to assess the patient's degree of immunosuppression.

Finally, Ig measurement is one of the protein measurements used in the so-called "screening" or "immune" protein profile.

INFORMATION

■ SAMPLE

Preferably into a dry tube, without separator gel (serum). Heparinised tubes can also be used.

Haemolysed and lipaemic samples must not be used.

■ QUESTIONS FOR THE PATIENT

Clinical and laboratory context (particularly if measurements form part of an investigation strategy for a monoclonal immunoglobulin).

State the patient's age and sex.

Current treatment: Drugs causing a large reduction in Ig:

– L-asparaginase reduces Ig concentrations by 30 to 60%

– Oral contraceptives reduce concentrations by 10 to 15%

– Treatments which may cause a slight fall in Ig: Phenytoin, glucocorticoids, immunosuppressants, chemotherapy and radiotherapy.

■ SAMPLE STORAGE AND TRANSPORT

Can be stored for 4 days at + 4°C and for 3 months at – 20°C.

Transport at + 4°C or at – 20°C if the sample is already frozen.

ASSAY METHODS

"Mass" measurement of polyclonal Ig by immunoprecipitation, immunonephelometry or immunoturbidimetry.

The best assay method (quantification) for a monoclonal immunoglobulin is currently protein electrophoresis (when the peak is measured by densitometry).

Technical difficulties:

– Specificity problem with immune sera

– Risk of antigen excess (zone effect) due mostly to the presence of occasionally very large amounts of a monoclonal component

– Non-specific reactions (occur with polymerised IgM).

NORMAL EXPECTED VALUES

International standard: CRM 470.

Work is ongoing in the IFCC *Committee for Plasma protein Standardization* to determine reference values.

Pending the results of this group, the reference values in Caucasian adults and adolescents based on CRM 470 are:

– IgG: 7 to 16 g/l

– IgA: 0.7 to 4 g/l

– IgM: 0.4 to 2.3 g/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Age:

IgG: Newborn babies only produce a small amount of antibodies. The IgG measured is mostly maternal in origin (crosses the placenta) and concentrations are the same as those in adults at approximately 7 to 16 g/l. Thereafter they fall until 3 months old reaching values of between 2.5 and 5 g/l. Levels at 1 year old are between 5 and 9 g/l and return to adult levels from the age of 6 years old.

IgM: Low concentrations at birth (0.05 to 0.30 g/l) increasing gradually (0.15 to 1 g/l at 3 months) reaching "adult" levels at approximately 9 months old.

IgA: Very low concentrations at birth: 0.0 to 0.20 g/l; 0.01 to 0.45 g/l at 3 months; 0.15 to 1.10 g/l at 1 year, then rising gradually reaching adult levels from the age of 14 years old.

Sex:

Serum IgM concentrations in women are higher than in men from the age of 7 years old onwards, whereas serum IgA concentrations in men are approximately 2 to 30% higher than those in women.

IgA falls by approximately 20% during the first 6 months of pregnancy in women and then rises in the 3rd trimester. IgG and IgM often fall in the 3rd trimester.

Geographical area:

Mean serum IgG and IgM concentrations are approximately twice as high in people living in tropical areas than those living in temperate areas (this rise is attributed to a higher incidence of infections).

PATHOLOGICAL VARIATIONS

Generally, a fall in one or all of the immunoglobulin classes reflects humoral immunodeficiency, which predisposes to extracellular infections. This deficiency may be congenital (rare) or acquired, particularly in malignant blood dyscrasias, viral infections (measles, rubella, herpes, cytomegalovirus, etc,) and autoimmune diseases (some forms of rheumatoid arthritis) or excessive Ig loss (nephrotic syndrome, exsudative enteropathy and extensive skin burns) *(cf. summary table)*.

An increase in the concentration of one or all classes of immunoglobulins indicates activation of the immune system, which may reflect infection (a very large number of bacterial infections are accompanied by a rise in Ig, particularly pulmonary infections and some parasitic diseases such as malaria, leishmaniasis or filariasis), vasculitis and connective tissue diseases (IgG in particular, rises in systemic lupus erythematosus, Gougerot-Sjögren's syndrome, some cancers and malignant blood dyscrasias *(cf. summary table)*.

Pathological variations in Ig *(non-exhaustive list)*

	Fall	Rise
IgG, IgA and IgM	• Congenital deficiencies (rare). • Acquired deficiencies: malignant blood dyscrasias (CLL, some lymphomas or myelomas), exsudative enteropathies, some viral infections (rubella, herpes, CMV, EBV), drugs and toxins (chemotherapy, high dose corticosteroids, immunosuppressants), severe malnutrition, (Kwashiorkor etc), depression.	• Polyclonal with 2 or 3 types of Ig with inflammatory reaction: infectious bacterial, viral or parasitic diseases, connective tissue diseases and vasculitides, some malignant blood dyscrasias dyscrasias (T lymphomas, Hodgkin's disease: rise not invariable). • Polyclonal with 2 or 3 types of Ig without inflammatory reaction (or with slight inflammation): chronic liver disease.
IgG alone	• Congenital IgG deficiency, IgA myeloma (by suppression of synthesis), Lyme's disease.	• Monoclonal: myelomas +++, monoclonal dysglobulinaemia of the elderly, some cancers or some severe chronic infections. • Polyclonal: humoral response secondary to infections, auto-immune disease, allergic reactions.
IgM alone	• Very elderly people (> 85 years, not invariable), IgG or IgA myeloma (by suppressed synthesis), some connective tissue, diseases (Gougerot-Sjögren's, Raynaud's syndrome), Wiskott-Aldrich's syndrome.	• Monoclonal: Waldenström's disease +++, monoclonal dysglobulinaemia of the elderly, some cancers or some severe chronic infections. • Polyclonal: infectious diseases (primary response), auto-immune haemolytic anaemias, primary biliary cirrhosis.
IgA alone	• Congenital IgA deficiency (1/700) or acquired deficiency (after treatment with some anti-epileptics or in ataxia telangiectasia)	• Monoclonal: IgA myeloma, monoclonal dysglobulinaemia of the elderly, some cancers or some severe chronic infections. • Polyclonal: infectious diseases localised to the respiratory, gastro-intestinal or skin mucosa, coeliac disease, Crohn's disease, cholecystitis, some cancers, Berger's disease, AIDS (poor prognosis), alcoholic cirrhosis (poor prognosis)

FOR FURTHER INFORMATION:

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IMMUNOREACTIVE TRYPSIN

DEFINITION

The term immunoreactive trypsin (IRT) groups together a set of compounds recognised by different antibodies, which develop against cationic human trypsin or trypsin-1, the protease produced from activation of the trypsinogens produced by the pancreas in human beings.

INTRODUCTION

Trypsin is produced by the pancreatic acinar cells as a pro-enzyme, which inactivates trypsinogen. Only minimal amounts of trypsin are found in the circulation, mostly bound to two protease inhibitors, alpha-1 antitrypsin and alpha-2 macroglobulin. These two forms of trypsin bound to the inhibitors are recognised differently by antitrypsin antibodies (the first 5% and the second 60%). Trypsin–alpha-1 antitrypsin complexes have been found in acute pancreatitis and in some patients with cystic fibrosis but they are not specific for any of these diseases. For this reason the indications for measuring immunoreactive trypsin in children and adults are very limited. Measurement of immunoreactive trypsin in the newborn however is particularly useful in the neonatal screening strategy for cystic fibrosis, firstly because very low volume blood samples can easily be taken onto blotting paper ("Guthrie" test) and easily transported and secondly because the assay method is suitable for mass screening.

INDICATIONS FOR MEASUREMENT

Measurement is mostly used in the newborn on day four after birth to screen for cystic fibrosis.

INFORMATION

■ SAMPLE

The conventional approach to mass neonatal screening for cystic fibrosis is to take the sample onto blotting paper. The test can also be performed on serum, although then requires a larger volume of blood collected into a dry tube.

For the blotting paper sample, capillary blood is taken on the fourth day after birth at the same time as for other neonatal screening tests (phenylketonuria, hypothyroidism, 21-hydroxylase deficiency) by making a microlance incision on the posterolateral surface of the heel. Drops are placed on blotting paper and dried at room temperature for two or three hours before being sent to the laboratory. The diameter of the blood spot cut out by the punch ranges from three to six millimetres and represents a blood volume of 2.5 to 10 µl. Usual recommendations apply when conventional samples are taken into a tube.

■ SAMPLE STORAGE AND TRANSPORT

Serum samples must be stored within an hour of sampling. Samples can be stored for several weeks under these conditions.

ASSAY METHODS

Radioimmunoassay or immunofluorimetry.

NORMAL EXPECTED VALUES

Reference values are < 55 µg/l. Any value over 55 µg/l should be confirmed by a second measurement.

PATHOPHYSIOLOGICAL VARIATIONS

The screening test is considered to be positive if the IRT concentration is > 65 µg/l. Card samples are then used to test for mutations for the gene involved in cystic fibrosis. A few false negatives can occur in cases of cystic fibrosis, which are not associated with hypertrypsinaemia at birth. False positives have also been described in 1 to 2% of cases. Confirmation with a sweat test is essential.

FOR FURTHER INFORMATION

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INFLUENZA

DEFINITION

Influenza is an epidemic viral illness caused by a group of viruses belonging to the *Orthomyxoviridae* family. The influenza viruses make up the *Influenzavirus* genus (single chain 8 stranded RNA viruses). They are divided into 3 types: A, B and C.

The Influenzaviruses A include human viruses and similar animal viruses (causing horse, swine and avian flu) and are the only ones that can cause pandemics. They are classified into subtypes depending on the antigenic determinants of their surface glycoproteins: Haemagglutinin (H) and Neuraminidase (N). To date, 15 H (H1 to H15) and 9 N (N1 to N9) have been identified in migratory birds. Only 3 H (H1 to H3) and 2 N (N1 to N2) are recognised to cause influenza epidemics in human beings. Each strain has a specific name: type/place of origin/number and year of isolation.

Examples: A/New Caledonia/20/99 (H1N1), A/Moscow/10/99 (H3N2), B/Hong Kong/330/2000.

INTRODUCTION

EPIDEMIOLOGY

Influenza develops in winter as annual epidemics (particularly the A and B viruses) and sometimes even as pandemics, which develop because of genetic rearrangement between animal and human strains.

The virus is easily transmitted between people by aerial spread through microdroplets and particles excreted by infected people, when they cough or sneeze. The influenza virus enters the body through the rhino-pharynx and symptoms develop 1 to 4 days after infection. Affected people become infectious a day before symptoms develop and remain so for 7 days.

The disease spreads rapidly particularly where populations are concentrated and the virus survives for longer times outside of the body when the weather is cold and dry, explaining why seasonal epidemics occur in winter in temperate zones.

MECHANISMS BY WHICH INFLUENZA VIRUSES EVOLVE

Whereas the B and C types are relatively stable, the A virus continually evolves through two main mechanisms.

The first is antigenic ***drift:*** mutations of the genes coding for surface proteins produce minor changes in the virus. The new variant remains very close to the previous such that the immunity conferred by previous influenza protects against the new variant. For the last twenty-five years the circulating viruses have been descendants of the Hong Kong virus (1968). Two types of influenza A virus, H3 N2 and H1 N1, currently co-exist in the world.

The second method of variation, called ***shift*** can be more serious. Pronounced changes in the viral antigenic proteins (often due to recombination between H and/or N genes from human and animal viruses) produce a new virus, which is entirely different to the virus circulating until that point. This new virus emerges and spreads to all continents causing a ***pandemic***.

1918-1919: "Spanish" influenza pandemic: A (H1N1) virus: 20 million deaths throughout the world.

1958-1959: "Asian" influenza pandemic: A (H2N2) virus, caused by the simultaneous shift of H and N (H2 N2 replacing the previous H1 N1 virus): 70,000 deaths in the United States.

1968-1970: Hong Kong influenza, A (H3N2) virus: more than a million deaths throughout the world including 18,000 in France by a single shift in the H (H2 to H3).

1977-1978: "Russian" influenza A (H1N1) virus: with re-emergence of the virus, which was present between 1947 and 1957 causing the Spanish influenza.

SYMPTOMS

The virus replicates in respiratory tract ciliated columnar cells.

After an incubation period of 1 to 2 days, the symptoms, which are non-specific, develop and include high fever, muscle stiffness, headaches, myalgia and arthralgia. Slightly later, a cough beginning in the larynx-trachea or bronchi develops and may or may not be accompanied by signs of conjunctival irritation. The fever abates at around the 3rd or 4th day when patients may feel they are recovering and then often rises again immediately afterwards falling completely on around the 6th day. The patients recover although remain feeling weak for some time.

Serious forms of the disease occur with complications mostly in frail "at risk" people: patients with respiratory insufficiency, asthma, bronchitis, the immunosuppressed, diabetics and the elderly.

The respiratory complications may be due to the virus itself (pulmonary oedema, acute respiratory failure) or to bacterial superinfections (bronchitis, otitis, pneumonia) and also to neurological and cardiovascular complications.

SEARCH INDICATIONS

– In people with severe, acute respiratory infection.

– In national or international epidemiological studies.

– In monitoring antigenic changes and the emergence of new variants of the virus in order to plan for appropriate effective vaccination.

INFORMATION

SAMPLE

Respiratory samples: naso-pharyngeal secretions collected either by aspiration or by deep nasal swabs, bronchial secretions or bronchio-alveolar lavage. Throat swabs are not recommended.

Respiratory samples for a rapid guide to diagnosis: these should be taken within 2 days of the onset of clinical signs before any treatment. Diagnosis becomes far less sensitive beyond this period.

Blood samples: collected into a dry tube (serum). The first sample should be taken in the acute phase of the infection and the second 2 weeks or 3 weeks later.

QUESTIONS FOR THE PATIENT

Age?

Vaccination?

Concomitant disease?

SAMPLE STORAGE AND TRANSPORT

Influenza viruses are fragile and therefore:

– Respiratory secretions and swabs must be transported to the laboratory within 48 hours of collection. The samples can be kept in a moist environment by adding a few drops of physiological saline to the bottom of the tube.

– Smears on slides should be transported at room temperature.

– Swab samples intended for cell culture or PCR require a special viral transport medium and should be stored at + 4°C for a maximum of 72 hours, failing which they should be frozen.

– Fluid samples do not require a transport medium.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

The viruses are found in the respiratory system from the start of the disease onwards. Viral antigens may be tested for by immunofluorescence (IF) or ELISA, or the virus itself by cell culture or RT-PCR.

In view of the high risk of infection it is important that the actual cause of a flu-like syndrome is established as soon as possible (acute episode of fever, sudden onset with respiratory signs), particularly in high-risk communities (the elderly, frail children, the immunosuppressed). Diagnosis can reduce prescription of antibiotics and further investigations and even enable anti-neuraminidase treatment to be started.

It is therefore important that in conjunction with these communities the laboratories are equipped with rapid "bar" or test strip diagnostic devices, which can diagnose influenza infection from a nasal secretion swab or aspirate simply, quickly and sufficiently reliably (positive predictive value: 90%, negative predictive value: 60%).

Detection of viral antigens

By IF: this is performed on the respiratory sample from a slide smear using a monoclonal antibody either labelled directly with a fluorochrome or unlabelled (when the sample is developed using a labelled anti-mouse antibody).

Commercial kits are available allowing combined identification of influenza viruses and other respiratory viruses such as the Adenoviruses, respiratory syncitial virus and the parainfluenzae viruses.

IF provides a rapid diagnosis although is theoretically less sensitive than culture (60 to 70%).

By ELISA: influenza antigens can be detected by a capture method using a monoclonal antibody developed immunoenzymatically. The ELISA membrane ("bar") test is available. These tests are more sensitive although non-specific reactions may occur particularly at low signal values.

Cell culture

This is a reference method reserved for specialist virology laboratory and provides full antigenic identification (type, sub-type and variant) of the influenza virus. It remains a method of choice for developing vaccines. It is limited by its response time (> 4 days).

The sample is inoculated on embryonated chick egg or MDCK cells (continuous dog renal cell line). The virus is revealed by haemadsorption using suspensions of red blood cells or IF with identical reagents to those used for direct viral testing.

If positive this is followed by identification of the virus by haemagglutin inhibition or a fluorescence neutralisation method.

PCR

Detection of the influenza virus RNA (influenza A + B) by gene amplification following reverse transcription (RT-PCR). The sensitivity and specificity of this method are > 95 % and the response time is 36 hours.

INDIRECT DIAGNOSIS

Complement fixation reaction:

This identifies total anti-influenza A or B virus antibodies as these are directed mostly against the internal virus antigen (NP), which is specific to type A or B. These develop slowly and are no longer detectable 3 months after infection.

A titre of 64 or higher may indicate recent infection although it is the increase in antibody titres in the 2nd sample by at least 2 dilutions, which confirms recent infection.

Haemagglutinin inhibition (HI):

This identifies antibodies against the envelope antigens, which are therefore specific for the types, subtypes and variants.

Other serological methods are available: **ELISA and neutralisation reactions**, which together HI, are reserved for specialist laboratories for use in epidemiological studies.

INTERPRETATION

Rapid direct diagnosis is useful in an emergency situation: such as influenza with acute respiratory infection, which may lead to hospitalisation. PCR provides an unequivocal diagnosis and identifies the strain. Culture remains essential to characterise strains for developing vaccines. The serological diagnosis is only retrospective and the CFR is useful if the diagnosis is late after the onset of the initial clinical signs. This is generally used to monitor the progression of an epidemic in a community or in an at risk population.

TREATMENT

The progression of A and B virus infection in the respiratory system can be slowed by anti-neuraminidases provided that these are administered within the first 24 to 48 hours of the disease.

Spread of influenza is only limited by vaccination. This is strongly recommended in at risk patient groups and in health staff. It must be given annually in view of the variability of circulating strains of virus.

Following the A/H1N1v influenza virus pandemic in 2009, several inactivated monovalent influenza vaccines have been developed against the fragmented or unfragmented vH1N1 virus, Celvapan®, Focetria®, Pandemrix®, Panenza®, with a lipid adjuvant (Focetria®) or ASO3 adjuvant (Pandemrix®) intended to amplify the immune response.

New cell culture production methods have also been developed using different cell lines, which are now available to produce anti-influenza vaccines although other production methods are also available particularly insect cell culture via recombination in a baculovirus. Research is currently being conducted to obtain a universal multivalent influenza virus against the M2 protein (which is constant).

An additional advance has been the development of new routes of administration: transdermal patches, intradermal administration (Intanza® 15 µg) and mucosal administration (nasal instillation). This is important as because vaccination needs to be repeated annually the hindrance of an injection can be avoided. In addition, the intradermal route is very good for vaccination as the dermis is of constant thickness regardless of age, sex or body mass index and phase 3 studies have already shown that the immunogenicity of ID is superior to IM in that it is well tolerated.

FOR FURTHER INFORMATION

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INHIBINS

DEFINITION

Inhibins are protein factors secreted by the gonads which have an inhibitory effect on FSH secretion, hence their name. They are glycoprotein dimers of approximately 32 kDa formed from an alpha sub-unit, common to the two inhibins A and B, and a specific beta subunit, βA and βB. The same β subunits can also form homo- or heterodimers, known as activins.

PHYSIOLOGICAL ROLE

Inversely to inhibin, activins stimulate FSH production in cultured pituitary cells, hence their name. The receptors are known to be transmembrane proteins with intrinsic serine /threonine kinase activity.

The inhibin receptors have not yet been completely characterised. The current belief is that the inhibins only exert their negative feedback control on FSH by blocking the access of activins to their receptors.

In any event, many experiments have demonstrated the physiological inhibitory role of inhibins; *in-vivo* or *in-vitro* inhibition of FSH production is still the only biological assay available for inhibin preparations.

Inhibin A is secreted by granulosa cells at the end of growth of the dominant follicle and during the luteal phase. It is secreted by the placenta from the end of the first trimester of pregnancy and is one of the indices used for placental growth.

Inhibin B is secreted in women from the start of the follicular phase, although its secretion stops at the beginning of the luteal phase.

Only inhibin B appears to be secreted in men, by the testes.

INDICATIONS FOR MEASUREMENT

The indications for measurement follow from the above.

1) Inhibin A measurement is used firstly during pregnancy as a maternal marker of foetal trisomy 21 risk, and secondly in the assessment of ovarian tumours, in which it is present in 20 to 80% of cases depending on histological type.

2) Inhibin B measurement is used as an index of ovarian reserve, measured on the 3rd day of the cycle. It is also part of the post-operative assessment of ovarian tumours.

3) Inhibin B is an excellent index of seminiferous tubule function in men and very low concentrations are invariably an adverse prognostic indicator for fertility.

4) In children, inhibin B measurement is indicated for:

– Ambiguous genitalia, all types
– Pseudohermaphroditism
– True hermaphroditism
– Cryptorchidism
– Feminising testes.

INFORMATION

SAMPLE

The assay is performed on serum or plasma.

The sample may be taken at any time during the day.

QUESTIONS FOR THE PATIENT

Date of last period?

Is the test for monitoring after surgery?

SAMPLE STORAGE AND TRANSPORT

The sample must be frozen promptly and the separated serum or plasma frozen within an hour of sampling until assay if the assay is not performed immediately.

Transport frozen.

ASSAY METHODS

Immunometric assay (sandwich) with enzymatic label.

EXPECTED VALUES

Age related expected values for inhibin B have been published by N. Lahlou and M. Roger (*Semin Reprod Med*. 2004, 2: 165-75) and are shown in the table below.

Boys		Girls	
Age	***Pg/ml***	***Age***	***Pg/ml***
0 to 15 days	75 – 540	0 to 15 days	<6 – 25
16 to 30 days	110 – 575	16 to 30 days	6 – 40
1 to 3 months	125 – 570	1 to 3 months	10 – 100
4 to 8 months	70 – 350	4 to 8 months	10 – 120
9 to 23 months	30 – 220	9 to 23 months	8 – 90
2 to 3 years	17 – 94	2 to 3 years	<6 – 12
4 to 5 years	6 – 72	4 to 5 years	< 6 – 15
6 to 8 years	7 – 125	6 to 8 years	<6 – 40
9 to 10 years	30 – 220	9 to 10 years	8 – 70
11 to 14 years	60 – 300	11 to 13 years	10 – 150
15 to 19 years	125 – 330	**>15 years old, with periods**	
20 to 49 years	135 – 350	1st week	10 – 300
50 to 59 years	135 – 300	2nd week	30 – 200
60 to 69 years	28 – 294	Pre-ovulatory phase	100 – 400
70 to 79 years	20 – 270	Luteal phase	<6 – 35
>79 years	15 - 240	Menopause	<6

Reference values for inhibin B established by Najiba LAHLOU*, Saint -Vincent-de-Paul Hospital, Paris*

Reference inhibin A values during the menstrual cycle have also been established by N. Lahlou and are shown in the table below.

INHIBIN A (pg/ml)		
- Follicular phase:	1st week	< 20
	2nd week	8 to 40
- Pre-ovulatory peak:		20 to 80
- Luteal phase (22nd – 25th day):		15 to 100

It should be noted that inhibin A is undetectable in men.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Inhibins A and B are generally undetectable before puberty in girls. Inhibin B concentrations begin to rise at around 7 years old at the same time as FSH. Inhibin A is only detectable after the first luteinisation, even if ovulation has not yet occurred.

Peak inhibin B values are seen after cycles have begun, at around day 8 of the follicular phase, and become undetectable after the menopause.

A postnatal secretory wave of production occurs in boys at the same time as the testosterone secretory wave. Concentrations at around the age of 3 months are higher than those in adult men. Beyond 2 years old, inhibin B is often undetectable and then rises progressively from the age of 8 years old, reaching adult levels at around 18 years old. Inhibin B concentrations fall in elderly men as seminiferous function deteriorates.

PATHOLOGICAL VARIATIONS

Inhibin B

Raised inhibin B in women may indicate androgen insensitivity (feminising testes) or tumour secretion (granulosa cell tumour). Low concentrations at the start of the cycle are considered to be an adverse prognostic indicator for fertility. Concentrations are extremely low in primary ovarian failure.

Very low concentrations in adult men indicate profound seminiferous tubule dysfunction as in Klinefelter's syndrome and in the "Sertoli Cell Only" syndrome and *particularly* in anorchidism. Reduced concentrations are seen in hypogonadotropic hypogonadism.

Inhibin A

Outside of pregnancy, inhibin A concentrations are generally raised in granulosa cell tumours and may also be moderately raised in other types of ovarian tumours.

During pregnancy, inhibin A concentrations may be reduced in placental disease but the measurement is used particularly in some countries, as a maternal serum marker of foetal trisomy 21 risk, as inhibin A concentrations are statistically lower in this situation.

FOR FURTHER INFORMATION

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INSULIN

DEFINITION

Insulin is a 51 amino acid protein (5808 Da) consisting of an A chain (21 amino acids) and a B chain (30 amino acids), connected by two disulphide bridges.

It is derived from a precursor molecule synthesised in the islet of Langerhans beta cells, pre-proinsulin, which itself is converted very rapidly into proinsulin (86 amino acids). Proinsulin is transported in the Golgi apparatus where it is cleaved enzymatically to produce insulin and a C peptide (the connective peptide) in equimolar amounts. The Golgi apparatus extrudes vesicles which become secretory granules containing small amounts of proinsulin together with insulin and C peptide.

Insulin secretion into the portal vein is accompanied by the release of an equimolar amount of C peptide and small amounts of proinsulin and another hormone known as amylin (or *Islet amyloid polypeptide*, IAPP).

Insulin is metabolised in the liver, whereas C peptide and proinsulin are catabolised mostly in the kidney. For this reason the half- life of insulin is short (approximately 4 minutes), whereas the half-life of C peptide is considerably longer (20 to 30 minutes). Because of this difference in their metabolism, insulin and C peptide concentrations are different in peripheral veins.

Insulin is the only blood glucose lowering hormone; it exerts its activity mostly in the liver, muscle and adipose tissue, increasing glucose uptake into insulin sensitive tissues, stimulating hepatic and muscle glycogen synthesis and inhibiting glycogenolysis and gluconeogenesis. It also promotes triglyceride storage in adipocytes and inhibits lipolysis.

INDICATIONS FOR MEASUREMENT

Insulin measurement is useful in the investigation of hypoglycaemia:

– Functional hypoglycaemia in which patients must be investigated for reactive hypoglycaemia at the 4th or 5th hour following an oral glucose tolerance test (OGTT).

– Organic hypoglycaemia or hyperinsulinism (e.g. insulinoma).

On the other hand, measurement is of no value in the diagnosis of diabetes mellitus, which is based purely on the finding of chronic hyperglycaemia. Insulin measurement is however indicated to demonstrate insulin resistance or an abnormality of pancreatic β cell secretory products.

Finally, measurement is also recommended in experimental pathophysiological studies on diabetes and impaired glucose tolerance.

INFORMATION

SAMPLE

Insulin can be measured in serum or plasma. Blood can therefore be collected into a dry tube or one containing heparin or EDTA. EDTA however must be avoided for some immunoassays.

In any event and regardless of sample type, it must not be haemolysed, as erythrocytes contain an enzyme which specifically degrades insulin. Even slight haemolysis causes a large fall in insulin of up to a third, after one hour at 20°C. To avoid any risk of haemolysis. Blood samples must be promptly centrifuged and the serum or plasma then separated and frozen at -20°C until assayed.

The sample is generally taken fasting in the morning, except on a specific request from the physician. The blood glucose is, of course, always measured simultaneously in order to interpret the result.

NECESSARY INFORMATION

Fasting status must be reported as should the sampling time and clinical context (pregnancy, diabetes, obesity, Cushing's syndrome etc.) and treatment being taken.

Use of insulin or oral anti-diabetic agents must be reported.

The request should also state whether measurement is part of a dynamic test, such as a fasting test, oral glucose tolerance test (OGTT),intravenous glucose tolerance (IVGTT) or glucagon test.

SAMPLE STORAGE AND TRANSPORT

Insulin is stable in plasma for 3 days at + 20°C, for 2 weeks at + 4°C and for several months at –20°C. Freeze-thaw cycles have little effect on insulin stability.

ASSAY METHODS

Insulin is usually measured immunologically. The first immunological methods proposed were radioimmunological with polyclonal antisera, which also recognised proinsulin. This caused over-estimation in cases, in which proinsulin was raised. Subsequently, monoclonal antibody development enabled immunoradiometric (IRMA), immunoenzymatic (IEMA) or immunofluorometric (IFMA) sandwich methods to be designed. Some of these methods can also be run on automated analysers.

It is important to note that regardless of the method used, the presence of anti-insulin antibodies produces erroneous results. Insulin results can be either over or under-estimated depending on the method. These antibodies may be autoimmune or may develop after insulin treatment, even with human insulin and have been found in approximately 30% of people with type 1 diabetes before any insulin has been administered. These antibodies therefore need to be removed and the free insulin measured. This is performed by precipitating the antibodies with polyethylene glycol (PEG) and centrifuging. The insulin measured in the supernatant is therefore the free non-antibody bound fraction. PEG immunoglobulin precipitation must be performed promptly after sampling in order to minimise the equilibrium between insulin and the antibodies.

DYNAMIC INVESTIGATIONS

Basal insulin measurement is usually not sufficient to assess insulin secretion and stimulation tests are therefore required.

STIMULATION TESTS

- **Meal test:** This is a potent stimulant but lacks standardisation.
- **OGTT:** During the three days before the test the subject should follow their normal physical activity, an unrestricted diet and in particular avoid oestrogen-progesterones, salicylates, diuretics and corticosteroids. After fasting overnight and a blood sample taken at time 0, the patient drinks 75 g of glucose in 200 to 300 ml of water. Blood samples are then taken every 30 minutes for 3 hours or later to investigate for hypoglycaemia.

  This test has the disadvantage of not being physiological and both intra and inter-individual results are therefore extremely variable.

  Compared to subjects of normal weight with normal glucose tolerance this test shows:

  – In obese patients: Hyperinsulinism in the first response phase

  – In people with impaired glucose tolerance: Hyperinsulinism and a delayed response

  – In type 2 diabetes: A delayed response but similar blood insulin concentrations to those in normal subjects.
- **The intravenous glucose tolerance test (IVGTT):** This test is used to assess the early insulin secretion peak as part of early screening for insulin-dependent diabetes in relatives. After injecting 0.5 g/kg of glucose intravenously, blood samples are taken at 1, 3, 5, 10, 20, 30, 45 and 60 minutes. The early insulin peak (EIP) is obtained by adding the blood insulin concentrations at 1 and 3 minutes and must be at least 45-50 mIU/l. A reduction in this peak is associated with an increased risk of developing type 1 diabetes.
- **Glucagon test:** After a baseline sample taken at time 0, 1 mg of glucagon is injected IV, and a second sample is taken after 4 or 6 minutes. This test has the advantage of being quick, directly stimulating insulin and minimising the risk of hypoglycaemia in insulinomas because of the hyperglycaemic effect of glucagon.

SUPPRESSION TESTS

Suppression tests, such as the fasting test or insulin administration, cause a reduction in blood insulin. In the latter of these tests, suppression is better monitored by measuring C peptide.

MEASUREMENT OF INSULIN RESISTANCE

Insulin sensitivity may be altered in various physiological, pathological and pharmacological situations. It is usually reduced. This insulin resistance causes a compensatory rise in insulin to maintain glucose homeostasis.

In order to measure insulin resistance the reference method is the euglycaemic hyperinsulinaemic clamp. This involves infusing a continuous rate of insulin in association with a variable rate glucose infusion in order to maintain constant blood glucose. It is complex to perform and different indices have also been proposed for the investigation, the two most widely used of which are the HOMA-IR *(Homeostasis Model Assessment Insulin Resistance)* and the QUICKI *(Quantitative Insulin Sensitivity Check Index)* which take account of blood insulin and fasting blood glucose concentrations.

The HOMA is calculated using the equation:

Insulin (mIU/l) x Glucose (mmol/l)/22.5

The QUICKI by the equation:

1 / [log (Insulin, mIU/l) + log (Glucose, mg/dl)].

Whilst these indices correlate well overall with insulin sensitivity measured using the glycaemic clamp, their use in patient subgroups and individual's raises problems. Considerable variations in correlations are seen in different subgroups, such as in controls, obese, type 2 diabetes and patients with polycystic ovarian syndrome. This heterogeneity may be due to differences in the pathophysiology of insulin resistance in the different populations.

UNITS

Insulin concentrations are usually expressed in mIU/ml or mIU/l.

Depending on the method, results are calibrated using the WHO international standard IRP 83/500 for which 1 IU = 6 nmol = 38.4 µg, or with the 1st IRP 66/304 (WHO standard), in which case µU/ml are converted to pmol/l by multiplying mU/ml by 6.945.

REFERENCE VALUES

The values obtained depend on the reagents used and the ability of the antibodies only to recognise insulin and not related molecules (C peptide and proinsulin).

For reference, in a healthy fasting person: 2-20 mU/l.

PHYSIOLOGICAL VARIATIONS

Blood insulin concentrations vary in complex rhythms, one of low amplitude (1 to 3 µU/ml) and with a periodicity of 10 - 15 minutes and the other of high amplitude with a periodicity of one to three hours.

They are lower (by 50 to 60%) in children under 6 years old than in adults and then rise regularly until the end of puberty.

During the first trimester of pregnancy, insulin action is stimulated by oestrogens and progesterone, and glucose concentrations tend to fall. Conversely, during the 3rd trimester, glucose tolerance is slightly reduced and insulin concentrations increase suggesting insulin resistance. This resistance is partly related to the human placental lactogen hormone (hPL), an insulin antagonist, secretion of which increases in the last part of pregnancy.

PATHOLOGICAL VARIATIONS

Blood insulin concentrations must be interpreted against the blood glucose. In the fasting state they are used as a reflection of insulin resistance, whereas after stimulation they reflect the secretory capacity of the pancreas.

HYPOGLYCAEMIA

Endogenous hyperinsulinism

Insulinoma

This is the commonest cause of hypoglycaemia as a result of endogenous hyperinsulinism. The tumours are generally single, although multiple insulinomas or micro-adenomas have been reported. It is a rare disease affecting both sexes equally, which may occur at any age. In the great majority of cases the tumour is intra-pancreatic, although it may lie outside of the pancreas. The tumours are malignant in a small proportion of cases (5 to 10%).

Insulinomas may form part of the type I multiple endocrine adenomatosis syndrome or Wermer's syndrome. This familial disorder is autosomal dominant in transmission and characterised by multiple adenomatous proliferation in the endocrine glands (the parathyroids, pituitary and pancreas). The laboratory diagnosis is based on the independence of insulin secretion demonstrated by persistent normal or increased blood insulin in the presence of hypoglycaemia.

The insulin suppression test does not produce a significant fall in C peptide (normally less than 50%). The diagnosis can therefore be confirmed by a fasting test from an increase in the blood insulin/glucose ratio during the fast, whereas this should normally fall.

In addition to insulin, some insulinomas secrete other hormones, including ACTH, hCG, somatostatin, serotonin, glucagon, and VIP.

Hypoglycaemia secondary to extra pancreatic tumours

The most common of these are the mesenchymal tumours (fibrosarcoma, neurofibroma and lymphosarcoma) usually located retro-peritoneally. Other tumours involved include hepatocellular carcinomas and adrenal gland tumours. With these tumours, episodes of hypoglycaemia are associated with low concentrations of insulin C peptide and proinsulin. The factor responsible has been found to be IGFII secreted by the tumour in a high molecular weight form (big IGFII).

Hypoglycaemia due to anti-insulin antibodies (Hirata's disease)

The anti-insulin autoimmunity syndrome which is common above all in Japan, is associated with the production of high titres of anti-insulin antibodies. The pathophysiology of the episodes of hypoglycaemia may involve binding of insulin secreted after a meal to auto-antibodies. When free insulin falls, passive insulin release results in hyperinsulinism. These kinetics may explain firstly the impaired glucose tolerance in an OGTT and secondly the late onset post-prandial hypoglycaemia which some patients develop. The diagnosis is based on the finding of hypoglycaemia combined with hyperinsulinaemia and high anti-insulin antibody concentrations.

Factitious hypoglycaemia

These mimic the clinical and laboratory features of hypoglycaemia secondary to insulinoma, although the hypoglycaemic episodes occur both fasting and in the immediate post-prandial period, which should raise the alarm. Practitioners may also be alerted by a past history of psychiatric disease which is present in approximately a third of these patients.

Factitious hypoglycaemia may occur secondary to administration of insulin or insulin secretagogues.

The diagnosis of hypoglycaemia due to insulin administration is based on the finding of inappropriate high or normal insulin concentrations, but above all, concentrations which are not low, associated with undetectable C peptide and proinsulin concentrations in the presence of a low blood glucose concentration of less than 0.45 g/l (2.5 mmol/l). The diagnosis is more difficult if the patient is seen outside of periods of hypoglycaemia although insulin suppression tests are normal.

Hypoglycaemia caused by a patient taking an insulin secretagogue is difficult to distinguish in laboratory terms from insulinoma. Blood insulin concentrations are inappropriately raised during episodes of hypoglycaemia as are concentrations of C peptide and proinsulin. The diagnosis in this situation is based on identifying the sulphonylurea in the patient's plasma or urine. HPLC capillary electrophoresis methods allow the different sulphonylureas to be measured.

SCREENING FOR DIABETES

The IVGTT can be used in at risk families (families with a type 1 diabetic parent or sibling), to assess pancreatic insulin content by determining the early insulin peak (EIP). A reduced EIP in a person indicates increased risk of developing type 1 diabetes, although this result must be associated with immunological and genetic markers in order to establish an overall risk of developing the disease. It should also be noted that a reduced EIP is not specific for type 1 diabetes, and is also seen in type 2 diabetes and indicates increased risk of developing diabetes in patients with impaired glucose tolerance.

INSULIN RESISTANCE SYNDROMES

Insulin resistance forms part of the pathophysiological picture of type 1 and particularly type 2 diabetes and of many other diseases, such as android obesity, cirrhosis, renal insufficiency and essential hypertension.

Major insulin resistance

These are rare diseases due to a mutation of the insulin receptor gene, involving and comprising of: type A insulin resistance, leprechaunism, lipoatrophic diabetes and the Rabson-Mendenhall syndrome. Hyperandrogenism is present in all of these diseases, although *acanthosis nigricans* is only seen in lipoatrophic diabetes and type A insulin-resistance. From a laboratory perspective these are characterised by hyperinsulinism.

Metabolic syndrome

The metabolic syndrome, also called syndrome X or the insulin resistant syndrome, can be defined as impaired glucose tolerance or type 2 diabetes, and/or insulin resistance associated with at least two of the following abnormalities: hypertension, hypertriglyceridaemia, android obesity, HDL cholesterol of less than 0.9 mmol/l or microalbuminuria.

Other abnormalities are commonly seen including hyperuricaemia, coagulation disorders and fibrinolytic abnormalities.

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INTERFERONS ALPHA AND GAMMA

DEFINITION – INTRODUCTION

The interferons (*to interfere* = to interfere, hinder, oppose) are polypeptides of approximately 160 amino acids secreted naturally by the body in response to assault from pathogenic agents, such as viruses, bacteria, parasites or tumour cells.

Two types of interferons are distinguished according to their biological properties and cell origin:

Types	Family	Abbreviation	Number
Type I	Interferons α	IFN-α	12
"antiviral action"	Interferon β	IFN- β	1
	Interferon ω	IFN-ω	3
	Interferon λ		
Type II			
"immune action"	Interferon γ	IFN-γ	

Type 1 interferons (IFN) mostly comprise IFN-α and IFN-β, secreted primarily by macrophages (IFN-α) and fibroblasts (IFN-β). IFN-α and IFN-β have antiviral and anti-tumour activity, explained through an anti-proliferative action (activation of synthesis of enzymes which inhibit viral DNA or cell replication) and activation of cell mediated immunity (activation of macrophages and NK cells increasing the expression of major histocompatibiilty complex class 1 antigens and stimulation of T_H1 and cytotoxic T $CD8^+$ lymphocytes).

Type 2 Interferon or IFN-γ is secreted primarily by activated T lymphocytes (T_H1 and $CD8^+$) and *Natural Killer* cells (NK cells), the synthesis of which, is stimulated by IL18 (or *IGIF Interferon-gamma inducing factor*). IFN-γ is involved in the regulation of immune and inflammatory responses, has antiviral and anti-tumour activity and potentiates the effects of IFN-α and IFN-β. It stimulates macrophage phagocyte activity, increases the class 2 antigens expression of major histocompatibility complex activates NK cells and simulates the humeral mediated immune response (antibody synthesis). IFN-γ is produced by T_H1 lymphocytes and contributes to leukocyte recruitment at the site of infection, leading to the inflammatory response.

IFN-γ is used therapeutically as an immunomodulator and is indicated in patients with chronic septic granuloma to prevent serious infections. Recombinant alpha interferon is used in antiviral therapy (hepatitis B and C, Kaposi and in AIDS) and anti-cancer therapy.

Alpha and gamma Interferon can be measured in serum and in CSF. As alpha Interferon is induced by the presence of viral genome, its detection indicates ongoing viral infection, although does not inform as to the nature of the virus involved. More generally, IFN alpha and gamma can be detected at significant levels in serum and/or CSF, in viral, parasitic and bacterial infections. Interferon gamma in particular is raised greatly in children with severe (often fatal) mycobacterial infections including after BCG vaccination, caused by a congenital IFN gamma receptor deficiency.

INDICATION FOR MEASUREMENT

Alpha interferon is offered in association with specific further investigations to diagnose encephalitis, particularly due to herpes virus, meningitis and neurological complications of AIDS (cytomegalovirus, varicella zoster virus super-infection) and systemic lupus erythematosus (SLE) or in the diagnosis of congenital rubella. IFN gamma measurement is of less use in these situations but it is used in clinical research to study IFN gamma receptor deficiencies (partial or total deficiency of the IFNγR1 or IFNγR2 receptor chains).

INFORMATION

SAMPLE

IFN alpha: Serum (Dry Tube).

IFN gamma: Serum or EDTA plasma (non-haemolysed).

CSF: 500 µl minimum.

SAMPLE STORAGE AND TRANSPORT

IFN alpha in serum can be stored for one week at + 4°C.

To measure IFN gamma in blood: Centrifuge for 10 minutes at 1,000 g within 30 minutes of sampling. Separate and store the serum or plasma at + 4°C (up to one week).

CSF can be stored at +4°C, for 1 week.

ASSAY METHODS

Immunoenzymatic or biological method.

NORMAL EXPECTED VALUES

For reference: IFN gamma (EIA): Serum or plasma < 16 pg/ml; CSF < 18 pg/ml.

IFN alpha (biological method): Serum and CSF < 2 kIU/l.

INTERPRETATION

Interferons alpha can be detected in serum (> 2 kIU/l):

– In viral infections (excluding hepatitis B, C and acquired rubella)

– In exacerbation of auto-immune diseases (SLE and scleroderma)

– After SC, IM or IV injection of IFN alpha.

Interferon is present in more than 90% of serum samples from foetuses infected with the rubella virus, more than 70% of those infected by CMV and 50% of those infected with parvovirus B19.

When microbiological examinations are negative and/or if encephalitis or meningitis is suspected in a clinical picture of infection, a high IFN alpha concentration in CSF can be a guide to further investigations.

Synthesis of IFN alpha in CSF can be detected (> 2 kIU/l) as soon as clinical signs appear in the following situations:

– Herpes encephalitis (95% of cases) and other primary viral encephalitides.

– Most cases of viral meningitis, particularly due to Coxsackie, Echo or mumps virus.

– Neurological complications of AIDS, (CMV, VZV superinfection) or SLE.

– Familial encephalopathies associated with central grey nucleus calcifications.

Conversely, IFN alpha remains undetectable (< 2 kIU/l) in the following situations:

– Normal CSF.

– Most cases of bacterial meningitis.

– Neurological diseases (multiple sclerosis, Guillain-Barré syndrome and Creutzfeld Jacob disease).

– Post-infectious encephalitis.

NB: IFN alpha measurements should preferably be performed in parallel in blood and CSF in the same patient. The diagnosis of herpes encephalitis, another viral encephalitis or viral meningitis is suggested when IFN alpha concentrations are higher in CSF than in blood, indicating intrathecal synthesis. The result may however be difficult to interpret if the blood-brain barrier is damaged and caution is recommended when serum values are high and values in CSF are low or undetectable (probable non-specific reaction not associated with central nervous system viral infection).

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IODINE

DEFINITION

Iodine is a trace element (15 to 20 mg) in the human body. It can be measured in either blood or urine.

In human beings, iodine is mostly obtained from the diet, the main sources being seafood, eggs, meat, milk and cereals. Food iodine content, however, varies greatly by region and season particularly for milk. Iodine is converted in the gastrointestinal tract into iodide which is rapidly absorbed and then distributed throughout the general circulation. It is then taken up in the thyroid gland and removed in urine. Under steady state nutritional conditions the amount of iodide ingested is the same as the amount removed in urine. Urine iodide is in fact the best indicator of iodine intake and is widely used in population epidemiological studies.

INTRODUCTION

Iodine is essential for the synthesis of thyroid hormones. It is taken up in the thyroid through an active transport system. Iodide is organified by peroxidise and then incorporated into thyroglobulin, the precursor of the thyroid hormones. Its ability to be bound and concentrated in the thyroid and its involvement in thyroid disease produced by iodine deficiency or overload (mostly iatrogenic) occasionally make it an essential component of the diagnosis.

In severe deficiency iodide uptake by the thyroid is inadequate, the intra-thyroid pool falls and thyroglobulin iodination is reduced. This is reflected in the circulation by a reduction in the concentration of T3, a reduction in T4 and an increase in TSH. Chronic thyroid stimulation by TSH produces an increase in thyroid mass, and in the long-term, goitre. Defects of thyroid function are generally more serious in new-born babies and children than in adults.

Sudden iodine overload has an antithyroid action, known as the Wolff-Chaikoff effect, with a reduction in organification and therefore in the synthesis of thyroid hormones. Beyond 48 hours, however, an escape phenomenon occurs, protecting people from secondary hypothyroidism. Chronic iodine overload may cause dysthyroidism (hypo- or, usually hyperthyroidism) and is reflected by an increase in serum iodine and in particular urine iodine and a white thyroid scintigram.

INDICATIONS FOR MEASUREMENT

– Individual screening for iodine deficiency in the presence of suggestive signs (congenital hypothyroidism, cretinism or mental retardation in children) or usually on a population scale (epidemiological studies, possibly triggered by increased incidence of endemic goitre; increase in miscarriages and in congenital hypothyroidism).

– Testing for iodine overload in the investigation of dysthyroidism (aetiological investigation, aid to interpretation of thyroid scintigrams which may be hindered by iodine excess) or testing for the absence of iodine overload before radioactive iodine therapy.

– Monitoring for iodine overload in patients treated with amiodarone.

– Monitoring for iodine overload in patients treated with iodinated antiseptic on skin or mucosal membranes. Iodine may cross through healthy skin depending on the surface area disinfected and the extent of any skin damage.

INFORMATION

SAMPLE

Blood: Preferably dry tube (serum); plasma is also acceptable (unless the anticoagulant contains iodine).

Urine: Urine iodide is ideally measured in a 24 hour urine collection collected without stabiliser. In occupational medicine, the sample is taken from an end of shift urine (end of week).

QUESTIONS FOR THE PATIENT

Are you taking (or have you taken) any of the following medicines: Iodinated contrast media for a radiological investigations, amiodarone and iodine containing skin antiseptics or thyroid replacement hormones?

For 24 hour urine measurements, please state the urine output.

SAMPLE STORAGE AND TRANSPORT

Serum: Store at + 4°C. Transport: + 4°C.

Urine: Store at + 4°C.

ASSAY METHODS

– Colorimetric methods: Particularly those based on the Sandell and Kolthoff reaction (measurement of ceric ammonium sulfate reduction by arsenic acid catalysed by iodide);

– Reverse phase liquid chromatography, potentiometry with "specific" electrodes and spectrophotometric methods (unsuitable for routine use and reserved for urine).

– ICP-MS: *Inductively Coupled Plasma Mass Spectrography* or plasma torch.

NORMAL EXPECTED VALUES

These can vary depending on the method used. For reference:

Urine iodide: 100 to 300 µg/24H or 787 to 2360 nmol/24H.

These reference values were obtained from a population sample although the WHO considers that iodine requirements in an adult and school age child population are satisfactory when the medium urine iodine concentration is 100 µg/l or 787 nmol/l or above.

Serum iodine: 34 to 80 µg/l or 267 to 629 nmol/l.

– Conversion factor: 1 µg/l = 7.88 nmol/l.

INTERPRETATION

FACTORS TO CONSIDER WHEN INTERPRETING RESULTS

– Iodine exists in the body in the form of iodide (the main form in urine) or bound to organic compounds. To measure it, it needs to be mineralised to return it to the iodide state and remove it from organic substances or minerals which may interfere with the assay.

– Urine iodide: Because of the large intra-individual variations, the best measurement is one performed on a 24 hour urine collection. In occupational medicine and when measured on a single urine sample, it must be accompanied by a urine creatinine measurement.

– Serum iodine: Total serum iodine (or protein iodine) is the sum of iodine contained in T4 molecules (the greatest proportion) and nutritional organic iodide. The patients' thyroid function should therefore be known in order to interpret a serum iodine result.

PATHOLOGICAL VARIATIONS

Reduction in serum or urinary iodine concentrations and identification of iodine deficiency

Iodine deficiencies are nutritional in origin. On a population scale, nutritional iodine intake is estimated from the daily urinary iodine excretion. According to the WHO, deficiency is not present if the median population urine iodine is > 100 µg/l. Mild deficiency is referred to when median values are between 55 and 99 µg/l, moderate for values between 20 and 49 µg/l and severe deficiency if values are < 20 µg/l.

Severe iodine deficiency causes thyroid dysfunction (mental retardation, cretinism and goitre), a reduction in fertility and an increase in miscarriages and perinatal mortality.

Large scale dietary iodione supplementation studies have been used to prevent problems due to iodine deficiency, such as iodination of salt or drinking water or either oral or parenteral administration of iodinated oil. Since the systematic supplementation of table salt in 1952, cretinism has almost disappeared in France; mild or moderate deficiencies remain in some mountain areas.

Increased serum or urine iodine concentrations and identification of iodine overload (urinary iodine > 400 µg / 24 hours)

Iodine overload is usually secondary to medicinal products which are rich in iodine, particularly amiodarone, iodinated contrast media and to a lesser extent, iodinated antiseptics (amiodarone, for example, causes hyperthyroidism in approximately 10% of patients treated, particularly in men). Iodine overload may cause laboratory or clinical dysthyroidism or may reveal underlying thyroid disease.

For occupational protection, people exposed to iodine undergo laboratory monitoring with a combination of urinary iodine measurements, thyroid function tests and thyroid scintigraphy.

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IRON

DEFINITION

Iron is the principal oligoelement in man, with a normal adult holding between 3 to 5 g. It has an essential role in the maintenance of life. It is the basic component of haemoglobin and myoglobin, as well as several enzymes and cytochromes. It is also present in the circulation, bound to transferrin, and in body stores (ferritin) mainly in the liver.

Iron homeostasis is controlled by a recently identified hormone, hepcidin.

INDICATIONS FOR MEASUREMENT

Investigation of iron status is used to diagnose states of iron deficiency (relatively common) and those of iron overload (rarer). Serum iron measurement is also one of the components of the tests of iron status employed to monitor iron replacement treatment. Serum iron results alone are not useful. To be properly interpreted they have to be combined with other measurements, particularly transferrin, transferrin saturation and ferritin.

INTRODUCTION

SAMPLE

Heparinised serum or plasma. Do not use EDTA or citrate tubes. Discard haemolysed specimens.

For monitoring purposes, it is advisable to standardise the time of sampling as there are circadian variations in serum iron levels. Ideally fasting blood should be taken in the morning without venous stasis.

QUESTIONS FOR THE PATIENT

Medication:

– All iron containing drugs, but also complex vitamin preparations (containing iron) and food prepared

with iron enrichment, which can cause substantial increases in serum iron levels (up to + 50 µmol/l).

– Progesterone can increase serum iron.

– Desferrioxamine treatment: as the iron is bound, it cannot be measured directly.

SAMPLE STORAGE AND TRANSPORT

The sample can be stored at laboratory temperature for up to 7 days or 3 weeks at + 4°C. The serum or plasma can also be stored for several months at – 20°C.

Transport at + 4°C or frozen if the sample is already in the frozen state.

ASSAY METHODS

Direct colorimetric techniques on automated biochemical analysers suitable for iron assay.

For total iron binding capacity (binding to transferrin) and calculation of the saturation coefficient, direct methods using iron measurement after saturation of the serum and removal of unbound iron should have been abandoned.

Total iron binding capacity (TIBC) is derived from the formula: TIBC (µmol/l) = Measured transferrin (g/l) x 25.

The percentage saturation of iron binding capacity = {serum iron (µmol/l) / TIBC (µmol/l)} x 100.

NORMAL EXPECTED VALUES

	Iron (µmol/l)	Saturation coefficient (SC)
Birth	10 – 36	0.55 – 0.65
Infant	9 – 20	0.10 – 0.30
Child	11 – 24	0.10 – 0.30
Adult male	12 to 30	0.20 – 0.40
Adult female	9 to 28	0.15 – 0.35

These values can vary according to the method employed.

Conversion factor: µg/l x 0.018 = µmol/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Varies with age and gender (see above).

Over the menstrual cycle, serum iron is lowest after menstruation: variation of 10 to – 30%.

During pregnancy the serum iron can increase under the influence of elevated progesterone or fall because of iron deficiency.

There is considerable circadian variation dependent on sleep pattern. Serum iron peaks in the morning, and then the value falls steadily throughout the day reaching the lowest point between 21.00 hrs and 05.00 hrs. This rhythm is inverted in night-shift workers. Mean variation is between 20 to 30 % but the changes can be as high as 100 to 300%. However, these changes are less marked in patients with low or high serum iron.

PATHOLOGICAL VARIATIONS

Combined with assay of transferrin and total iron binding capacity, the measurement of serum iron is more useful for demonstrating overload than deficiency.

Low serum iron reflects iron deficiency. The main causes are:

In the child: most frequently, poor intake. In the infant the risk factors are prematurity and being a twin.

In the adult: deficient intake, increased requirements (pregnancy) and increased loss.

– Causes of increased loss include:

- Gastro-intestinal loss (gastro-duodenal ulcers, piles, etc,), uterine fibroids in the woman and all blood loss, leading to iron deficiency anaemia..

- Low serum iron is also a feature of many states of anaemia due to iron sequestration by monocytes and macrophages (anaemia of infection; cancer; anaemia secondary to inflammation, in rheumatoid arthritis, for example...), during treatment of pernicious anaemia, anaemia of chronic renal failure or that due to Vitamin C deficiency

■ **Elevated serum iron** reflects iron overload, which can be genetic or acquired.

Genetic iron overload: Haemochromatosis.

Acquired overload results from:

- Either a disturbance of haematopoiesis (secondary haemochromatosis). The iron overload can be due to a variety of causes, such as haemolytic anaemia, haemoglobinopathies (thalassaemia major: ineffective erythropoiesis + increased intestinal absorption), sideroblastic anaemia (ineffective erythropoeisis) and transfusion overload.

- Or from liver cell cytolysis (hepatitis) or due to other causes, such as chronic alcoholism and porphyria cutanea tarda.

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IRREGULAR AGGLUTININ TEST

DEFINITION

The purpose of an irregular agglutinin test (IRT) is to detect and identify antibodies directed against erythrocytes. The test involves incubating the subject's serum or plasma with phenotyped O-type erythrocytes with respect to the main blood groups (Rh, Kell, Duffy, Kidd, MNS, P1, Lewis, Lutheran etc.; as defined by the regulations).

The presence of the antibodies in question is detected through an agglutination reaction (spontaneous or induced, depending on the nature of the antibody).

Antibodies (Abs) directed against erythrocytes can be divided into two classes:

– Natural Abs: These antibodies are present without any need for stimulation on cells from foeto-maternal or transfused blood. They usually belong to the IgM class and pose no danger to the foetus. They have negligible impact on transfusion unless they are haemolytic *in vitro* (anti Lewis, anti-P1) and/or their titre is high.

– Immune Abs: These are elicited by exposure to one or more antigens on the membrane of a non-self erythrocyte during gestation or as a result of transfusion. They are usually IgGs, are dangerous in transfusion and can pose a danger to the foetus (depending on the nature of the antigen recognised and the titre of the antibody).

SEARCH INDICATIONS

An IRT is a basic test in the prevention and diagnosis of immune haemolysis in blood transfusion as well as in screening for incompatibility between mother and foetus. An IRT is indicated in:

– Any patient likely to be transfused in the near future (before any transfusion or series of transfusions) and following transfusion to monitor for potential haemolytic problems

– Pregnant women, to screen for incompatibility between mother and foetus. The official regulations stipulate that such a test is to be performed in pregnant women with the phenotype Rh:-1 (D negative), at least four times in the course of the pregnancy (Months 2, 6, 8, and 9) and again after delivery; in first-time mothers (who cannot have been immunised) with the phenotype Rh:1 (D positive), at Month 2; and in pregnant women who have already given birth or with a history of miscarriage and/or artificial abortion with the phenotype Rh (D) positive, at Months 2, 6, 8, and 9

– Any patient scheduled for graft or transplantation.

INFORMATION

SAMPLE

Blood should be drawn into a dry tube (serum) and on EDTA (whole blood).

Enough blood should be drawn for the test, complementary examinations and for storage in a serum bank.

The test must be performed within 72 hours of blood drawing. In the context of transfusion, the IRT result is valid for three days.

Identification is carried out on an unopened, undecanted sample if it is being performed by a laboratory other than that which is responsible for the screening.

QUESTIONS FOR THE PATIENT

Date and result of the last IRT.

Prior blood transfusion(s)? If yes, then when?

Previous pregnancies? Miscarriages? Artificial abortion?

Medical history (notably leukaemia and bone marrow grafts)?

Concomitant drugs?

Injection of anti-D immunoglobulin? If yes, when and how much?

STORAGE

Blood samples can be stored at +4°C for up to 72 hours.

The serum should be stored at +4°C.

ASSAY METHODS

An IRT involves two steps:

Screening, based on a panel of at least three phenotyped O-type erythrocytes permitting distinction between Abs directed against the following antigens: D (RH1), C (RH2), E (RH3), c (RH4), e (RH5), K (KEL 1), k (cellano : KEL2), Kpb (KEL 4), Fya (FY1), Fyb (FY2), Jka (JK1), Jkb (JK2), M (MNS1), N (MNS2), S (MNS3), s (MNS4), P1, Lea (LE1), Leb (LE2), Lub (LU2). The antigen profile of the screening panels is defined by the regulations.

Identification is obligatory if the screen gives a positive result. It involves determining the specificity of the Ab(s) present using a panel of at least ten test erythrocytes (phenotyped O-type erythrocytes including the types Cw +, Lua + Kpa + in addition to the specificities of the screening panel). This result should allow the identification of any common single antibody, and provide identification information for mixtures of antibodies.

In an IRT, two different methods are used to detect the reaction between antigen and antibody:

– The indirect antiglobulin test (or indirect Coombs' test) which uses a polyvalent human antiglobulin or an anti-IgG

– Tests based on proteolytic enzymes (e.g. papain, bromelain or trypsin) which partially strip the red cell membrane to expose certain antigens.

Classic tube methods are being abandoned in favour of methods based on gel filtration, microscopic beads or microplates, as these are more sensitive and have the advantage that they can be automated.

VALIDATION AND INTERPRETATION OF RESULTS

The identification of one or more antibodies directed against erythrocytes necessitates:

– Checking the specificity of each of the antibodies

– Ruling out the possibility of supplementary antibodies

– Phenotyping to check the absence of the antigen corresponding to each identified alloantibody.

The result of the screen is negative: No antibodies directed against erythrocytes.

The result of the screen is positive: The specificity of the Ab(s) should be determined in the identification step. Identifying a single Ab does not usually pose any problem but identifying a mixture of Abs is far more complicated and requires complementary approaches. The most commonly encountered antibodies are those directed against antigens associated with the Rh, K, Fya, Jka, S, s systems.

In all cases, ABO grouping and Rh-Kell phenotyping are essential since any patient who has or has had an Ab must be transfused with phenotyped and characterised blood.

The presence of anti-erythrocyte antibodies and the date of their detection should be recorded on the subject's Blood Group Card.

FOR FURTHER INFORMATION

■ BIOFORMA N° 26 (2002): *Immunohématologie et groupes sanguins*.

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■ Y. Brossard, F. Parnet-Mathieu, M. Larsen, *Diagnostic et suivi prénatals des allo-immunisations érythrocytaires*, Feuillets de Biologie, 2002-vol. XXXXIII- N° 245.

ISONIAZID

DEFINITION

Isoniazid is the hydrazide of isonicotinic acid which is indicated for use in the curative treatment of active pulmonary or extra-pulmonary tuberculosis and very occasionally in the treatment of sensitive atypical mycobacterial infections. It is also the recommended chemoprophylaxis for tuberculosis.

It is used in two treatment regimens for the treatment of tuberculosis:

– Administration of Isoniazid, rifampicin and ethambutol for 2 months, followed by Isoniazid and rifampicin for 7 months

– Administration of Isoniazid, rifampicin, ethambutol and pyrazinamide for 2 months followed by Isoniazid and rifampicin for 4 months.

The usual dosage is 5 mg/kg/d in adults and 10 mg/kg/d in children (maximum dose 300 mg/d). It is recommended that it be taken in the morning fasting.

Synonym: INH.

PHARMACOKINETICS

Bioavailability (oral)	100%
Plasma peak	1 to 2 hours (after administration of a single fasting dose of 300 mg)
Steady state reached	Varies between 5 and 30 hours (approximately 5 half-lives)
Protein binding	Weak
Metabolism	Mostly by acetylation* by an N-acetyl-transferase in the small intestine and liver
Elimination	More than 75% in urine mostly in the form of metabolites. The renal excretion of active INH varies from10% in fast acetylators to 30% in slow acetylators
Half-life of elimination**	Bi-modal: – Fast acetylators: 35 to 110 min – Slow acetylators: 110 to more than 400 min*

**The metabolites have no anti-tuberculus activity. Acetylation capacity is genetically controlled, the population being divided into two phenotypes: fast acetylators and slow acetylators (acetylation rate ranges by a factor of 4 to 5 between the two groups). Fast acetylators make up 40% of white subjects, 60% black subjects and 90% of yellow skinned subjects*

*** The half-life of elimination is increased in hepatic insufficiency and in renal insufficiency.*

Determination of acetylation phenotype:

The I3 inactivation index is calculated from the INH concentration measured 3 hours post-dose (C3 and mg/l):

I3 = C3 (mg/l) + 0.6 / dose (mg/kg)

Fast acetylators	Slow acetylators
I3 < 0.5	I3 > 0.6

INDICATIONS FOR MEASUREMENT

The measurement is particularly useful because of the existence of two population types (fast acetylators and slow acetylators). In order to avoid under-dosing (lack of therapeutic effect), or overdose with signs of toxicity developing, such as gastrointestinal problems (nausea, vomiting and epigastric pain), fever, myalgia, arthralgia, anorexia, neurological disorders involving peripheral neuropathy and hepatotoxicity with raised transaminases.

In addition, serum concentrations vary greatly for the same dose of INH.

In the treatment of tuberculosis INH is associated with rifampicin, an enzyme inducer which may alter the metabolism and serum concentrations of INH. In addition, other pharmacokinetic therapeutic interactions occur and should be taken into account:

Medicinal products which may accelerate the hepatic metabolism of INH (potentiation of the hepatotoxicity of INH due to increased production of toxic metabolites; reduction in plasma INH concentrations): Volatile halogenated anaesthetics and glucocorticoids.

Finally, tuberculosis treatment is a long term ambulatory treatment (6 or 9 months) and measurements may be used to confirm good adherence to treatment.

INFORMATION

■ SAMPLE

Serum or heparinised plasma. Avoid tubes with separator gel.

The sample must be taken 3 hours post dose (C3).

■ QUESTIONS FOR THE PATIENT

– All requests for drug measurements must include the reason for requesting (investigation for efficacy or toxicity), the sampling time, treatment start date and/or any change in dosage, dosage information (amount administered, frequency and administration route) and the age, height and weight of the person wherever possible.

– Concomitant treatments which may interfere with INH metabolism *(cf. list above).*

– For determination of acetylation phenotype and optimal dosage, the person's weight and the dosage must be known.

■ SAMPLE STORAGE AND TRANSPORT

If testing is not being performed immediately, then centrifuge, separate and freeze the serum or plasma within an hour of sampling. Store serum or plasma at - 20°C. Transport the separated sample at - 20°C .

ASSAY METHODS

HPLC with UV detection after sample deproteinisation.

NORMAL EXPECTED VALUES

Therapeutic INH values 3 hours post-dose should be between 1 and 2 mg/l in all treated people.

Conversion factor: 1 mg/l = 0.137 µmol/l.

Vivien et al. (*Rev Fr Mal Respir* 1973; 1: 753-72) developed a dosage adjustment method based on calculating the I3 index from the INH concentration measured 3 hours post dose (C3).

The INH dose enabling the desired concentration (DC) to be achieved, i.e. between 1 and 2 mg is:

Dose (mg) = Weight (kg) x (0.6 + DC) / I3

This method has been validated for use in paediatrics. The adjusted doses are approximately 6 mg/kg/day for fast acetylators and 3 mg/kg/day for slow acetylators.

FOR FURTHER INFORMATION

■ Dictionnaire Vidal®.

■ Compagnon P., Bouquet S., Houin G., *Suivi thérapeutique de l'isoniazide*. In : Suivi thérapeutique pharmacologique pour l'adaptation de posologie des médicaments, Collection OptionBio, Ed Elsevier, Paris, 2004: 97-104.

JAK2 (MUTATION TESTING)

DEFINITION – INTRODUCTION

The discovery of the *JAK2*, *(Janus Tyrosine Kinase 2)*, mutation in 2005 completely turned around the pathophysiological understanding of myeloproliferative syndromes (MPS) outside of chronic myeloid leukaemia (CML).

The myeloproliferative syndromes (MPS) are characterised by clonal proliferation of myeloid haemopoietic stem cells, expressed clinically by disease involving one or more myeloid cell lines (erythroblast, granular and/or megakaryocyte).

The MPS classification was revised in 2008 by the World Health Organisation, based on 2 criteria:

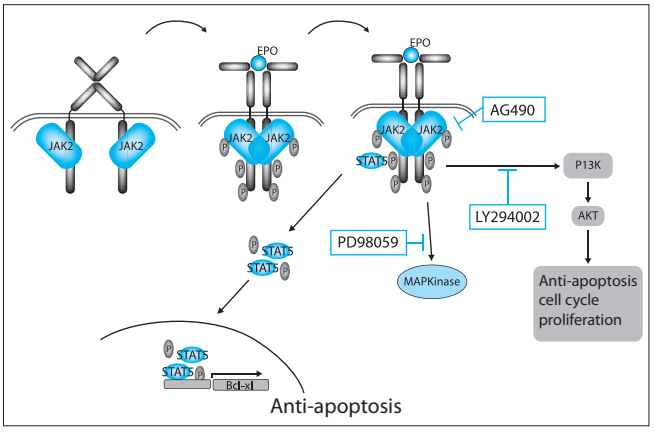
– Characterisation of molecular clonality markers with determination of the *BCR- ABL transcripts and the V617F JAK2* mutation

– Introduction of new histological criteria for unclassifiable MPS.

Whilst presence of the *BCR-ABL* transcript is specific for chronic myeloid leukaemia, the *JAK2 V617F* mutation appears to be a key marker for several clinical entities, as it is seen in polycythaemia rubra vera, essential thrombocythaemia and osteomyelofibrosis.

– JAK2 mutation and MPS

Janus kinase 2 (JAK2) is a tyrosine kinase protein involved in several signalling pathways *(JAK-STAT)*, particularly the haemopoietic cell proliferation pathway. *JAK2* binds to receptors for the cytokine family, which can be activated by cytokines and growth factors such as EPO-R, TPO-R, GM-CSF-R, GH-R and PRL-R.



From Ann biol Clin 64;1 2006

A point mutation in exon 14 of the *JAK2* gene results in replacement of a valine by a phenylalanine in position 617 *(V617F)*. This mutation has negative control on the kinase activity of the JAK2 protein, making haemopoietic progenitor cells hypersensitive to and independent of cytokines.

From a clinical perspective the mutation has a prevalence of 80 to 97% in polycythaemia rubra vera (PRV) and is seen in more than 50% of cases of essential thrombocythaemia (ET) and osteomyelofibrosis (or primary myelofibrosis = PMF).

Although the mutation is not specific to MPS, its determination is an important diagnostic tool, as seen in the new decision algorithms proposed recently in the WHO classification of myeloid diseases.

– JAK2 exon 12 mutations in MPS and MPL mutations

New mutations have been described in exon 12 of the *JAK2* gene in 5% of *JAK2 V617F*-negative PRV. Correlations have been shown between the presence of these mutations and the clinical picture of PRV, with preferential proliferation of the erythroblast cell line.

Eight new mutations have been reported to date in polycythaemia rubra vera. These exon 12 mutations do not appear to be involved in ET and PMF.

In 2006, a new mutation was implicated in JAK2 V617F negative MPS in the transmembrane domain of the ***MPL gene*** (coding for the transmembrane thrombopoietin (TPO) receptor). This mutation is located on codon 515 of the transmembrane domain and results in the replacement of a leucine or lysine tryptophan *(MPLW515L/K)*.

The mutation results in activation of a *JAK2* independent signalling pathway.

These mutations are seen in 1 to 2% of cases of ET and in 5 to 9% of cases of osteomyelofibrosis with preferential expansion of the platelet line and myelofibrosis.

– WHO diagnostic criteria for MPS

The WHO has defined a series of major and/or minor criteria for the diagnosis of polycythaemia rubra vera, essential thrombocythaemia and osteomyelofibrosis.

The presence of the *JAK2V617F* mutation is a major criterion in the diagnosis of these three conditions.

– Diagnosis of polycythaemia rubra vera

The following are needed:

2 major criteria and 1 minor criterion or 1 major criterion and 2 minor criteria.

– Major criteria

- Haemoglobin of 18.5 g/dl in men or 16.5 g/dl in women, or any other diagnosis indicating an increase in red cell mass.
- *JAK2 V617F mutation or JAK2* exon 12 mutation.

– Minor criteria

- Precise histological appearance.
- Low serum EPO concentration.
- *In-vitro* endogenous formation of erythroid colonies.

– Diagnosis of osteomyelofibrosis

3 major criteria and 2 minor criteria are required for this diagnosis.

– Major criteria

- Precise histological criteria.
- No factors supporting an alternative MPS – PRV, MDS or other MPS.
- Presence of the *JAK2V617F* mutation or of another clonal marker such as *MPLW515L/K*.

– Minor criteria

- High leukocyte count and/or Hb.
- High LDH.

- Anaemia.
- Splenomegaly.

– Diagnosis of essential thrombocythaemia

The following 4 criteria are needed for the diagnosis:

- Platelet count > 450 x 10^9/l.
- Precise histological criteria.
- No other criteria for MPS.
- Presence of the *JAK2V617F* mutation or another clonal marker and no reactive thrombocytosis.

The JAK2 V617F mutation is also described in approximately 60% of cases of Budd-Chiari syndrome (supra-hepatic vein thrombosis). Testing for this genetic abnormality may therefore be offered for the investigation of thrombosis.

INDICATIONS

– Diagnosis of myeloproliferative syndromes.

– Thrombosis investigation (2nd line).

INFORMATION

■ SAMPLE

– 2 x 5 ml tubes of whole blood collected into EDTA.

– Results of the Full Blood Count must be attached.

■ SAMPLE STORAGE AND TRANSPORT

Sample stability: 24 h after sampling at room temperature or for 4 days at + 4°C.

ASSAY METHODS

The method principle is based on real-time quantitative PCR (RQ-PCR). This method discriminates between alleles, through the use of 2 diagnostic primers at the 5' end, corresponding to the wild type and V617F mutated type respectively and a common 3' primer. PCR amplification only occurs when the primers and target are 100% complementary. If not, the PCR cannot amplify. This method also uses a fluorescent probe common to the wild and mutated alleles. The use of a common hydrolytic probe for the two (wild and mutated) alleles with a 5' "reporter" and a 3' "quencher" allows the RQ-PCR analysis to proceed. The probe is hydrolysed by the 5'>3' exonuclease activity of Taq polymerase. In the absence of a target sequence (no PCR amplification), the probe remains intact as the distance between the two molecules (the *reporter* and the *quencher*) prevents the reporter emitting fluorescence. If the target sequence of interest is present, the probe binds specifically and is degraded by the 5'-3' exonuclease activity of the Taq Polymerase, allowing fluorescence to be emitted *(Figure 1)*. The increase in fluorescence is directly proportional to the number of target copies present in the sample when amplification begins. A standard curve can be constructed and the number of target copies present in the sample established using standards with a number of copies of the desired cDNA being tested

The increase in fluorescence is directly proportional to the number of target copies present in the sample at the beginning of amplification.

A standard curve can be constructed and the number of target copies present in the sample can be established using standards with a known number of molecules.

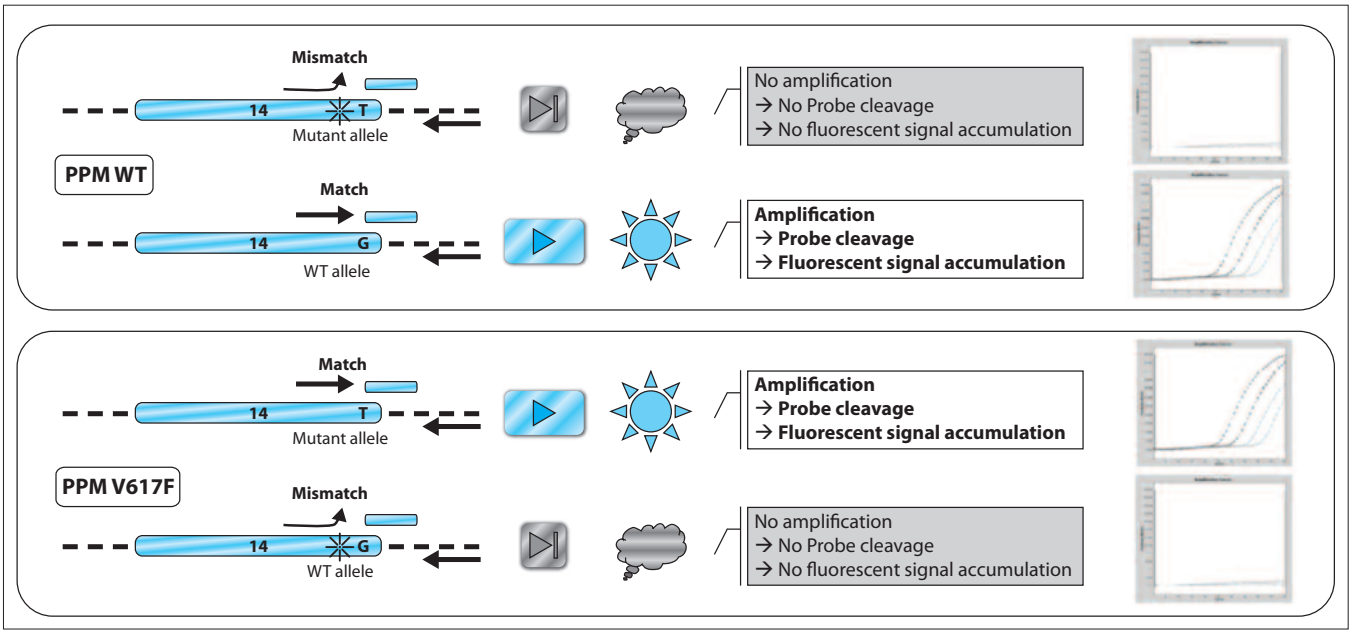


Figure 1: Diagrammatic representation of detection of the V617F mutation by specific allele PCR.

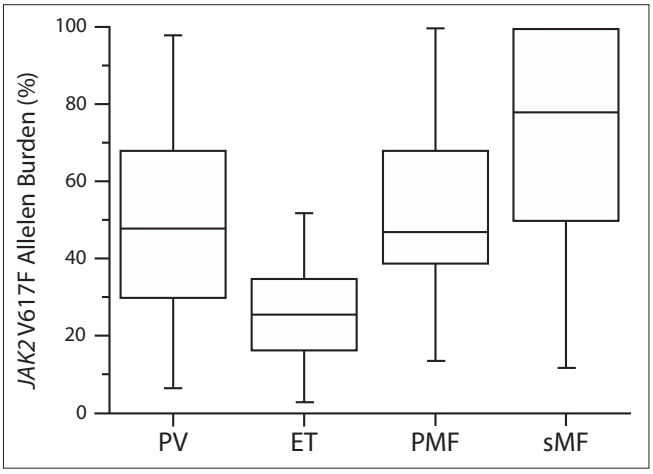
INTERPRETATION

The percentage variation in *JAK2 V617F* is related to the differences in the number of mutated alleles in the cell.

Current sensitivity is 0.21%.

– Mutation burden and diagnosis

A recent study has shown that more than 50% of alleles are often mutated in PRV and PMF, whereas less than 50% are mutated in ET, although there may be overlaps between the different diseases, as shown in the study by Vannuchi AM in 2008.



Study of 893 patients suffering from MPS, PRV (n=297), ET (n= 382), MPF (n = 168) and transformed MPS (sMF) (post PRV and post TE) (n = 46).

Median values are very low for ET (26 +/-15) compared to the other diseases such as PRV (48 +/-26), MPF (72 +/-24) and sMF (46 +/- 30).

– Mutation burden and prognosis

The benefit of quantification is that it provides an assessment of tumour mass, which is indirectly related to the prognosis of the MPS, explaining the high percentage mutated allele rate in PRV and MPF compared to ET.

However, no studies have actually demonstrated a correlation between tumour mass and the risk of transformation of PRV or MPF or in acute leukaemia.

Similarly, results on the risk of thrombosis have been contradictory. These differences are to a large extent related to the often very different methods used in these studies and the fact that they are generally retrospective.

New prospective studies are ongoing to better define prognostic indicators.

– Mutation burden and monitoring for residual disease

The initial clinical studies on targeted anti-JAK2 treatments which are currently ongoing showed rapid clinical responses from the outset, both in mutation + and mutation - patients. These results suggest that quantitative analysis of the *V617F JAK2* mutation is not a target for response to treatment.

Overall, the presence of a JAK2 V617F mutation in *BCR-ABL1* negative MPS has completely changed the diagnostic decision algorithms for MPS.

The existence of this clonal marker has led to the introduction of targeted therapies.

In addition, the existence of genuine clinical presentations of MPS without the *JAK2* mutation, suggests that other factors are probably involved in the pathophysiology of these cases of MPS, such as the *TET2* gene.

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KETONE BODIES

DEFINITION AND SYNONYMS

The ketone bodies comprise β-hydroxybutyrate (β-hydroxybutyric acid), and acetoacetate (acetoacetic acid). β-hydroxybutyrate and acetoacetate are synthesised in liver cell mitochondria and are produced as a result of lipolysis or from ketogenic amino acids (leucine). The ratio of the concentrations of the two ketone bodies depends on the NAD/NADH system. High hepatic NADH concentrations promote the formation of β-hydroxybutyric acid (this occurs in hypoxia, fasting and diabetic or alcoholic ketoacidosis).

INTRODUCTION

Ketone bodies are alternative energy substrates to glucose for the brain, nervous system and muscles used during periods of fasting or intense physical exercise. Acetone is only produced from acetocetate and it is formed by the spontaneous decarboxylation of acetoacetate in urine or in alveolar secretions. Total serum ketone body concentrations are a result of an equilibrium between hepatic ketogenesis and peripheral consumption. Outside of the context of any disease these concentrations are very low.

Raised total ketone body concentrations are harmful as they result in metabolic acidosis (ketoacidosis).

INDICATIONS FOR MEASUREMENT

Diagnosis of ketonaemia and ketoacidosis, which have many causes, such as diabetes mellitus, alcoholism, stress, prolonged fasting, metabolic diseases, (Von Gierke's), gastro-intestinal problems and childhood acidaemias. Measurement can also be used to confirm isopropanol poisoning.

INFORMATION

■ SAMPLE

Blood: Purified sample, supernatant, frozen within an hour of sampling. Take capillary or venous blood without using a tourniquet. Measure precisely 0.6 ml of blood and add immediately to a tube containing 0.8 ml of 1 M perchloric acid (provided by the laboratory performing the analysis and kept in a refrigerator). Shake vigorously and freeze the supernatant immediately.

Urine must be collected into a sealed container stored at +4°C to reduce evaporation of acetone.

■ QUESTIONS FOR THE PATIENT

Many drugs can interfere with the assay methods. The patient should be asked about all recent treatment including vitamin C.

■ SAMPLE STORAGE AND TRANSPORT

Purified supernatant frozen within an hour of sampling, and urine stored in a sealed container for a maximum of 3 days at +4°C.

ASSAY METHODS

Assay of total ketone bodies: The most widely used method is the Legal test strip method using ammonium and nitroprusside. This only reveals the presence of acetoacetate and acetone in biological fluids. Drug interferences can cause false positive reactions: 8-hydroxyquinolene, L-dopa, enalapril, D penicillamine and dimercaprol. The presence of phenylpyruvic acid (phenylketonuria) also produces false positive results. False negative results are seen with hyperketonaemia or hyperketonuria with a predominance of β-hydroxybutyrate.

Interpretation of test strip results using the Legal method

Positivity level	Concentration of acetoacetate (mg/l)	Concentration of acetoacetate (mmol/l)
+	100 to 300	1 to 3
+ +	300 to 800	3 to 8
+ + +	> 800	8

For specific measurement of acetoacetate and β-hydroxybutyrate the biological fluid must be deproteinised (with 1M perchloric acid). Different assay methods are described, such as colorimetry, gas chromatography, capillary electrophoresis and enzymatic. The Williamson enzymatic method is the most widely used routinely (quantification of the NADH produced when β-hydroxybutyrate is converted into acetoacetate by hydroxybutyrate dehydrogenase).

Performance of assay methods for ketone bodies

	Analytical range	Limit of detection
Nitruprusside / Legal colourimetric method	3-8 mmol/l	0.5 mmol/l
Hydroxybutyrate / Williamson enzymatic method	0.02-5 mmol/l	0.01 mmol/l

NORMAL EXPECTED VALUES

These vary depending on age, fasting status and method used. Refer to the normal values reported by the laboratory.

PATHOPHYSIOLOGICAL VARIATIONS

Physiological variations in ketone bodies

Serum ketone body concentrations depend on the patient's age and fasting status (blood ketone concentrations are raised by a factor of 6 and the β-hydroxybutyrate/acetoacetate ratio rises to above 2.5 to 3.5 during a 15 hour fast). Raised ketonaemia is seen in the following situations: prolonged fasting, intense physical exercise, fat-rich diet, dietary deficiency in pregnancy or neonatology, repeated vomiting and fever in young children.

Pathological hyperketonaemia

Hyperketonaemia in a normally fed patient is pathological. Ketone body concentrations rise in the following situations: decompensated type I diabetes, alcohol poisoning, salicylate poisoning, growth hormone deficiency, corticosteroid deficiency and some metabolic diseases (acetoacetyl CoA thiolase deficiency, succinyl CoA transferase and pyruvate carboxylase deficiencies).

In type I diabetes, semi-quantitative monitoring of ketone bodies is sufficient particularly in situations at risk of decompensation (stress, pregnancy, infections, etc). Blood

ketone monitoring is recommended before starting insulin therapy. Raised serum concentrations in a diabetic patient or in a patient with persistent hyperglycaemia suggests diabetic ketoacidosis which is a medical emergency. In this situation, β-hydroxybutyrate concentrations are raised. Other laboratory and clinical indices can be used to confirm the diagnosis, such as bicarbonate < 17 mmol/l, arterial pH < 7.3 and plasma glucose > 14.9 mmol/l (2.5 g/l).

Without these signs and particularly without hyperglycaemia, raised β-hydroxybutyrate concentrations can be seen with fever in young children (due to depletion of glucogenic reserves) and in alcoholic ketoacidosis through deficient hepatic function.

Specific measurement of each ketone body is recommended in children to diagnose certain constitutional mitochondrial cytopathies and to monitor exclusive parenteral nutrition.

Hypoketonaemia in metabolic diseases

Hypoketonaemia associated with hypoglycaemia is always pathological and may suggest hyperinsulinism or constitutional fatty acid diseases (multiple mitochondrial acyl CoA dehydrogenase deficiency, carnitine palmitoyl transferase II deficiency, etc).

β-hydroxybutyrate/acetoacetate ratio in hepatic disorders and metabolic diseases

A raised ratio may suggest defective mitochondrial oxidation. The ratio is used successfully to assess liver transplantation or the severity of liver disease in chronic liver diseases. A raised ratio is supporting evidence in favour of specific pyruvate carboxylase deficiency.

FOR FURTHER INFORMATION

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■ Vassault A., *Corps cétoniques*, Encyclopédie Médicale et Biologique, 2003.

KETOSTEROIDS (17-)

DEFINITION

The 17-ketosteroids (17KS) or 17-oxosteroids are a group of steroids derived from androstane (19 carbon atoms) with a ketone group at carbon 17, giving them their name. They consist mostly of 7 steroids commonly called: Dehydroepiandriosterone (DHEA), etiocholanolone (E), androsterone (A) and the 11β-OH and 11-oxo derivatives of etiocholanolone and androsterone. They are metabolites formed in the liver from both gonadal (testosterone, androstenedione and dehydroepiandrosterone) and adrenal (DHEA sulphate and 11β¬hydroxyandrostenedione) androgens. A small proportion of the 17-KS also arise from cortisol catabolism.

Testosterone and DHEA, in equilibrium with its sulphate, are converted firstly in the liver into androstenedione, which undergoes two successive reductions, producing the two main isomers which are found in the urine: Androsterone (3α-hydroxy-5α-androstan,-17-one) (A) and etiocholanolone (3α-hydroxy-5β-androstan-17-one) (E). 11-hydroxyandrostenedione is also metabolised by successive reductions producing the 11 oxygenated derivatives of A and E. These are also formed from the catabolism of cortisol, the side chain of which undergoes oxidative cleavage followed by reduction of the C4 double bond and then of the C3 ketone group.

All of these metabolites are excreted in conjugated form in the urine as sulphates or glucosiduronates. DHEA sulphate is excreted in its native state. DHEA is almost exclusively sulphoconjugated. The reduced 5α- derivatives are predominately sulpho-conjugated whereas the reduced 5β derivatives are predominantly glucurono-conjugated.

INDICATIONS FOR MEASUREMENT

As this is a heterogeneous group originating from both gonadal and adrenal, or only adrenal steroids, measurement of the 17-KS has now lost much of its value as the active androgens and cortisol can be measured in the serum. Measurement may still however be indicated to diagnose adrenal and particularly adrenal cortical tumours. Measurement may also be recommended to monitor the treatment of congenital adrenal hyperplasia (11β-hydroxylase or 21-hydroxylase deficiencies). They may also be measured as part of the hormonal assessment of Cushing's syndrome.

INFORMATION

A 24 hour urine sample is collected without preservative and stored at + 4°C. Creatinine is measured in parallel to assess the quality of the urine collection.

ASSAY METHODS

The 17-KS are extracted with dichloromethane following hot acid hydrolysis. The extracts are purified with sodium hydroxide and assayed with colorimetric assay with the Zimmermann reaction. The intensity of red-purple colour which the 17-KS develop with alkaline metadinitrobenzene is measured spectrophotometrically at 520 nm. A standard curve is constructed in parallel with the unknown samples under the same conditions using increasing concentrations of DHEA. The results for the unknown samples are obtained from calibration with the standard curve and are expressed in DHEA equivalents.

INTERFERENCE FACTORS

The Zimmermann colorimetric reaction is not particularly specific for the 17-KS, with many substances, particularly numerous medicinal products, interfering in the reaction. Some medicinal products therefore result in overestimation of 17-KS concentrations, such as chlorpromazine, meprobamate, penicillin, nalidixic acid, spironolactone, tiaprofenic acid but others such as reserpine, chlordiazepoxide, progestogens and propoxyphene produce a false underestimate.

Apart from these drugs, urine contains various steroidal and non-steroidal compounds which produce the characteristic red-purple colour of the 17-KS with Zimmermann's reagent. These therefore increase 17-KS results. Various methods including aluminium column chromatography have been proposed to improve the specificity of the reaction although because of the non-specificity of the colorimetric reaction and the heterogeneity of the urinary 17-KS, their measurement is increasingly being abandoned in favour of measurement of the active hormones in the serum.

UNITS

Results are generally expressed in mg/24 hours or µmol/24 hours. Conversion to µmol/24 hours is achieved by multiplying mg/24 hours by 3.47 (in DHEA equivalents).

PHYSIOLOGICAL VARIATIONS

Urinary 17-KS concentrations vary with sex and age. Before puberty, 17-KS concentrations in children are due to adrenal secretions and no sex differences are seen. Concentrations remain almost at a plateau up to the age of three and then increase linearly with age up to the age of approximately 9. The change in concentrations then accelerates markedly, only reaching adult values at around the age of 15 *(cf. table)*.

In adults, concentrations are generally higher in men than in women. There is however an overlap between the distribution curves in men and in women at lower values in men.

In addition to day-by-day intra-individual variations, variations in 17-KS excretion occur during the 24 hour cycle.

17-ketosteroid excretion depending on sex and age

Age	Male		Female	
(years)	mg/24 hours	µmol/24 hours	mg/24 hours	µmol/24 hours
< 2	0.15 – 0.75	0.52 – 2.60	0.15 – 0.75	0.52 – 2.60
2 - 4	0.20 – 0.90	0.69 – 3.12	0.20 – 0.90	0.69 – 3.12
5 – 9	0.50 – 3.00	1.74 – 10.41	0.50 – 3.00	1.74 – 10.41
10 - 12	1.20 – 6.20	4.16 – 21.51	1.10 – 6.20	3.82 – 21.51
13 - 14	1.60 – 8.70	5.55 – 30.19	1.60 – 9.80	5.55 – 31.00
Adults	7.00 – 17.00	24.29 – 59.00	5.00 – 14.00	17.35 – 48.58

17-KS excretion falls progressively with age. This fall becomes increasingly marked from 75 years old onwards in both men and women.

PATHOLOGICAL VARIATIONS

Urinary 17-KS concentrations are reduced in adrenal insufficiency, either primary or secondary, to long-term corticosteroid therapy. Concentrations are also reduced in hypogonadism, both primary and secondary to pituitary dysfunction.

17-KS excretion is raised in hypercorticism, both in Cushing's disease and Cushing's syndrome. Concentrations are also raised in congenital adrenal hyperplasia due to 21 or 11β-hydroxylase deficiency.

A discrepancy is also seen between the rise in 17-KS concentrations and the fall in 17¬-hydroxycorticosteroids (17-OHCS) in 21-hydroxylase deficiency because of a deficiency in the biogenesis of cortisol, the precursor of 17-¬OHCS.

Large rises in 17-KS are seen with adrenal tumours, particularly adrenal cortical tumours.

In gonadal diseases, raised concentrations of 17KS are excreted in testicular Leydig tumours or leydigomas, in virilising ovarian tumours and in the polycystic ovarian syndrome.

In conclusion, as described above, gonadal dysfunction and adrenal diseases are more reliably investigated using measurements of the active steroids in the plasma rather than urinary 17KS.

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KRAS MUTATION STATUS

DEFINITION – CLINICAL USE

Colorectal cancer (CRC) is the second most commonly diagnosed cancer. CRC represents the second most common cause of deaths world wide. Approximately 30 % of patients with CRC have metastatic disease at the time of diagnosis (mCRC).

KRAS mutations screening is performed in cases of metastatic colorectal cancer (mCRC) for therapeutic decision. Several studies showed the presence of a hotspot mutational site in codons 12, 13, 61 and 63 in *KRAS* oncogene. *KRAS* mutation status allows for the identification of patients who might benefice from anti-EGFR therapies and avoid a costly and potentially toxic administration of this treatment in non responsive patients.

- Wild-type *KRAS* status: responsive to anti-EGFR therapies
- Mutated *KRAS*: non responsive to anti-EGFR therapies.

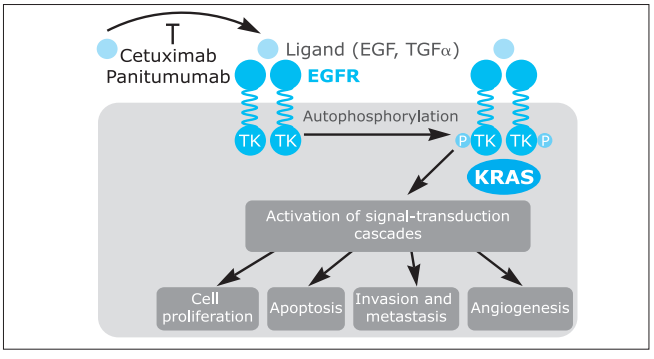
SCIENTIFIC BACKGROUND

Somatic mutations in the *RAS* oncogene familly (*HRAS, KRAS* and *NRAS*) are observed in a variety of various malignancies, including colorectal cancer (33~53 %), pancreatic cancer (~80 %), lung adenocarcinoma (~30 %), ovarian and endometrial cancer, gall bladder cancer, bile duct cancer (~45 %), thyroid cancer (~55 %) and hematological malignancies.

The *KRAS* gene is located on chromosome 12 and encodes for a G protein involved in colorectal carcinogenesis. The KRAS protein plays a central role in tumor development, regulating downstream proteins involved in proliferation, survival, metastasis and angiogenesis via the EGFR signalling pathway.

The *KRAS* protein regulates PI3K/AKT and RAS/MEK/ERK signalling pathways located downstream of many growth factor receptors, including EGFR. When bound to it's ligand, EGFR stimulates tyrosine kinase activity leading to activation of KRAS and signalling pathways.

Genetic alterations of the intracellular effectors involved in EGFR-related signalling pathways may have an effect on response to this targeted therapy. The presence of an activating mutation in codons 12 and 13, the KRAS protein is permanently turned on, even without being triggered by EGFR mediated signalling and the therapies targeting EGFR are ineffective.



INDICATIONS: SPECIFIC TARGET-DIRECTED THERAPIES

Three monoclonal antibodies have been approved for colorectal cancer therapy including monoclonal antibodies against epidermal growth factor receptor (EGFR) and vascular endothelial growth facter (VEGF).

The new therapies targeting EGFR are cetuximab (Erbitux®; Merck Serono) and panitumumab (Vectibix®; Amgen). The action of cetuximab or panitumumab is the blockage of ligand binding receptor and thereby causing the inhibition of ligand mediated pathway.

The European Commission has granted a market authorization for cetuximab and panitumumab for the treatment of patients whose tumors harbor normal, non-mutated (wild type; WT) *KRAS* gene.

SAMPLE PREPARATION PRE-ANALYTICAL REQUIREMENT

Molecular biology for KRAS status is available on:

Paraffin embedded tumoral tissue

Formalin - not Bouin

or

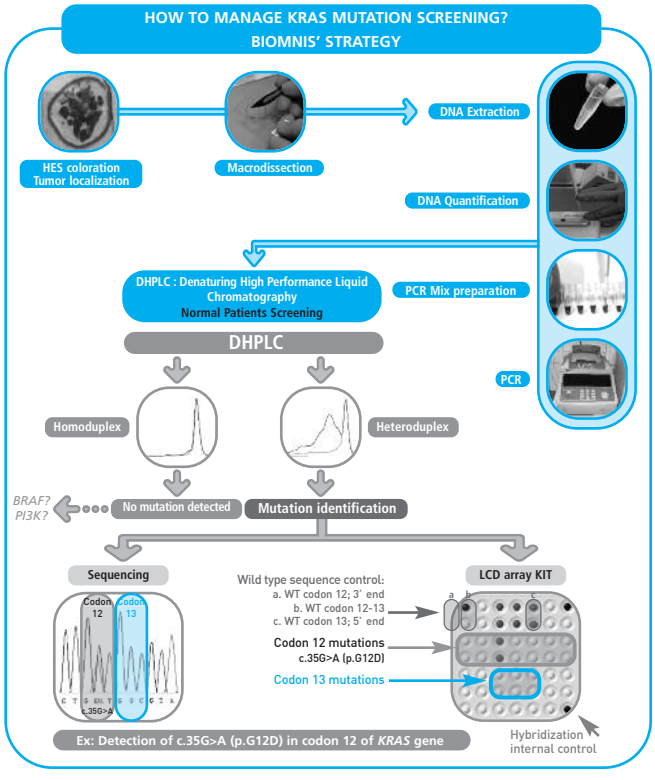
Unstained slides (10-20 µm thick)

Paraffin embedded (or cryo) mass tumor: 5 unstained slides

Paraffin embedded (or cryo) biopsy: 10 unstained slides

Histology report is mandatory.

METHODS



	ROUTINE KRAS MUTATIONS ANALYSIS METHODS					
	DIRECT SEQUENCING	PYROSEQUENCING	DHPLC	HRM High Resolution Melting	Real time PCR	LCD array
SENSITIVITY (MT/WT, %)	15-20 %	5 %	2-5 %	2-5 %	1-2 %	<1 %
ADVANTAGES	• Detects all genetic variations in the sequenced amplicons	• Sensitive • Detects only specific mutations tested	• Sensitive • Detects all genetic variations in the amplicon tested	• Sensitive • Detects all genetic variations in the amplicon tested	• Sensitive • Rapid TAT (1 week) • Detects only specific mutations tested	• Sensitive • Rapid TAT (1 week) • Doesn't require any technical background • Only 1 PCR to identify all the mutations tested • Presence of Wild Type suppressor sequence (WSC) to enhance sensitivity of the mutation detection • Detects only specific mutations tested
DISADVANTAGES	• Lack of sensitivity • Technical background required • Extended TAT (4 days to 2 weeks)	• Technical background required • Extended TAT (4 days to 2 weeks)	• High technical background required • Sequencing confirmation required • Extended TAT (4 days to 2 weeks)	• High technical background required • Sequencing confirmation required • Extended TAT (4 days to 2 weeks)	• Not convenient: detects only a single specific mutation per reaction • Expensive (2 primers and 2 probes for each specific mutation in each PCR reaction)	

RESULTS

– KRAS mutations in exon 2:

Kras mutations Distribution in metastatic colorectal cancer		
p.G12V; c.35G >	T 21,7 - 28,1%	Codon 12
p.G12A; c.35G >	C 6,4 - 8,2%	80%
p.G12D; c.35G >	A 35,7 - 38%	
p.G12S; c.34G >	A 7,6 - 9,9%	
p.G12C; c.34G >	T 5,3 - 7,6%	
p.G12R; c.34G >	C 1,2 - 1,6%	
p.G13D; c.38G >	A 11,7 - 15,8%	Codon 13
p.G13C; c.37G >	A 0,6%	15%
Others (codons 61 and 63)		< 5%

– Additional advances in bio marker use for direct target therapies:

BRAF and PI3K mutations are being explored and seem to give promising data for a more accurate therapeutic approach.

FOR FURTHER INFORMATION

■ KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. Lièvre A et al - J Clin Oncol. 2008 Jan 20; 26(3): 374-9.

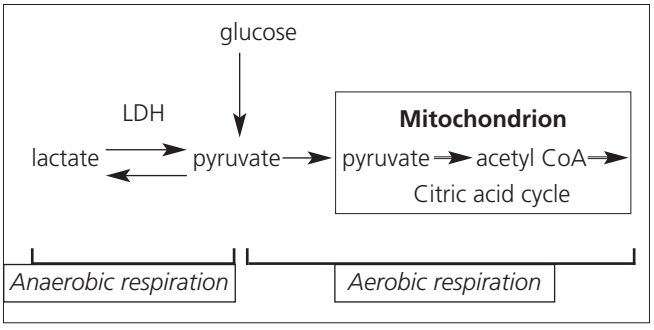
■ Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer.Amado RG et al - J Clin Oncol. 2008 Apr 1; 26(10): 1626-34.

■ Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with nonsmall-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. Eberhard DA et al - J Clin Oncol. 2005 Sep 1; 23(25): 5900-9.

LACTIC ACID

DEFINITION

Lactic acid is the end-product of anaerobic glycolysis, a process which occurs in the cytoplasm of the cell.



It is generated by the catalytic conversion of pyruvate by lactate dehydrogenase (LDH) in muscle tissue, the brain, red blood cells, the gut and the skin. The organs that consume lactic acid are the liver and kidneys.

Lactic acid is a metabolic dead-end and large quantities of it can build up when the rate of anaerobic glucose catabolism is high.

Synonym: lactate.

INTRODUCTION

PHYSIOLOGICAL ROLE

Lactic acid constitutes the main energy source in certain tissues. In the fasting state, it is converted into glucose in the liver and kidneys; after eating, it is oxidised in mitochondria to generate energy for direct consumption in the muscles, heart and kidneys.

INDICATIONS FOR MEASUREMENT

The lactate concentration is of interest when investigating certain metabolic problems and this test is used in the diagnosis and monitoring of diseases that lead to tissue hypoxia (including shock and severe acute asthma) as well as diverse poisoning situations (notably with carbon monoxide, biguanides or salicylate). When taken together with the blood glucose concentration, the result of this test is very useful in the differential diagnosis of metabolic acidosis. Finally, it is also used in sports medicine to estimate effort capacity in athletes.

A lactic acid assay can be ordered on its own or together with a pyruvate test, or it may be carried out in the context of metabolic challenge testing. Lactate can be assayed in venous or arterial blood, cerebrospinal fluid (CSF) or some other aspiration biopsy fluid. In CSF, it yields information about metabolic status in the brain.

INFORMATION

SAMPLE

Venous blood samples should be drawn from a patient who is fasting and has been resting for two hours. Do not use a tourniquet (to preclude venous stagnation). Any muscular exertion, even minimal, should be avoided.

The only anticoagulants that can be used are ***fluoride/heparin or heparin/iodoacetate*** (in whole blood, the lactate concentration in a sample can rise by 20% in 3 minutes and by 70% in 30 minutes if glycolysis is not adequately inhibited).

After drawing, the blood must be immediately cooled on ice and centrifuged at +4°C. The plasma should be decanted off within 30 minutes and frozen, if the test is to be performed later. Icteric and haemolysed samples should be rejected.

QUESTIONS FOR THE PATIENT

Have you ensured that you have fasted appropriately? Blood lactate concentrations vary with nutritional status *(see below)*.

Are you taking any of the following medications? Drugs that can induce a high blood lactate concentration are anti-epileptics, nucleoside analogues (antiretrovirals), salicylate (at toxic doses), isoniazid and biguanides.

SAMPLE STORAGE AND TRANSPORT

The decanted plasma can be kept for two days at between +2°C and 8°C, and for weeks at –20°C. If the sample is being sent to a specialist laboratory, it should be frozen within one hour of drawing.

In CSF, lactic acid is stable for 24 hours at +4°C; if the sample is to be kept for longer before testing, it should be frozen and kept at –20°C. If it is to be transported, it should be frozen within one hour of drawing.

ASSAY METHODS

The most common methods are enzyme-based assays with spectrophotometric read-out.

NORMAL EXPECTED VALUES

Venous blood: New-born babies (< 3 days): 0.90-2.70 mmol/l
Children - Adults: 0.50-1.70 mmol/l

Arterial blood: Children – Adults: < 1.25 mmol/l

CSF: New-born babies (< 3 days): 1.60-3.10 mmol/l
Children - Adults: 1.10-2.20 mmol/l

Conversion factor: 1 mg/l = 0.011 mmol/l.

In a healthy subject, the lactate/pyruvate ratio is about 10.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Age-dependent variations:

cf. see Normal Expected Values.

- **Variations with physical activity and nutritional status:**

The blood lactate concentration rises after physical exertion and eating. It also increases in alcoholics and in patients on parenteral alimentation after the administration of carbohydrate. It decreases in fasting patients.

PATHOLOGICAL VARIATIONS

In adults, lactic acidosis is defined as a plasma lactate concentration over 4 mmol/l (NB: lactic acidosis is often associated with hyperuricaemia and hyperphosphataemia).

Hyperlactataemia can be caused by a number of pathologies; the Cohen & Woods classification system (1976) is still in use:

- **Type A lactic acidosis: Anoxia due to tissue hypoperfusion:**

Endotoxic, cardiogenic and haemorrhagic shock, severe anaemia, left ventricular insufficiency, epileptic convulsions, severe acute asthma, methemoglobinaemia, and poisoning with carbon monoxide or cyanide.

- **Type B lactic acidosis: Secondary to systemic or metabolic disease and poisoning:**

B1: Systemic diseases

Diabetes mellitus, kidney failure, hepatic insufficiency, blood diseases (leukaemia, lymphoma), cancer.

B2: Toxins and drugs

Ethanol, methanol, ethylene glycol, infused sugars (fructose, sorbitol, xylitol), cyanide, Salicylate, biguanides (in subjects with kidney failure), barbiturates, nucleoside analogues (antiretroviral drugs).

B3: Congenital and acquired enzyme deficiencies

Vitamin B1 deficiency and deficiencies in various enzymes involved in: hepatic glycogen metabolism—glycogen synthase, amylo-1-6-glucosidase, hepatic phosphorylase; gluconeogenesis—glucose 6-phosphatase, fructose 1,6-diphosphatase, phosphoenol pyruvate carboxykinase; the pyruvate crossroads—pyruvate kinase, pyruvate dehydrogenase; the citric acid cycle—fumarase, alpha-ketoglutarate dehydrogenase; and the respiratory chain.

- **Sports medicine**

Lactic acid is a marker for exertion. In the course of increasing exertion, there is a close relationship between the degree of effort and the plasma lactate concentration which can be used to define the borderline between "aerobic" exercise and anaerobic respiration in the muscles (at which point the lactate concentration rises).

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LAMOTRIGINE

DEFINITION

Lamotrigine is a phenyltriazine derivative used as an anti-epileptic. It is marketed in the form of 25, 50, 100 and 200 mg dispersible tablets and is indicated for use in the treatment of generalised epilepsy (clonic, tonic, tonic-clonic seizures, absences, myoclonic and atonic jerks and Lennox-Gastaut syndrome) and in partial epilepsies (partial seizures with or without secondary generalisation). It is prescribed as monotherapy or in association with other anti-epileptics in adults and in children between 2 and 12 years old in association with another anti-epileptic treatment when the other is not sufficiently effective. The dosage must always be started low and increased gradually because of the high risk of a skin rash.

Lamotrigine acts on the voltage-dependent sodium channels by stabilising neuronal receptors and inhibits the release of excitatory neuromediators, particularly glutamate, which play an important role in the genesis of epileptic seizures.

Its benefits are its good global tolerability, non-sedative nature, absence of induction and lack of teratogenicity. The major limitation to its use is the risk of skin rashes, which usually occur during the first 8 weeks of treatment (this risk is 1 per 1000 in adults and 3 per 1000 to 1% in children). Whilst the majority of these rashes are mild and transient, they can be serious and life-threatening (Stevens-Johnson or Lyell's syndrome).

PHARMACOKINETICS

Bioavailability	Approximately 98%
Plasma peak (Tmax)	2.5 hours
Plasma protein binding	55%
Metabolism	Approximately 80% hepatic by glucuronide conjugation (inactive metabolites)
Plasma half-life of elimination	23 to 37 hours
Renal elimination	(8% in the unchanged form); also excreted in milk.

INDICATIONS FOR MEASUREMENT

Lamotrigine measurements are justified because of the inter-individual variability in its pharmacokinetics and the lack of relationship between dosage and serum/plasma concentrations. Measurements are indicated if treatment is ineffective or if signs of toxicity develop, particularly when lamotrigine is associated with other drugs which may alter its metabolism (enzyme inducers or inhibitors, *cf. below*).

INFORMATION

SAMPLE

Serum or plasma taken into EDTA or heparin. Avoid tubes with separator gel.

Take sample immediately before the next dose (trough concentration), usually before the morning dose.

QUESTIONS FOR THE PATIENT

Are you taking other medical treatment? Rifampicin, phenytoin, carbamazepine and phenobarbital increase the metabolism of lamotrigine (risk of poor efficacy) because of their enzyme-inducing effect. Sodium valproate reduces its metabolism (it doubles the half-life of lamotrigine), carrying a risk of overdose with increased plasma lamotrigine concentrations and hypersensitivity reactions. If the association is necessary, close clinical monitoring is required.

SAMPLE STORAGE AND TRANSPORT

Store separated plasma/serum for up to 24 hours at room temperature, for a few days at + 4°C; 15 days at – 20°C; if analysis is to be delayed, it is recommended that the sample be transported frozen.

ASSAY METHODS

High performance liquid chromatography with UV detection or capillary electrophoresis.

NORMAL EXPECTED VALUES

For reference, therapeutic range: 2.50 to 15.0 mg/l i.e. 10 to 60 µmol/l.

If massive doses are taken (10 to 20 times the maximum therapeutic dose), the symptoms described are nausea, vomiting, nystagmus, ataxia, altered consciousness and coma. Treatment is symptomatic in the absence of any specific antidote.

FOR FURTHER INFORMATION

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LDH

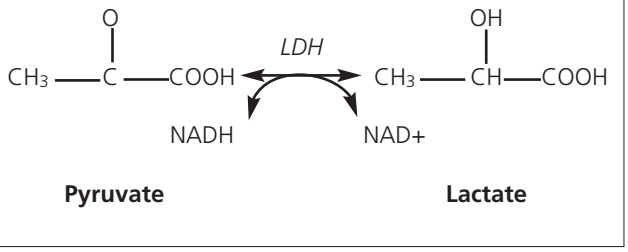
DEFINITION

Lactate dehydrogenase (LDH) is a ubiquitous intracellular enzyme which catalyses the reversible conversion of pyruvate to lactate in the presence of NAD+/NADH. It is a 135 kDa tetramer, consisting of two types of subunits, H (Heart) and/or M (Muscle). The sub-unit composition defines five isoenzymes which have a specific tissue distribution.

Alpha-hydroxy-butyrate dehydrogenase (HBDH) describes the activity of several LDH isoenzymes (mostly LDH-1).

INTRODUCTION

LDH is an intracellular enzyme contained in most tissues (myocardium, liver, kidney, brain and striated muscle) and blood cells (mostly erythrocyte and myelocyte cell lines). LDH catalyses the conversion of lactate to pyruvate and vice versa. In an aerobic environment it catalyses the conversion of lactate to pyruvate, which enters the glucogenesis pathway. In an anaerobic environment it takes part in glycolysis by hydrolysing glucose into lactate.



As LDH is an intracellular enzyme it is released into the blood circulation when cells lyse or tissue is damaged. LDH isoenzymes are differentiated by the type of subunits which make up the tetramer and by their tissue distribution. The H subunit has a high affinity for lactate, promoting energy formation in the aerobic Krebs cycle. The M subunit is more effective in an anaerobic environment.

Isoenzyme	Subunits	Tissues / cells
LDH-1	HHHH	Myocardium, brain, red blood cells
LDH-2	HHHM	Myocardium, brain, red blood cells, reticulo-endothelial system
LDH-3	HHMM	Striated muscle, brain, kidney
LDH-4	HMMM	Liver, striated muscle, kidney, brain
LDH-5	MMMM	Liver, kidney, striated muscle

HBDH (mostly the activity of LDH-1) is found in high concentrations in red blood cells and myocardial tissue. Its measurement has been proposed as a late marker of myocardial infarction.

INDICATIONS FOR MEASUREMENT

Measurement of serum LDH concentration (and studying the distribution of its isoenzymes) was historically offered for a late diagnosis of myocardial infarction and is now used to monitor liver and muscle diseases and cancers.

INFORMATION

SAMPLE

LDH measurement: Serum or heparinised plasma. Haemolysed samples must be discarded (intracellular red blood cell LDH concentrations are 100 times serum concentrations). Anticoagulants such as sodium fluoride or potassium oxalate may influence the accuracy of measurement. A fasting sample is not required.

Isoenzyme measurement: Unhaemolysed serum (minimum 2 ml).

QUESTIONS FOR THE PATIENT

Main disease type: Cancer, past history of myocardial infarction, liver or pulmonary disease, etc?

Recent medicines taken?

Does the patient have a mechanical cardiovascular prosthetic valve?

Recent physical activity?

Pregnancy?

SAMPLE STORAGE AND TRANSPORT

The sample must be centrifuged promptly and the serum separated to avoid haemolysis. It may be stored at room temperature or at +4°C for up to 5 days. When the isoenzymes are being investigated, the sample must not be stored for more than 48 hours at 4°C (LDH-4 and LDH-5 are relatively unstable) and the samples should not be frozen.

ASSAY METHODS

LDH and HBDH are enzymatic at 340 nm measuring the formation or consumption of NADH, H+ (depending on pH).

The isoenzymes are distinguished electrophoretically on agarose or acetate gel and revealed by the initial consumption of NAD and pyruvate substrates and then by reduction of tetrazolium. The isoenzymes are classified by their electrophoretic mobility (closest to the anode: LDH-1).

Immunological reactions have been proposed for specific measurement of LDH-1 concentration. This isoenzyme has the specific feature of being heat stable at 65°C for 30 minutes, unlike the other isoenzymes.

NORMAL EXPECTED VALUES

Serum LDH concentrations in adults varies depending on the reagents used. In the pyruvate to lactate direction: between 100 and 190 U/l.

The serum distribution of isoenzymes is shown below:

Isoenzyme	% serum
LDH-1	17 – 27%
LDH-2	27 – 37%
LDH-3	18 – 25%
LDH-4	9 – 15%
LDH-5	8 – 20%

The LDH-1/LDH-2 ratio is normally less than 0.8 and the LDH/HDBH ratio is between 1.2 and 1.6. These ratios are altered in myocardial infarction (increase in LDH-1/LDH-2 and fall in LDH/HBDH).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL OR IATROGENIC VARIATIONS

Serum LDH concentrations are generally higher in children and return to adult levels in adolescence.

– Newborn ≤ 1 week: + 400 to 600% compared to adults

– Infants ≤ 1 month: + 300 to 400%

– Children at 3 years old: + 200 to 300%

– Children between 4 and 13 years old: + 150 to 200%

Recent physical activity can transiently increase LDH concentrations, particularly LDH-1, 2 and 5, (up to + 30 to 45% with vigorous exercise). LDH-1 and LDH-2 concentrations rise in any haemolytic disease.

Some drugs or substances can alter serum LDH concentrations. They are increased by alcohol, aspirin, fibrates and anaesthetics, mithramycin, procainamide and the anti-epileptics, and reduced by ascorbic acid. Patients with a cardiovascular mechanical prosthesis have constantly elevated serum concentrations. Serum LDH concentrations are raised in the third trimester of pregnancy and are two to three times normal values at childbirth.

PATHOLOGICAL VARIATIONS

Magnitude of change	Causes
2N to 40N	*Megaloblastic anaemia* *Extension of solid cancer* *Hypoxia* *Cardiovascular/septic shock*
2N to 4N	*Myocardial infarction* *Pulmonary infarction* *Myelocyte leukaemia* *Hodgkin's Disease* *Infectious mononucleosis* *Muscular dystrophy*
< 2N	*Hepatitis* *Cholestasis* *Cirrhosis* *Myxoedema* *Nephrotic syndrome*

Macroenzymes

A macroenzyme may be seen on the electrophoretic trace. This is a complex between LDH and immunoglobulins IgA or IgG. The macroenzyme causes overestimation of serum LDH isoenzyme concentrations and is seen in patients with autoantibodies or light chain abnormalities. A macroenzyme has also been reported, consisting of LDH bound to streptokinase after fibrinolytic streptokinase treatment.

Myocardial infarction

LDH increases in myocardial infarction, particularly in the first 24 hours. It remains at elevated levels for 7 to 10 days reaching a peak between the 2nd and 3rd day after the onset of the infarction. The increase is mostly in LDH-1 and therefore HBDH. The LDH-1/LDH-2 ratio inverts and is over 2 in 80% of patients on day 2.

LDH concentration monitoring may be recommended for the late diagnosis of myocardial infarction.

NB: The LDH concentration is not affected by angina.

	Onset	Peak	Duration
HBDH	12 hours	3 days	10 days
LDH	12 - 24 hours	2 to 3 days	5 to 10 days
Ratio LDH-1/LDH-2	12 - 24 hours	2 days	-

Lung disease

Serum LDH concentrations raise in disease of the pulmonary system, such as pulmonary embolism, pulmonary infarction, pneumonia and heart failure with cor pulmonale. Lung disease usually increases skeletal muscle action and is usually associated with a rise in the LDH-5 isoenzyme. Serum LDH concentrations (specifically LDH-2 and LDH-3) rise in pulmonary embolism, without a rise in CK or ASAT.

Liver disease

Diseases associated with hepatitic changes can cause a rise in serum LDH concentrations, such as hepatitis, active cirrhosis, hepatocellular carcinoma and liver disease in infectious mononucleosis. LDH is only moderately raised in viral hepatitis. Investigation of the LDH-5/LDH-4 ratio has been proposed to monitor liver diseases (the ratio raises in this situation).

NB: The raise in serum LDH-5 concentrations in hepatic disease is independent of CK concentrations unlike what is seen in muscle disease.

Muscle disease

Serum LDH and HBDH concentrations rise if muscle cell integrity is damaged, in muscle dystrophy and in trauma. This rise (generally LDH-5) is accompanied by a rise in serum CK concentrations. Isoenzymes LDH-1 to LDH-3 rise in Duchenne-Erb dystrophy (no LDH-5 isoenzyme).

Renal disease

Renal parenchymal disease also produces a rise in LDH concentrations. This may occur in glomerulonephritis, nephrotic syndrome, acute tubular necrosis and renal transplant rejection. The isoenzymes involved are generally LDH-3, LDH-4, and LDH-5.

Haematological disease

Serum LDH and HDBH concentrations may rise in anaemia, particularly peripheral haemolytic anaemia and megaloblastic anaemia. The main isoenzymes involved in haemolysis are LDH-1 and LDH-2. Treatment may be monitored by following a return of concentrations to normal. Serum LDH concentrations do not raise in iron-deficiency anaemia or due to bone marrow failure. Serum LDH raises are also seen in myelocytic leukaemia, Hodgkin's disease and reticulo-endothelial system diseases. Monitoring of LDH concentrations has been proposed as a prognostic index in non-Hodgkin's lymphomas.

Malignant disease

Serum LDH concentrations may be raised in local or metastatic extension of a solid cancer. In abdominal and pulmonary tumours the LDH-5 isoenzyme rises preferentially, whereas the LDH-1 isoenzyme raises in testicular (seminomas) or ovarian (dysgerminomas) cancers. The LDH-5/LDH-1 ratio has been proposed to monitor the activity of some cancers, such as prostate cancer. In breast cancer, LDH is the most consistently raised enzyme and it is used to screen for recurrence after surgery. A specific LDH isoenzyme running between LDH1 and albumin on the protein electrophoresis strip has been reported in these cancers.

Other diseases

Rises in serum LDH concentrations have been seen in bowel ischaemia, pancreatitis and in heat stroke, connective tissue disease, hypotension and cardiovascular or septic shock.

LDH in other biological fluids (other than serum)

Urinary LDH measurement can be used to monitor urinary tract disease (cancer or damage). Measurement of the LDH-4 isoenzyme in semen has been proposed to monitor fertility. LDH measurement in pleural fluid can be used to distinguish an exsudate (an accumulation of fluid due to inflammation) from a transudate (accumulation of fluid due to underlying stasis). Pleural concentrations may exceed 1000 IU/l in empyema.

	Exsudate	Transudate
Pleural LDH	> 200 IU/l	< 200 IU/l
Ratio Pleural LDH /Serum LDH	> 0.5	< 0.5

LDH isoenzyme distribution analysis has also been proposed for cerebrospinal fluid. Normally, isoenzyme concentrations decrease from LDH-1 to LDH-5, although in bacterial meningitis or neurological disorders this may be inverted with decreasing concentrations from LDH-5 to LDH-1. Generally, a rise in LDH in CSF is a sign of encephalitis and a poor prognostic indicator.

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LEAD

DEFINITION AND SYNONYMS

Lead (Pb) is a very soft, malleable, grey-bluish metal which is insoluble in water but soluble in nitric acid and hot sulphuric acid. Traditionally used pure in printing and metallurgy, it is also used in the form of various alloys in numerous other activities (building, lead capacitors, enamelling, "tin" soldering, lead glasswork and pigments for some paints). Finally, lead is a constituent of petrol, although this source of contamination has fallen due to the distribution of "lead free" petrol.

INTRODUCTION

Inorganic lead is absorbed through the lungs and gastro-in–testinal tract. Absorption through skin is generally low. Absorbed lead passes into the systemic circulation and more than 90% is bound to red blood cell haemoglobin and membranes. The remaining lead circulates in serum and is distributed between different organs and tissues. Three main compartments can be distinguished:

– The blood mass and a few rapid exchange tissues in which the half-life of lead is approximately one month

– The central and peripheral nervous system, together with the liver, kidneys and muscles, in which the half-life is 40 to 60 days

– The skeleton, which is the largest compartment, and in which lead content increases over time by transfer from the above two compartments. The half-life of lead in this compartment is 20 to 30 years.

Lead does not accumulate in the lungs, although it crosses the placenta and may accumulate in foetal tissue (blood, liver, brain and skeleton). Inorganic lead is not metabolised by the body. Unabsorbed inhaled lead is removed to the gastro-intestinal tract and then, like ingested lead, is absorbed or directly removed in faeces. Absorbed lead is mostly removed in urine (80%) and in bile (15%), gastro-intestinal secretions, sweat and the integument. It is also secreted in breast milk.

Lead poisoning or saturnism is one of the oldest diseases known. In parallel, childhood lead poisoning is a major public health problem, as chronic poisoning even at low dose, can impact on height and weight, intellectual and psychomotor development in the child and secondly because oral exploratory behaviour in children increases the risk of poisoning, old lead paints (white lead) being the major source of poisoning particularly in the socially disadvantaged.

Occupational lead poisoning may occur after inhaling (dust or smoke) or ingesting (regurgitation or skin hygiene problem) lead or its mineral compounds. Acute occupational toxicity is rare. Lead poisoning is characterised by gastro-intestinal problems (oesophagitis and gastritis causing vomiting), epigastric and abdominal pain, diarrhoea and black stools, and renal problems. Laboratory tests show haemolytic anaemia, a raised serum creatinine and occasionally hepatitic changes. Chronic poisoning is characterised by normochromic, normocytic anaemia followed by microcytic, hypochromic anaemia, when associated with iron deficiency. In some cases this is the first sign of poisoning and begins at blood lead concentrations in the region of 500 µg/l. Gastro-intestinal toxicity produces extra-cellular deposition of lead in the gums (Burton line), Gübler spots on the cheeks and abdominal pain of varying severity which may cause "lead colics", sometimes promoted by infection or alcohol. Neurological damage is characterised by deterioration in cognitive function which may develop at blood lead concentrations of 400 µg/l. Sensory motor neuropathy, the classical form of which is pseudo-radial paralysis may also be seen, together with amyotrophic lateral sclerosis, which resolves or stops progressing when exposure ceases. Lead also causes interstitial tubular nephropathy, characterised by low or absent proteinuria, glycosuria, amino aciduria and disturbances of ion transport. Hyperuricaemia may occur. Ovulatory dysfunction with sterility has been reported, whereas the toxic effect of lead on male reproductive function is disputed.

As children are more sensitive to the toxic effects of lead, acute toxicity may be characterised by severe neurological disturbance with an association of encephalopathy, signs of raised intracranial pressure and occasionally convulsive coma occurring at blood lead concentrations in excess of 800 µg/l, and may leave major complications. Encephalopathy occurs commonly in children who have ingested only small amounts of lead and involves serious clinical signs (convulsive coma) and deterioration in higher functions.

INDICATIONS FOR MEASUREMENT

Blood lead is the best indicator of lead exposure in the weeks following stable exposure. The blood lead-atmospheric lead concentration relationship is linear at least when atmospheric lead concentrations are less than 50 µg/m^3. Blood lead is an indicator of recent exposure and does not measure the lead load in the body. When performed distant to any contact with lead it underestimates the lead pool, whereas in the days following excessive exposure, it over-estimates the pool. It rises from the beginning of exposure (D1), varying depending on exposure peaks, reaching a steady state three months after first exposure and then falling for a month after exposure ceases.

Random urine lead measurements are rarely used as they are influenced by diet, the environment, drinking water and renal function. Conversely, the calcium-sodium EDTA stimulated urine lead test can be used to confirm the diagnosis of lead poisoning and is the best laboratory indicator of the internal pool. It assesses the amount of biologically active mobilisable lead and correlates well with neuromuscular symptoms.

δ-aminolevulinic acid dehydratase (ALA dehydratase) is an erythrocyte enzyme involved in the synthesis of haem components which condenses two ALA molecules into porphobilinogen. The activity of this enzyme is reduced in some forms of intermittent porphyria and also in heavy metal, particularly lead, poisoning.

N.B. Urinary delta-aminolevulinic acid (U ALA) and zinc protoporphyrin (ZPP) measurements are described in separate sections.

INFORMATION

SAMPLE

Samples for the measurement of blood lead or ALA dehydratase are collected into anticoagulant (EDTA or heparin).

When monitoring exposed workers, the sample must be taken before the start of the shift; the day of the week is unimportant. In view of the risk of sample contamination, specific precautions are required: samples must be taken away from the work premises, from people who have showered and are not wearing their work clothes (the sample should preferably be taken on the morning of the first work day of the week). The skin must be thoroughly washed before the sample is taken and a vacuum aspiration device collecting into a guaranteed lead free tube (including closure) is used. It is essential that the occupational physician contacts the laboratory that is to conduct the analysis and the laboratory that organised the sample, if different, in order to establish the sampling and transport conditions.

In a request for childhood blood lead, practitioners should assess for risk factors for lead exposure (outdated housing and paints, proximity to a source of industrial exposure, parents' occupation and hobbies, the child's tendency towards pica behaviour, poisoning in neighbouring children and socio-economic status).

Stimulated urine lead tests are designed to identify people who require chelator therapy. A 24 hour urine collection is taken on D-1 and stored at + 4°C for baseline urine lead measurement. On D0, the patient empties his/her bladder and is given 1 g of calcium-sodium ethylene diaminetetracetate (EDTA Ca Na2), reserved for hospital use, as the test must be performed in hospital by slow intravenous administration. The urine is collected over a period of 5 hours and the urine lead is calculated in this collection. Urine must be collected into a bottle which has been pre-rinsed with nitric acid HNO3, 10% v/v and then carefully rinsed with distilled water.

For ALA dehydratase measurement, medical drugs and alcohol should preferably be restricted during the two weeks before the investigation.

QUESTIONS FOR THE PATIENT

Is the patient occupationally exposed to lead?

When was the sample taken vis-à-vis any exposure to lead?

Has the patient showered and changed his/her clothes since the exposure period?

Does the patient live in an environment at risk of lead poisoning?

Does the patient have a person with lead poisoning amongst his/her close contacts?

Does the patient smoke?

For ALA dehydratase measurement: is the patient diabetic and what medicinal products is he/she taking?

SAMPLE STORAGE AND TRANSPORT

Whole blood samples can be stored for one week at + 4°C and beyond this period, at –18°C.

Urine is stable for several weeks at + 4°C and beyond that, frozen at –18°C.

ASSAY METHODS

Assay methods use atomic absorption or better, induction coupled plasma-mass spectrometry (ICP-MS) which is a far more sensitive technique and which under the correct conditions can achieve the limits of detection and quantification required particularly to diagnose childhood lead poisoning.

ALA dehydratase activity is measured by incubating a lysate of red blood cells with δ-aminolevulinic acid which acts as the substrate, and porphobilinogen formation is measured.

NORMAL EXPECTED VALUES

Blood lead measurements are expressed in µg/l or in µmol/l (1 µmol/l = 2 µg/l) and more rarely in µg/100 ml. Reference values for blood lead in the general population in France are < 90 µg/l in men and < 70 µg/l in women. Poisoning is deemed not to be present if the blood lead is < 100 µg/l in children. Random urinary lead in the general population in France is < 25 µg/g of creatinine.

Lead exposure can be confirmed in children if excretion is more than 170 µg/5 hours or 2750 µg/g of creatinine or if the 5 hour urine lead (µg)/EDTA administered (mg) ratio is greater than 0.65. Lead exposure is confirmed in adults at values of more than 600 µg/5 hours, 1600 µg/g of creatinine or a urine lead/EDTA ratio of > 0.60.

Normal values for ALA dehydratase vary between reagents and depending on the temperature at which the reaction is performed. For reference, a normal value may be taken as: > 14 µmol/min/l of red blood cells.

PATHOPHYSIOLOGICAL VARIATIONS

When interpreting results it is important to take account of non-occupational sources of exposure due to the environment (gas escape) or diet (soft drinking water in lead pipes, acid foods stored in enamel containers, excessive consumption of wine and hobbies such as shooting). Active and passive smoking can increase blood lead through hand-to-mouth contamination of lead contained in cigarettes, or raised haematocrit, causing an increased blood-lead transport capacity.

For exposure to elemental lead and inorganic lead salts: blood lead = 300 µg/l. Women of childbearing potential with a blood-lead concentration in excess of 100 µg/l are at risk of giving birth to children with a blood-lead concentration in excess of the limit value of 100 µg/l recommended by the Centre of Disease Control (CDC). Developmental defects may occur if this value remains raised.

The 2003 ANAES Consensus Conference in France produced recommendations for monitoring blood lead in at risk children up to the age of six years old *(table I)*.

Blood-lead (µg/l)	Recommendations for monitoring blood lead in children
< 100	*No poisoning.* *Monitor blood lead every six months to one year until the age of six years old if the child belongs to an at risk group.* *Remove sources of poisoning.*
100 to 249	*Check blood lead every six months.* *Remove sources of poisoning.* *Mandatory declaration.*
250 - 449	*Check blood lead every three to six months.* *Refer child to a centre able to assess poisoning and discuss the indication for chelator therapy.* *Remove sources of poisoning.* *Mandatory declaration.*
≥ 450	*Urgent referral of child to a centre able to assess poisoning and treat the child.* *Remove the sources of poisoning.* *Mandatory declaration.*

Table I: Recommendations for monitoring blood lead in children

(Source: Conférence de consensus "Intoxication par le plomb de l'enfant et de la femme enceinte - prévention et prise en charge médico-sociale - novembre 2003")

FOR FURTHER INFORMATION

- Conférence de consensus, *Intoxication par le plomb de l'enfant et de la femme enceinte – prévention et prise en charge médico-sociale – novembre 2003*, www.anaes.fr rubrique publications.
- Fiche toxicologique n° 59, *Plomb et composés minéraux*, INRS. www.inrs.fr
- Fiche Biotox, *substance «plomb»*, www.inrs.fr

LEGIONELLOSIS

DEFINITION

Legionellosis or legionnaire's disease derives its name from how it was first described in 1976 from the development of clusters of cases of acute pneumonia in *"American legion" veterans* in a meeting at Philadelphia. This new air-borne infection was due to a newly identified respiratory pathogen, known as ***"Legionella pneumophila"***. *Legionella* are Gram negative bacilli which belong to the *Legionellaceae* family. Forty-two species are currently described. *L. pneumophila* ***serogroups 1 and 6*** are those most often responsible for human infections, 80% of cases being caused by serotype 1. Infections caused by other species *(L. micdadei, L. bozemanii, L. Longbeachae, etc.)* are far less common and are mostly isolated cases in immunosuppressed patients.

EPIDEMIOLOGY

Legionella are bacteria from a water environment which cause acute pneumonia. They can be found in natural fresh water, cold or warm water distribution systems and in air conditioning systems. *Legionella* survive and multiply in water (they multiply particularly well in amoebae). This intracellular parasitic infestation allows them to multiply in macrophages and in particular in pulmonary alveolar macrophages.

The legionelloses are transmitted by droplets (water jets, shower heads, whirlpool baths and air conditioning systems). No human-to-human transmission occurs. Most cases are sporadic, although legionella infections may also occur in clustered cases in communities (hotels, hospitals, thermal stations, etc.) as a result of infection from the same droplet source.

In France, *Legionella* are responsible for approximately 4% of acute adult pneumonias.

SYMPTOMS

Legionella can cause legionnaire's disease (or legionellosis) and Pontiac fever.

A) ***Acute legionella pneumonia*** pneumonia is characterised by sudden onset respiratory disease with a high temperature, dry cough, severe dypnoea, headache and diarrhoea after an incubation period of 2 to 10 days. The clinical picture becomes extremely severe after a few days (hyperthermia, disordered consciousness and renal insufficiency).

Radiological appearances are usually those of interstitial pneumonia with multi-lobe involvement, lesions which extend very rapidly and often, a pleural effusion (50% of cases).

The mortality rate is high (approximately 15%), particularly in the elderly and immunosuppressed. The diagnosis should always be considered when faced with any acute, severe pneumonia occurring in a person over 50 years old with risk factors (male, smoker, alcoholism, underlying disease and immunosuppression), in whom laboratory samples are negative (sputum, blood cultures, soluble antigens, etc.) in tests for usual organisms, and which is resistant to beta-lactam antibiotic therapy.

B) ***Pontiac fever*** is a benign disorder of the upper respiratory tract. After a short incubation period (36 hours), infection develops in the form of isolated fever or a flu-like syndrome which recovers spontaneously. This infection is very rarely diagnosed.

INDICATIONS FOR MEASUREMENT

In any pneumonia from a nosocomial or epidemic setting and in "at risk situations", such as travel, thermal stations, exposure to water droplets etc.

In the following very suggestive clinical situation (1/3 of case): severe pneumonia, acute onset, no ENT signs, *pulsus paradoxus*, bilateral disease particularly in a predisposed patient (cancer or blood dyscrasia) or after failure of initial treatment with beta lactam.

INFORMATION

SAMPLES

Broncho-alveolar fluid, sputum, bronchial aspirate, tracheal aspirate, biopsies, CSF, stored at + 4°C. Samples can be frozen at -20°C if they are not to be cultured for more than 3 days.

Serum: 5 ml of blood into a dry tube. Serum can be stored at + 4°C for 1 week and should then be frozen at - 30°C (1 year).

Urine: 10 ml kept at room temperature or + 4°C if stored for more than one day.

BIOLOGICAL DIAGNOSIS

DIRECT DIAGNOSIS

- **Direct immunofluorescence** is an easy to perform, fast method which can be performed on pathological bronchopulmonary products (sputum, bronchial aspirate and bronchio-alveolar lavage) and can visualise the organism microscopically using fluorescent polyclonal or monoclonal antibodies recognising *L. pneumophila*. This method is however poorly sensitive (30 to 50% positive reactions) with a detection limit of 10000 CFU/ml. Specificity is average at 90%.
- The reference method is **bacteriological culture,** which is essential to identify the source of the infection. This requires a good quality sample (best sensitivity, of 90%, is achieved from a BAL) and the use of a special BCYE *(Buffered Charcoal Yeast Extract)* medium spiked with cysteine, iron pyrophosphate, ketoglutarate, glycine and antibiotics.

  The characteristic colonies (ground glass appearance) are seen with a magnifying glass after incubating for at least 48 hours at 35 - 37°C in an atmosphere containing 2.55% of CO2. Differentiation and identification of *Legionella* colonies is conventionally based on examining their culture, biochemical and antigenic characteristics. Blood cultures may be produced (Isolator lysis centrifugation method) although the sensitivity of this method is only 10 to 30%.

Testing for soluble antigen in urine

Detection of soluble antigens in urine can provide an early diagnosis in the acute phase. The antigens develop within 2 to 3 days after the onset of clinical signs in 90% of patients and excretion is not influenced by antibiotic therapy. The excretion period varies depending on the patient (from a few days normally to occasionally several weeks). This method (micro titre plate ELISA or rapid membrane immunochromatography test), is simple and fast, and in principle is only valid for *L. pneumophila* serogroup 1.

Gene amplification

This diagnostic method does not form one of the defining criteria for the legionelloses. Molecular typing of human and environmental strains, however, can confirm clinical epidemiological findings if their identity is confirmed.

SEROLOGY

This provides only a late or even retrospective diagnosis. Testing is performed by an indirect immunofluorescence (IIF) method on a mixture of 6 L. *pneumophila* serogroups, or possibly by ELISA. The antibodies appear after a week (25% of cases), 2 weeks (50% of cases), 4 weeks (90% of cases) or up to 9 weeks.

Many cross-reactions have been described, particularly with *Pseudomonas, Haemophilus, Mycobacteria, Mycoplasma pneumoniae, Coxiella burnetii, Chlamydiae,* etc.

The significant antibody titre varies by IIF method depending on the antigen preparations used and is 256 for the French National Reference Centre, which offers a confirmatory test using different antigens: *Legionella pneumophila serogroups 1 to 10; Legionella micdadei, Legionella bozemanii, Legionella dumofii, Legionella jordanis, Legionella gormanii and Legionella longbeachae*.

The advantages and disadvantages of these methods are summarised in table I:

Methods	Sensitivity	Specificity	Advantages	Disadvantages
Soluble urinary Ag	56 – 80%(*)	99%	Fast + early, even on treatment, early diagnosis and treatment, reduced mortality	L.p. 1 (*L. pneumophila* type 1) Expensive Routine? Risk of under-diagnosis of other Legionnella
Culture	60%	100%	Gold standard All species 3 – 5 D	Specialised media Specific request Rapidly becomes negative Poor sensitivity on treatment
Serology	80%	97 - 99%	Epidemiological	Limited use in acute phase
DIF	25%	65%	Fast	Specialised labs Cross-reactions

INTERPRETATION

The confirmation of cases of legionellosis is based on clinical and/or radiological signs of pneumonia combined with the following laboratory findings:

– Either, identification of *Legionella* by culture or direct immunofluorescence in a clinically sample

– Or by the presence of soluble *Legionella* antigens in urine

– Or by a significant increase in antibody titre between 2 samples.

Probable cases

Clinical and/or radiological signs of pneumonia, associated with a single or repeated titres of antibodies at or above the significant titre for the method used.

Definition of trusted cases of legionellosis

– At least two cases occurring within a period of less than 6 months in people who have attended the same exposure site.

– If the time interval between cases is more than 6 months, we refer to related cases which have lesser epidemiological importance than clustered cases.

Definition of a case of nosocomial legionellosis

Definite case: A patient who has stayed in the establishment during the 10 days before the onset of clinical signs.

Probable case: A patient who has stayed in the establishment at least one day during the 8 days before the onset of clinical signs.

TREATMENT – PREVENTION

The diagnosis of legionellosis must be combined with testing for *Legionella* in the environmental water, which is the main source of infection. This testing can be used to apply disinfection and prevention measures, particularly in cases of epidemics.

The beta-lactams are not effective, as *Legionella* produces a beta-lactamase. Macrolides (high dose erythromycin for 21 days) is the treatment of choice. Rifampicin, which enters cells, can be used in association, together with the fluoroquinolones.

In health establishments, prevention is based on maintenance and regular control of water supplies.

FOR FURTHER INFORMATION

BEH, Bulletin épidémiologique hebdomadaire, n° 30-31/2002: 149-155: numéro spécial consacré à legionellosis: http://www.invs.sante.fr/beh

Jarraud S. et *al.*, *Les légionelloses*, Rev Fr Lab 2002; 32 suppl: 28-32.

LEISHMANIASIS

DEFINITION

The leishmaniases are parasitic diseases which affect human beings and many mammals. The agents responsible are leishmania, which are flagellated protozoa, belonging to the *Trypanosomatides* family and *Leishmania* genus. Two clinical forms of the disease are distinguished: visceral leishmaniasis and cutaneous or muco-cutaneous leishmaniasis.

Synonyms: Visceral leishmaniasis / kala-azar / dumdum fever.

INTRODUCTION

EPIDEMIOLOGY

The leishmania develop through two morphological stages during their life cycle: the immobile intracellular ***amastigote form*** which parasitically infests vertebrate host histiomonocyte cells and the flagellated, free, mobile ***promastigote form*** which is found in the digestive tract of the insect carrying the disease. Leishmaniasis is transmitted mostly by bites from small insects called *Phlebotomus*. These are found in tropical regions throughout the year but only emerge in summer in sub-tropical and Mediterranean regions. The reservoirs for the parasites vary depending on the species of leishmania and the regions concerned *(cf. table)*.

Clinical form	parasite	regions	reservoir
Visceral leishmaniasis	*L. donovani* *L. infantum* *L. chagasi*	India, China, Iraq, Syria, Kenya, Mediterranean basin, Central Asia, China, South America.	Human beings, Dogs, Wild dogs
Cutaneous leishmaniasis of the ancient world	*L. tropica* *L. killicki* *L. major*	Central Asia, Middle East, Greece, Morocco, Tunisia, Turkey, Central Asia, Middle-East, India, Africa.	Human beings, Dogs Rodents
Cutaneous leishmaniasis of the new world	*L. mexicana* *L. venezuenlis* *L. guyanensis* *L. panamensis* *L. peruviana* *L. lainsoni*	Central America Venezuela French Guyana Central America Peru Brazil	Rodents Unknown Sloths Sloths Dogs Agouti
Diffuse cutaneous leishmaniasis	*L. pifanoi* *L. amazonensis* *L. aethiopica*	Venezuela Columbia, Brazil, Ethiopia, Kenya, Tanzania.	Rodents Rodents Damans
Muco-cutaneous leishmaniasis	*L. braziliensis* *L. donovani*	Broad distribution (from Costa Rica to the Argentine) Chad, Sudan.	Unknown Human beings, Dogs.

From: Gentilini M. Les leishmanioses. In: Médecine tropicale. Paris Flammarion; 1993, 5th edition: p141.

SYMPTOMS

Visceral leishmaniasis (VL)

The infant form affects children between 2 and 3 years old. Following an incubation period of 1 to 2 months, it develops insidiously with rapid deterioration of general health. The active disease state involves irregular fever associated with anaemia, causing extreme pallor with hepato-splenomegaly and lymphadenopathy. The disease is fatal if untreated.

The adult form which is increasingly common has a more sudden onset than the childhood form. The active disease state is similar apart from a less pronounced hepato-splenomegaly and lymphadenopathy but is combined with skin signs, occasionally with gastrointestinal signs and/or haemorrhage. Atypical forms are common (gastro-intestinal, respiratory or cutaneous), particularly in the immunosuppressed (AIDS and immunosuppressant treatments).

Cutaneous leishmaniasis (CL)

Of the Old World, the incubation period ranges between 1 and 4 months and is followed by single or multiple skin lesions which develop on the face, hands, forearms or lower limbs. A dry form exists with central ulceration covered with a crust *(L. tropica)* and an excavating, inflammatory form called "wet" where more lesions are present *(L. major)*. Nodular, eczematiform or lupoid lesions are also described. These lesions heal spontaneously, although leave permanent scarring.

Of the New World, the localised skin lesions are generally ulcerated and delineated by an inflamed peripheral circumference, often covered with a crust. They are more severe than the Old World form, the severity being due to the diffuse nature of the disease progressing to chronic disease and mutilation.

Diffuse cutaneous leishmaniasis (DCL)

This is due to defective host cellular immunity, resulting in nodular lesions distributed throughout the body, suggestive of lepromatous leprosy. If untreated, DCL is fatal.

Muco-cutaneous leishmaniasis (MCL)

The skin lesion resembles that of CL, although on recovery the parasite may reach the facial mucosal membrane secondarily (the nasal or ear cartilages, lips or oropharyngeal mucosa), resulting in facial mutilation and occasionally being fatal.

INDICATIONS FOR MEASUREMENT

Diagnosis of VL, particularly in the immunosuppressed.

Diagnosis of Old or New World CL.

Diagnosis of the MCL.

Differential diagnosis between VL and malignant blood dyscrasia or infectious febrile splenomegaly.

Differential diagnosis between CL or MCL and other skin disorders (lupus, sarcoidosis, fungal infections, etc.).

INFORMATION

SAMPLES

Visceral leishmaniasis

Peripheral blood: Collected into EDTA for direct diagnosis and into a dry tube for serological diagnosis.

Bone marrow:: Collected into EDTA by sterile puncture in adults or from the iliac crest in children.

Other samples: Gastrointestinal, lymph node biopsies and BAL.

- **Cutaneous leishmaniasis**

  The sample is obtained by scratching the skin with a vaccine needle or curette on the periphery of the lesion, spreading the serous material or by the injection-aspiration method with sterile physiological saline at the edge of the lesion. Any bleeding must be avoided as this makes the sample more difficult to read. A skin biopsy may also be taken.

- **Muco-cutaneous leishmaniases**

  Biopsy forceps sample.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

Stay in area endemic for leishmaniasis?

Immune status (HIV co-infection, etc.)?

SAMPLE STORAGE AND TRANSPORT

Serous samples spread onto a slide should be sent to the laboratory within half a day. A hypodermic syringe used for aspiration should be sent within an hour, under sterile conditions. Lymph node aspiration fluids should also be sent sterile.

Serum can be stored for a few days at + 4°C. Whole EDTA blood or bone marrow collected into EDTA for PCR analysis can be stored at + 4°C for 2 weeks.

DIAGNOSTIC METHODS

PARASITOLOGICAL DIAGNOSIS

- **Direct examination:** Testing for the parasite on a May-Grünwald-Giemsa smear in the intracellular or extracellular amastigote form. Direct examination is performed by leukocyte concentration (LCC) for a peripheral blood sample or lymph node aspirate.
- **Culture:** Performed on NNN (Novy-Nicolle-McNeal) medium or on liquid media spiked with foetal calf serum, incubated at 24-26°C. Incubation is slow and should only be considered to be negative after 6 weeks as it sometimes requires several sub-cultures. When positive, the mobile flagellated promastigote forms can be seen.
- **Molecular biology:** This is tending to replace culture. The parasite DNA is detected by PCR on blood, bone marrow or skin tissue. It also enables the species to be identified.

INDIRECT DIAGNOSIS

This is essential for the diagnosis of VL but far less useful for CL or MCL as the antibody titres produced are lower. The reference method is indirect immunofluorescence on cultured promastigotes. Cross-reactions occur with other malarial or trypanosome parasites and in some connective tissue diseases. Other methods are available, including ELISA, a direct agglutination test, passive haemagglutination and rapid immunochromatographic tests.

Immunoblotting is very sensitive and very specific and is used as the confirmatory serological method (always performed if the screening test is positive).

TREATMENT

The molecules available are pentavalent antimony compounds, amphotericin B in liposome form and pentamidine salts. First line treatment of VL is with antimony derivatives. In HIV co-infected patients loading treatment is less effective and recurrences occur more frequently and require maintenance treatment. The treatment of LC varies depending on the species responsible and clinical symptoms, from no treatment to local or systemic treatment.

FOR FURTHER INFORMATION

- Marty P., *Leishmania infantum agent de la Leishmaniose viscérale*, Encycl Med Biol. Elsevier Paris, 2003.
- Dedet J.P., *Leishmania (atteinte cutanée de l'Ancien Monde)*, Encycl Med Biol. Elsevier Paris, 2003.
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LEPTIN

DEFINITION

Leptin is a 16 kDa polypeptide hormone primarily produced by adipocytes and to a lesser extent by the placenta and mammary epithelial cells in breast-feeding women.

Synonym: OB protein.

INTRODUCTION

■ SYNTHESIS, SECRETION AND METABOLISM

Leptin is coded and formed by the *obese* or *ob* gene in human beings, which is located on chromosome 7. Nonsense mutations causing failure of leptin synthesis have been described in homozygous *ob/ob* obese mice. No *ob* gene mutation is seen in human beings and both thin and obese people have identical genes. The only mutations which are seen are, conversely, preserving. This appears to argue in favour of endogenous leptin resistance in obese people.

Leptin synthesis is regulated by many hormonal and non-hormonal factors. Stimulatory factors in both rodents and human beings are overfeeding, insulin and glucocorticoids. Inhibitors are fasting state, cAMP and adrenergic agonists.

Leptin is secreted in a pulsatile manner and follows an inverse circadian rhythm to that of ACTH and cortisol, with a peak slightly after midnight and a trough at the beginning of the afternoon. The serum half-life of leptin is approximately 1 hour and 30 minutes, although its plasma half-life is between 12 and 14 hours because of a different renal elimination mechanism, depending on whether the leptin is circulating free or partially bound.

■ PHYSIOLOGY

The first studies performed described leptin as a satiety factor able to inform the hypothalamus about the state of fat reserves and therefore contributing to controlling body weight and energy equilibrium. Leptin, however, is also involved in the regulation of gonadal function and bone metabolism.

Physiologically, leptin acts by binding to a receptor (Ob-R) coded for by a gene, splicing of which leads to the expression of several forms (genetic polymorphisms). The long form (Ob-Rb) is mostly found in hypothalamic centres, where it regulates eating behaviour and energy metabolism. Increased fat mass stimulates adipocyte synthesis of leptin and the rise in serum leptin concentration inhibits food intake and stimulates energy storage. Conversely, when the adipose mass falls, leptin secretion is reduced, and then hunger sensation develops and is associated with a fall in energy expenditure.

Short or truncated leptin receptors are mainly found in the lungs, kidneys and pancreatic islets of Langerhans. This could explain the frequent association of type 2 diabetes with obesity, although the relationship between insulin and leptin is complex. Insulin increases leptin production, whereas leptin inhibits the secretion of insulin induced by dietary glucose.

One hypothesis is that excess circulating leptin in the obese is involved in hyperglycaemia by contributing to the development of insulin resistance and possibly reducing insulin secretion.

Reduced serum leptin concentration secondary to a fall in fat mass, is associated with activation of the corticotropic axis and reduced thyroid function (by a fall in TRH synthesis) and reproductive function (defective pubertal development and fertility).

Leptin is involved in the regulation of haematopoiesis (stimulatory reaction) and the immune system. It is also a potent inhibitor of bone formation (anti-osteogenic action).

In pregnancy it is involved in foetal and placental growth, as leptin concentrations in cord blood correlate very closely with birth weight in both sexes.

INDICATIONS FOR MEASUREMENT

Leptin measurement is currently a research tool for the pathophysiological study of eating disorders, some forms of diabetes, infertility and delayed puberty.

INFORMATION

■ SAMPLE

Serum (dry tube) or plasma taken into EDTA, heparin or citrate. Note that excess heparin increases leptin concentrations. The sample must always be taken at the same time, preferably in the morning. Haemolysed or lipaemic samples should be discarded.

■ NECESSARY INFORMATION

The patient's height, weight and age must be stated together with pubertal stage for children. Results are interpreted against reference values which need to take account of this information.

■ SAMPLE STORAGE AND TRANSPORT

Serum or plasma samples can be stored for a few hours at + 4°C, although if the assay is not performed within this time they must be centrifuged, separated and frozen within an hour of sampling. Transport frozen at – 20°C.

ASSAY METHODS

Leptin is measured by an immunological method using an enzyme (immunoenzymatic assay) or radioisotope (radioimmunoassay) labelled tracer.

NORMAL EXPECTED VALUES

These must take account of sex, body mass index (BMI) and pubertal stage in adolescents.

As an indication, in people with a BMI of between 18 and 25, the reference values (RIA measurement) are:

– In women: Mean of 7.4 ng/ml with a standard deviation of 3.7 ng/ml,

– In men: Mean of 3.8 ng/ml with a standard deviation of 1.8 ng/ml.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Leptin concentrations vary according to an inverse circadian rhythm to that of ACTH and cortisol, with a peak shortly after midnight and a trough at the beginning of the afternoon. They vary by weight and correlate with body mass index (BMI) and fat mass index.

When leptin concentrations are adjusted with adiposity, a difference is seen between girls and boys. Similarly, for equivalent BMI, serum/plasma leptin concentrations are higher in women than men (suppressant effect of testosterone or stimulatory effect of estrogens). Concentrations increase 2.5 times more per BMI unit in women than in men.

Leptin does not vary post-prandially either in thin, obese or diabetic people and does not appear to be stimulated by acute insulin secretion. It does not therefore change during a glucose tolerance test. Conversely, hyperinsulinism produces a rise in leptin.

PATHOLOGICAL VARIATIONS

Serum/plasma leptin concentrations are generally approximately 50% higher in obese people than in the non-obese. Leptin resistance appears to be a key factor, as defective leptin production has not to date been shown in the obese.

In view of its many physiological actions, leptin is not only involved in obesity. Some pubertal abnormalities are associated with genetic anomalies of the leptin or leptin receptor gene. The same applies to some cases of unexplained infertility which may be due to leptin.

Reduced serum/plasma leptin concentrations may be seen in thin women associated with amenorrhoea, causing subfertility. Conversely, dysmenorrhoea with subfertility may be seen in some obese women associated with high circulating leptin concentrations.

Increased serum leptin concentrations have been seen in infections which may explain the anorexia, with which it is generally associated.

Further studies are now required to examine the involvement of leptin in controlling numerous endocrine functions. Depending on the results of these studies, recombinant leptin may be used in human therapeutics in the future. A few studies are currently being developed, particularly in the treatment of obesity and in some types of diabetes.

FOR FURTHER INFORMATION

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LEPTOSPIROSIS

DEFINITION

Leptospira are bacteria belonging to the *Spirochaetales* order, *Leptospiraceae* family which contains only the genus *Leptospira*. Two species are conventionally recognised based on simple phenotypic criteria. *L. interrogans* groups together pathogenic strains responsible for leptospirosis. *L. biflexa* contains the "water-borne" saprophytic strains. Each species is divided into serovars which may be combined into serogroups using detailed antigenic criteria. This serotype classification has been questioned by gene studies. Leptospira are visible on dark background microscopy as thin spirochetes (4 to 20 µm long and 0.1 µm in diameter) with hooked extremities. Their mobility is complex (flexion, spin). They are heat and light-sensitive obligate aerobes. They may be cultured *in-vitro* on enriched medium but then rapidly lose their pathogenic activity. Hamster and young guinea pigs are sensitive to experimental infection.

INTRODUCTION

EPIDEMIOLOGY

The leptospiroses are endemic throughout the whole world, predominately in tropical zones. In mainland France the National Reference Centre lists around 200 to 400 cases per year, with a peak in summer and early autumn. The predominant serogroup is *icterohaemorrhagiae*, followed generally by *grippotyphosa*. Incidence varies year by year, depending on weather and environmental conditions. The reservoir for the organisms is mostly animals, particularly wild rodents which frequently excrete them in their urine. Cattle, horses and pigs can be infected as can dogs which may act as an asymptomatic carrier. Leptospira only last for a few days in water and in low salt-content shaded marshland. Infection occurs transcutaneously (occasionally conjunctivally), predisposed by excoriations or after the skin has been softened by standing in water for long periods of time. Leptospirosis is an occupational disease (drain workers, farmers, butchers, miners, abattoir workers and paddy field workers, etc.) Infection is occurring increasingly from water hobbies (fishing, freshwater bathing, etc.).

SYMPTOMS

The typical form of leptospirosis is infectious jaundice with recurrent fever (Mathieu and Weil recurring jaundice, or Weil's disease). After an incubation period of 8 to 12 days, a picture of severe infection develops with violent myalgia, frank meningism and conjunctival congestion. Pronounced jaundice develops on day six with acute hepatonephritis. This phase lasts for 4 to 5 days and the signs then generally resolve. A relapse of fever occurs in the third week and then disappears. It has a long convalescent period, generally without complications. This typical form is relatively rare and the isolated meningeal form of the disease is more common. Very severe and often fatal haemorrhagic forms of the disease occur. Ocular complications (uveitis) may develop late. Flu-like forms of the disease have been described under various names, such as swamp fever and pig farmer's disease. The infection may be latent or clinically silent. The main leptospiral diseases seen at present are listed in the table below.

Serogroup	Preferred host	Clinical
icterohaemorrhagiae	rat	Acute hepatonephritis - Meningism
grippotyphosa	various rodents	Marsh fever - Aseptic meningitis
canicola	dog	Jaundice – Febrile syndrome - Aseptic meningitis
australis	rat, wild rodents	Pig farmer's disease Febrile syndrome – Aseptic meningitis
pomona	Mice incl. fieldmice	Paddy field fever

INDICATIONS FOR MEASUREMENT

Aetiological diagnosis of hepato-renal syndrome or meningism, based on epidemiological criteria (contact with potentially soiled water, exposed occupation, etc.).

INFORMATION

SAMPLE

Whole blood drawn into heparin (preferably) or EDTA plasma, or serum during the first week of the disease.

CSF, urine and aqueous humour for direct examination and culture. The samples must be taken before antibiotic therapy.

Non-haemolysed serum into a sterile tube for antibody testing.

QUESTIONS FOR THE PATIENT

Date of onset and nature of clinical signs, epidemiological and occupational risks.

SAMPLE STORAGE AND TRANSPORT

Isolation of leptospira by culture requires prompt inoculation (within a few hours) of whole blood serum or plasma samples.

Urine (10 ml, with no preservative) and CSF must be transported promptly at + 4°C away from light. For PCR, whole blood collected into EDTA (serum or plasma EDTA are acceptable) or a urine sample can be stored (for 4 to 5 days) and transported at + 4°C; CSF must be frozen at -20°C. For serology, serum can be stored (4 to 5 days) and transported at + 4°C.

DIAGNOSTIC METHODS

The effectiveness of methods used depends on the stage of progression of the disease. During the first phase, leptospira spread throughout the body in the blood stream and a direct diagnosis may be made.

During the second week the organisms disappear from the blood but can be found in CSF and urine. Beyond this time, a retrospective diagnosis is made from seroconversion.

DIRECT DIAGNOSIS

Screening by direct examination:

This can be performed first line as soon as the sample is taken (blood, CSF or urine), and requires centrifuging in order to concentrate any leptospira present.

Dark background microscopy must be performed on very fresh samples in which the leptospira have retained their mobility. The silver impregnation staining method or above all, with acridine orange staining after ethanol fixation, is used to assess the morphology of the bacteria. Immuno-labelling (fluorescence or peroxidise) can also be performed using anti-leptospira antibodies. These methods require a trained observer and also cannot differentiate pathogenic from saprophytic leptospira and have poor sensitivity (10^4 bacteria/ml).

- **Testing by culture:**

  This is reserved for specialist laboratories and requires enriched media, supplemented with rabbit serum or currently, tween-albumin (liquid or semi-solid EMJH medium). The medium can be rendered partially selective to isolation by adding 5-fluorouracil. It is recommended that the samples be diluted 1/10 before being inoculated and that urine be alkalinised. They are incubated at 25-30°C in darkness and observed regularly by a microscope for two months. If a positive culture grows the strain is usually identified by agglutination with specific antisera. Genotypic identification methods are also increasingly being used.

- **Animal inoculation:**

  Young guinea pigs or better still, hamsters, which are sensitive to a larger number of serovars, can be used. The animals are observed clinically and autopsies are performed on those which die spontaneously or are humanely killed at 21 days (taking the necessary precautions to avoid the handler becoming infected). Leptospira may be seen and cultured from the liver, spleen and kidneys.

- **Detection of pathogenic leptospiral DNA "sensu lato" by PCR:**

  This can be used for a rapid diagnosis without the inherent constraints of bacterial culture. It may be detected in blood, plasma or serum during the first week and later in CSF, urine and where applicable, aqueous humour.

INDIRECT DIAGNOSIS

Leptospirosis is still usually diagnosed serologically. Two samples taken at 8 to 10 day intervals are required to make the diagnosis of acute forms of the disease.

- **TR antigen test**

  This is a slide macro-agglutination reaction using a temperature-resistant antigen (hence the name TR) prepared from a strain of *Leptospira patoc* (non-pathogenic). It is a very simple test, although has poor sensitivity and above all poor specificity, making it unsuitable for diagnosis.

- **ELISA Tests**

  An ELISA test using an antigen extracted from a strain of *Leptospira patoc* which detects IgG or IgM antibodies, can be used either on a microtitre plate or in individual stick tests (ELISA-dot). This test can be used for screening, although may produce false-negative results with infection from some serovars. Results are improved by using an antigen combining several serovars, although makes the reaction more complex to perform.

- **MAT (Martin and Pettit agglutination-lysis) or microscopic micro-agglutination test**

  This is the reference method and involves placing dilutions of the test serum in contact with living cultures of leptospira. The antibodies agglutinate the bacteria and the test is read on dark background microscopy. At least a dozen representative reference strains of the main sera-groups, together with a strain of *Leptospira patoc* which can be agglutinated by the antibodies induced by many pathogenic serovars need to be tested. A serum sample is positive at a given dilution for the test strain if at least 50% of the leptospira are agglutinated compared to a reference strain. This method is difficult to perform (laboratory leptospira strains need to be maintained) and is reserved for specialist laboratories.

- **Interpretation**

  The absolute diagnosis is made by finding pathogenic leptospira. Culture is not widely used in practice because of the operating conditions required and the fact that the results are usually slow to obtain. PCR can produce a reliable result in a few hours and is tending to become the method of choice, provided samples are taken early enough. IgM antibody detection by ELISA is useful as these appear at the beginning of the second week. The method, however, has limitations: false positive results and lack of sensitivity in infection with some serovars.

  The micro-agglutination reaction is delicate to perform but is the most reliable technique. Antibodies often reach high titres and antibodies agglutinating several serovars are often found at the start of the disease. Only a late serum can be used to specify the serovar responsible, hence the use of testing a third serum sample a few weeks after recovery. The MAT can be used with any animal species, although the positivity thresholds vary between species.

TREATMENT

CURATIVE

The haemorrhagic jaundice form requires hospitalisation in intensive care because of the risk to the kidneys. Antibiotic therapy (penicillin and penicillin derivatives, doxycyclin) reduce the risk of complications when administered early but do not appear to change the duration of the infection. Atypical flu-like forms only require symptomatic treatment.

PREVENTATIVE

- **Individual prevention**

  This is based on informing at risk people, combating rodents, good management of riverbanks, testing bathing water, cleaning infected sites and general hygiene regulations, particularly in occupations exposed to the disease (systematic hand washing, wearing gloves, not eating or drinking in the animal house). Prevention of infection of domestic animals also helps to avoid human infection. Sanitary prophylaxis is difficult because of the large number of species of animals which are liable to host leptospira and because of the survival of the bacteria in the external environment.

Medical prevention: vaccination

Inactive vaccines are used in potentially highly exposed people. As immunity is specific to the serovars, the protection conferred depends on the composition of the vaccine. The vaccine used in human beings is only effective against the single serovar *icterohaemorrhagiae*. It is generally well tolerated and administered as two injections at a 15 day interval with a first booster at 6 months and then every two years thereafter. The efficacy of the vaccine can be checked by measuring induced antibodies (MAT).

Vaccines intended for cattle, pigs and dogs are available in several countries. A vaccine is marketed in France for dogs and contains strains of the serovars *icterohaemorrhagiae* and *canicola* (2 injections at 3 week intervals followed by an annual booster, or ideally a booster every 6 months). It is limited in efficacy and protection only lasts for 6 to 12 months. In addition, it does not prevent either carrying or excreting the bacteria and a vaccinated dog may therefore cause infections in human beings.

FOR FURTHER INFORMATION

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LH

DEFINITION

LH *(Luteinising hormone)* is a glycoprotein hormone secreted by pituitary gonadotropic cells. It has a dimeric structure consisting of a specific β subunit and an alpha subunit which is common to FSH, TSH and HCG. The beta chain gives the hormone its specific immunological and biological features. Each of the subunits carries a carbohydrate part which guarantees their stability in plasma and enables their hormone action to take place. Dissociation of the two alpha and beta subunits results in loss of biological activity. The considerable heterogeneity of the circulating forms of LH is due to large variability in the carbohydrate part and in the extent of sulphation and sialylation of the gonadotropins, causing numerous analytical problems.

Synonyms: Luteotropin and Luteinising hormone.

PATHOPHYSIOLOGY

LH is secreted in a pulsatile manner in adults into the blood under the control of hypothalamic Gn-RH. The frequency and amplitude of pulses varies depending on the phase of the menstrual cycle in women. Pulses last approximately 90 minutes in the follicular phase and 2 to 3.5 hours in the luteal phase. LH circulates in plasma in the free form with a plasma half-life of between 20 and 30 minutes and is removed in the unchanged form in urine (5 to 20% of daily production). Serum LH concentrations vary during the menstrual cycle depending on estradiol and progesterone levels.

The gonadotropins (LH and FSH) are involved in gonadotropic function (fertility). In women, LH controls follicular maturation in the ovary, the triggering of ovulation and regulation of the synthesis and secretion of steroid and peptide hormones. During the first phase of the cycle beyond a critical threshold, the progressive rise in estradiol secretion by the maturing follicle triggers the secretory LH peak, which is responsible for triggering ovulation. In men, LH stimulates testosterone secretion by the testicular interstitial Leydig cells.

INDICATIONS FOR MEASUREMENT

IN WOMEN

- **Investigation of the gonadotropic axis:**

  Parallel measurement of LH and FSH in the initial assessment of subfertility:

  – In amenorrhoea, to distinguish ovarian hypogonadism (hypergonadotropic hypogonadism) from central hypogonadism (hypogonadotropic hypogonadism)

  – Dynamic investigations: LH-RH test to assess gonadotropic function after stimulation

  – In clinical signs of androgen excess: As an aid to the differential diagnosis of polycystic ovarian syndrome

  – In the peri-menopausal period (women from 45 years old and above): Combined with FSH (according to some authors).

- **Investigation of pubertal disorders:** FSH combined with LH, baseline measurements and after stimulation with GnRH.

- **Laboratory monitoring of induction of ovulation in Medically Assisted Pregnancy programmes (MAP):** Measurements of LH and estradiol every 3 to 7 days when treatment is started and then possibly at closer intervals as the pre-ovulatory peak approaches.

IN MEN

– Aetiological diagnosis of hypogonadism.

– Subfertility – semen analysis abnormalities.

INFORMATION

SAMPLE

Serum (dry tube) or either heparinised or EDTA plasma, depending on the manufacturer's recommendations (avoid dry tubes with separator gel). These samples should preferably be taken first thing in the morning. A fasting sample is not essential.

Day of sampling is dependent on the purpose of the measurement:

– In a woman with amenorrhoea: No specific day

– In a woman with menstrual periods between the 3rd and 5th day of the cycle

– In MAP: In the pre-ovulatory phase. In this situation, measurements must also be performed in the same laboratory and results must be sent on an urgent basis (within half a day)

– Dynamic investigations LH-RH test: At the start of the follicular phase (D2 to D5). Measurement at baseline and then 30, 60, 90 and 120 minutes after the injection of LH-RH.

QUESTIONS FOR THE PATIENT

In women of childbearing age: Are you having menstrual periods, date of last period, usual length and regulatory of periods, clinical signs such as acne or hirsutism, etc, length of previous cycles and the presence or absence of hot flushing (in the peri-menopausal period)?

Any current treatment, particularly hormone treatment (contraceptives and hormone replacement therapy for the menopause) andmanagement in a MAP (induction or stimulation of ovulation)?

SAMPLE STORAGE AND TRANSPORT

Centrifuge after the clot has completely retracted (approximately 30 minutes at room temperature).

Analyse within hours of sampling or store according to the manufacturer's recommendations. Generally this will be for up to a few days at + 4°C or at – 20°C for periods longer than this. The sample must be vortex mixed or preferably re-centrifuged before analysis if thawed.

ASSAY METHODS

Sandwich immunometric methods with monoclonal antibodies: Radio-isotopic, enzymatic, chemoluminescent, fluorescent or colourimetric label.

Standardisation: There are several types of standards. The most widely used standard is hLH 2nd IS 80/552.

NORMAL EXPECTED VALUES

Serum LH concentrations vary by sex, age and point of cycle in women. Also note disparities in results obtained between the different immunoassays (structural heterogeneity of circulating LH).

Reference values in adult women (in IU/l).

Examples

	Follicular phase	Pre-ovulatory peak	Luteal phase	Pregnancy	Menopause
Beckman Coulter Immunotech® IRMA method	0.5 to 5	5 to 30	0.5 to 5	< 1	> 20
Roche Elecsys® Chemoluminescence method	2.4 to 12.6	14 to 95.6	1 to 11.4		7.7 to 58.5

As an indication

In men (Roche Elecsys® Chemoluminescence): 1.7 to 8.6 IU/l.

In children (Roche Elecsys® Chemoluminescence):

LH (IU/l) Girls

1 to 7 days	8 to 30 days	1 to 12 months	1 to 5 years	6 to 10 years	11 to 13 years	14 to 17 years
< 0.1 – 6.4	<0.1 to 7.8	<0.1 to 0.4	<0.1 to 0.5	<0.1 to 3.1	<0.1 to 11.9	0.5 to 41.7

LH (IU/l) Boys

1 to 7 days	8 to 30 days	1 to 12 months	1 to 5 years	6 to 10 years	11 to 13 years	14 to 17 years
< 0.1 – 6.4	<0.1 to 7.8	<0.1 to 0.4	<0.1 to 1.3	<0.1 to 1.4	0.1 to 7.8	1.3 to 9.8

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATION

Puberty: The start of puberty is characterised by an LH peak > 5 IU/l with an LH/FSH ratio of > 1

Menstrual cycle: *cf. normal values above*.

Ageing: LH increases gradually from the age of 35-40 years old. This rise is faster and greater in the peri-menopausal period (between 45 and 55 years old) but is more difficult to identify in women taking hormone treatment (oral contraception or hormone replacement therapy for the menopause). In men the progressive fall in serum testosterone is associated with a rise in FSH and LH.

■ IATROGENIC VARIATIONS

Some oestrogen-progestogen oral contraceptives and some progestogens administered 15 to 20 days per month, anabolic androgens and high dose corticosteroids (see corticosteroid infiltrations) reduce circulating LH concentrations to a greater or lesser extent.

LH can be reduced markedly (or can even be completely suppressed) after 10 days' treatment with GnRH agonists or from 24 hours of treatment with GnRH antagonists.

Clomiphene citrate (200 mg/d) increases serum LH by approximately 85% in 6 to 10 days.

INTERPRETATION

■ DISEASE OF THE GONADOTROPIC AXIS

A large rise in serum LH and FSH concentrations in women indicates primary ovarian failure: Gonadal dysgenesis in primary amenorrhoea, and early or later menopause or castration (surgical, irradiation, radiotherapy or chemotherapy) in secondary amenorrhoea. A rise in serum LH in men suggests gonadal failure (the increase in LH is proportional to interstitial Leydig tissue damage). In Klinefelter's syndrome, for example, LH is increased (but proportionately less than the FSH) and correlates with the degree of Leydig tissue atrophy.

– A rise in LH and FSH with normal serum estradiol is commonly seen in the peri-menopausal period and may also occur in the very rare so-called gonadotropin resistant ovarian syndrome (due to an inactivating mutation of gonadotropin receptors).

– An isolated rise in LH (with normal FSH and estradiol) suggests polycystic ovarian syndrome. In this context the amplitude and number of LH pulses are increased.

– A large fall in LH and FSH in men and women usually indicates anterior pituitary failure.

– A fall in plasma LH and FSH concentrations in women, together with raised serum estradiol should trigger investigation for pregnancy. Low values associated with amenorrhoea suggest hypothalamo-pituitary gonadotropic insufficiency (psychogenic or pituitary tumour, etc.). Further investigations as a minimum with GnRH dynamic test are required to provide a conclusion.

■ BIOLOGICAL MONITORING OF INDUCTION OF OVULATION IN MEDICALLY ASSISTED PREGNANCY

The increase in plasma LH concentration is used to identify the approaching ovulatory period and therefore the best time for sexual intercourse.

FOR FURTHER INFORMATION

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LIPASE

DEFINITION

Lipase is a glycoprotein secreted primarily by the pancreas. It is present in small amounts in the gastric mucosa, leukocytes and erythrocytes.

INTRODUCTION

Lipase is an enzyme which hydrolyses bonds between triglycerides in the presence of co-lipase and low concentrations of bile salts.

INDICATIONS FOR MEASUREMENT

Serum lipase measurement is useful in the diagnosis and monitoring of pancreatic disease, particularly acute pancreatitis. It has no prognostic value.

INFORMATION

SAMPLE

Serum (dry tube) or heparinised plasma. Tubes containing EDTA, oxalate or citrate, must not be used, as calcium ions act as effectors of the lipase reaction. Discard lipaemic and/or haemolysed samples.

Severe hypertriglyceridaemia (approximately 4.5 g/l or more) interferes with blood lipase measurement.

QUESTIONS FOR THE PATIENT

Current treatment needs to be known, as some medicinal products that cause spasm of the sphincter of Oddi (opiates, cholinergics, etc,), may cause increased serum lipase.

SAMPLE STORAGE AND TRANSPORT

Lipase is stable for a few hours at laboratory temperature and for a few weeks at + 4°C. Freezing has no denaturing effect.

Transport at + 4°C (1 ml serum or heparinised plasma), if samples are already frozen, then transport in the frozen state.

ASSAY METHODS

Direct methods: Measurement of fatty acids released by lipase (titrimetry).

Indirect methods, various operating procedures:

– Measurement of the substrate degradation rate by nephelometry or turbidimetry

– Methods based on assaying the glycerol released or dimercaptoethanol produced in the lipase reaction (colourimetry, reflectometry).

NORMAL EXPECTED VALUES

Usual lipase activity in plasma depends on method used (it may vary by a factor of 1 to 10). As an indication, by a chemical method (spectrophotometry): 13 to 60 U/l at 37°C.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Some authors have reported slight age-related rises in serum lipase after the age of 60.

PATHOLOGICAL VARIATIONS

Acute pancreatitis:

Increased serum lipase (at least 3 times the upper limit of usual values) is good diagnostic evidence in favour of acute pancreatitis. In this situation, serum lipase rises between 8 and 30 hours after the onset of pain, reaching a peak at between 24 and 48 hours (approximately 15 to 20 times the upper limit of normal values) returning to normal over 5 to 10 days.

Compared to amylase, the rise in lipase in acute pancreatitis is generally earlier, larger and more prolonged.

Other causes of raised serum lipase:

– Chronic pancreatitis and pancreatic cancer.

– Obstructive, inflammatory or traumatic abdominal disease or inflammation of organs close to the pancreas, such as a perforated ulcer, upper bowel obstruction, mesenteric infarction, biliary tract obstruction, peritonitis, etc.

– Acute or chronic renal insufficiency.

– Alcoholics.

– Patients suffering from diabetic ketoacidosis.

Unlike amylase, lipase does not rise in mumps, head injury, ovarian cancers, bronchial cancers and non-neoplastic lung disease.

FOR FURTHER INFORMATION

Lessinger J.M., Le Cahier de formation Biochimie Tome I. Paris, Bioforma, 1992: 186-91.

LIPID ELECTROPHORESIS

DEFINITION

Lipid electrophoresis is the electrophoresis of the lipoproteins which are the transported forms of lipids and are formed from an association of the binding of cholesterol, triglycerides and phospholipids to apolipoproteins. The lipoproteins are very heterogeneous in nature and their migration in an electric field is explained by their surface charge.

INTRODUCTION

Chylomicrons and VLDL transport triglycerides from the diet to cells. LDL and HDL are involved in the transport of cholesterol and phospholipids. LDL carries lipids to the cell, whereas HDL binds cellular cholesterol, transporting it to the liver where it is metabolised. Lipoprotein metabolism occurs as a result of permanent exchange between lipid and protein constituents.

INDICATIONS FOR MEASUREMENT

For the investigation of hyperlipidaemia. In France its use is indicated for and restricted to the diagnosis of severe hypertriglyceridaemia, particularly type III or type V hyperlipoproteinaemia *(ANES recommendations, 2000)*.

INFORMATION

SAMPLE

The sample is taken into a dry tube without anticoagulant or additive.

The sample should be taken in the morning after a 12 hour fast.

QUESTIONS FOR THE PATIENT

Suspected disease and current treatment.

SAMPLE STORAGE AND TRANSPORT

The sample can be stored, when separated, for a few days at + 4°C.

Transport the separated sample at + 4°C.

ASSAY METHODS

Agarose gel electrophoresis at alkaline pH.

3% polyacrylamide gel electrophoresis.

NORMAL EXPECTED VALUES

By analogy to the protein electrophoresis, three main zones are identified:

– Alpha lipoproteins are the furthest from the loading point, spread out and weakly stained, representing HDL

– Pre-beta lipoproteins have intermediary mobility and have a tight weakly stained band corresponding to VLDL

– Beta lipoproteins are close to the loading spot, narrow and highly stained, representing LDL

– Chylomicrons which are normally absent from the serum of a fasting person remain at the loading point spot if present.

PATHOLOGICAL VALUES

HYPERLIPOPROTEINAEMIA

Classified according to Fredrickson from changes in the lipid electrophoresis.

- **Type I:** Severe hypertriglyceridaemia with or without hypercholesterolaemia. Corresponding to the accumulation of intestinal chylomicrons and occurring as a result of lipoprotein lipase deficiency.
- **Type IIa:** Hypercholesterolaemia due to the accumulation of LDL. This picture is associated with defective degradation of LDL, due to a genetic defect of the receptor membrane which recognises LDL.
- **Type IIb:** Severe hypercholesterolaemia combined with hypertriglyceridaemia. VLDL and LDL are raised.
- **Type III:** Hypercholesterolaemia associated with occasionally very severe hypertriglyceridaemia. The lipid electrophoresis reveals an increase in β and pre-β lipoproteins, which form a wide band known as broad beta band, corresponding to IDL *(intermediate density lipoproteins)*.
- **Type IV:** The commonest hyperlipoproteinaemia characterised by an isolated rise in pre-beta lipoproteins. Serum cholesterol is normal or slightly increased and triglycerides are increased.
- **Type V:** Rare, and an association of type I and type IV abnormalities, characterised by hyperchylomicronaemia and hyperpre-betalipoproteinaemia.

HYPOLIPOPROTEINAEMIA

– Absent HDL: Tangier's disease.

– Hypoα-lipoproteinaemia: In hepato-biliary disease the HDL is markedly reduced.

– Hypoβ-lipoproteinaemia: In the heterozygous form this results in a reduced intensity of the β zone. In the homozygous form it produces severe hypocholesterolaemia with absent β zone.

PRESENCE OF ABNORMAL LIPOPROTEINS

– Lp (x): β mobility is present in cholestasis and congenital lecithin cholesterol acyltransferase (LCAT) deficiency.

– IDL: Intermediary electrophoretic mobility between LDL and VLDL, characterising type III hyperlipidaemia.

FOR FURTHER INFORMATION

- *Modalités de dépistage et diagnostic biologique des dyslipidémies en prévention primaire*, Recommandations et références professionnelles, ANAES, October 2000.

LIPOPROTEIN (A)

DEFINITION

Lipoprotein (a) or Lp (a) is a glycoprotein synthesised in the liver, formed from a molecule analogous to low density lipoproteins (LDL) associated with one molecule of apolipoprotein (a). Like LDL, Lp (a) is formed from a lipid part, rich in cholesterol esters and a protein part, apolipoprotein B100. Apo (a) is bound to apo B100 by a disulphide bridge. Lp (a) exhibits considerable sequence homology with plasminogen, giving it prothrombotic properties in addition to its atherogenic properties.

Lp (a) also exhibits considerable size polymorphism (it has 11 isoforms of molecular masses between 300,000 and 700,000 daltons) controlled by a polyalleal system (19 co-dominant alleals). Its plasma concentration is therefore genetically determined, mostly (in at least 70% of cases) by is mass polymorphism.

Synonym: Lp (a).

PATHOPHYSIOLOGY

Lp (a) is a lipoprotein which has been shown to be of use as an independent atherogenic risk factor in many retrospective epidemiological studies (although some have not confirmed this). LDL-cholesterol and Lp (a) probably act harmfully in synergism. Its atherogenic role is due to its ability to bind strongly to arterial wall glycosaminoglycans. The Lp (a) glycosaminoglycan complexes which are formed accumulate and are taken up by macrophages. At high concentrations, Lp (a) may also prevent fibrinolysis of the formed thrombus and as such may be the primary link between lipoproteins and the coagulation factors.

INDICATIONS FOR MEASUREMENT

Assessment of atherogenic risk, particularly in people with at least one other cardiovascular risk factor (diabetes, hypertension, hypercholesterolaemia, etc.) and/or in patients with moderate hyperlipidaemia, to assess risk before starting drug therapy. Lp (a) must be measured in association with a lipid profile, including total cholesterol, triglycerides and HDL-cholesterol (with calculated or measured LDL).

As Lp (a) is genetically determined there is no merit in repeating measurements. Two consistent results are sufficient to assess a patient's risk.

INFORMATION

SAMPLE

Serum (dry tube): For a lipid profile the sample must be taken after fasting for at least 12 hours; fasting is not required for an isolated measurement.

QUESTIONS FOR THE PATIENT

Are you taking medical treatment? Oestrogen-progestogens (particularly hormone replacement therapy for the menopause) can reduce blood Lp (a) concentrations.

SAMPLE STORAGE AND TRANSPORT

Centrifuge within two hours of sampling.

Agarose gel electrophoretic methods are performed on fresh serum. For other methods, the serum may be stored for 7 days at + 4°C, and then at –20°C for several months. It may be thawed only once (beyond this, Lp (a) increases).

ASSAY METHODS

Electrophoresis or immunochemical methods (immuno-nephelometry, immunoturbidimetry and ELISA).

NORMAL EXPECTED VALUES

Depending on the author and/or method used, the normal limit is < 0.30 or < 0.45 g/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Lp (a) increases from birth up to the second year of life and then remains stable in males.

In females it increases during pregnancy in the 2nd and 3rd trimesters (by a factor of 2 to 3) and after the menopause. Three months should be left after childbirth before assessing lipid abnormalities in women.

PATHOLOGICAL VARIATIONS

Lp (a) is a risk factor for coronary and cerebral atherosclerosis when plasma concentrations are over 0.30 or 0.45 g/l (depending on the author). It is independent from other identified cardiovascular risk factors. High Lp (a) concentrations are also associated with acceleration in coronary artery restenosis after angioplasty and worsening of stenoses in venous bypass grafts

High plasma Lp (a) concentrations are not affected by a good diet or lipid lowering drug therapy.

Plasma Lp (a) is generally often raised in myocardial infarction or cerebrovascular accident in people with Apo B receptor deficiency, poorly controlled diabetics, in hypothyroidism (x 1.5), in haemodialysed patients (x 3 to 4) and to a lesser extent in inflammatory states.

It may be reduced in hyperthyroidism, chronic alcoholism, hepatic insufficiency and cirrhosis.

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- Legrand A., *Dyslipoprotéinémies et maladies cardio-vasculaires*, Biotribune 2004; 11 :34-6.
- Boutron A., Legrand A., *Fiches pré-analytiques*. www. sante. gouv. fr

LISTERIOSIS

DEFINITION

Listeriosis is an infectious disease common to human beings and many species of animal. It is caused by the bacterium, *Listeria monocytogenes (Lm)*.

Lm belongs to the *Listeria* genus (which contains 4 other similar species) and the Corynebacteria family. Infection is from food and mostly affects industrialised country, preferentially in people with a weakened or immature immune system, such as pregnant women, the newborn, elderly and people with immunodeficiencies due to immunosuppressant therapy or disease.

EPIDEMIOLOGY

Listeria monocytogenes is a ubiquitous saprophytic organism found widely in the external environment (water, earth and plants). It has a great ability to adapt to the environment and to variable temperatures, pH and salinity conditions. Its optimal growth temperature is between 20 and 37°C, although it can grow at refrigerated temperatures. The increasing use of refrigeration (household refrigerators and industrial refrigerated depots) has also promoted its spread.

Listeriosis is found widely throughout the animal kingdom and can be transmitted to human beings.

The most common method of infection is eating contaminated foods, such as dairy products (soft unpasteurised milk cheeses), cooked pork meats (patés and pork tongue, etc.) but also from poultry, raw vegetables and some smoked fish.

Cases of nosocomial infection occur in hospitals, in gynaecology departments and maternity units.

The disease has a worldwide geographical distribution. It is often not apparent and is under-estimated in frequency (there are 200 cases documented annually in France). It usually develops as the sporadic form, although true food epidemics occur.

SYMPTOMS

The bacterium enters through the gastro-intestinal tract, crosses the bowel through the lymphatic and blood system and reaches the liver. It then causes a bacteraemia with metastatic sites in the placenta or central nervous system.

The incubation period is extremely variable, from a few days to more than a month.

Lm infection is asymptomatic and transient in most cases. When it does become apparent because of reduced immunity the clinical symptoms vary depending on whether or not the disease is materno-foetal.

The materno-foetal and neonatal form of the disease is generally asymptomatic or involves mild flu-like symptoms in the mother, such as isolated fever or fever associated with headache, myalgia, rhinopharyngitis and occasional gastro-intestinal problems.

For this reason, it is often not recognised and is revealed by obstetric complications or by severe infection in a child.

It affects all stages of pregnancy, causing spontaneous miscarriage in the first 2 trimesters and *in-utero* foetal death or premature delivery of an infected child in the 3rd trimester.

Listeria monocytogenes can infect the child transplacentally, often following bacteraemia in the mother.

Neonatal listeriosis is seen:

– As a septicaemic form of disease (in the first days after birth) with disseminated septic granulomatosis in advanced disease. This is often fatal

– Or by a later acute meningeal form (within 3 weeks of birth) which is rare and less serious.

The adult form represents 75% of cases of listeriosis and almost always occurs in a context of immunosuppression. It results in ***septicaemia*** and predominantly ***central nervous system disease*** (isolated meningitis and meningo-encephalitis, which is fatal in 20 to 30% of cases or even isolated encephalitis). In the last few years, epidemics of ***Lm gastro-enteritis*** have also occurred.

SEARCH INDICATIONS

Any unexplained maternal fever during pregnancy or labour.

Following unexplained premature childbirth.

In the context of neonatal distress or acute foetal distress.

In an immunosuppressed or fragile adult with suggestive clinical signs.

In epidemiological studies.

INFORMATION

SAMPLE

Materno-foetal disease:

Mother systematic blood cultures for unexplained fever during pregnancy or during delivery, amniotic fluid sample, placental and lochia samples following childbirth.

In new-born babies: blood, CSF, nasal, pharyngeal and conjunctival secretions, meconium and gastric fluid.

Outside of materno-foetal disease the samples are guided by clinical signs: blood cultures, CSF in CNS disease, serum and more rarely stool samples.

QUESTIONS FOR THE PATIENT

Clinical features?

Immunosuppression and context?

Has the patient eaten potentially contaminated foods (dairy products, etc.)?

Term of pregnancy?

SAMPLE STORAGE AND TRANSPORT

Samples for *Listeria monocytogenes* testing are mostly taken from sterile sites and therefore must be cultured immediately or otherwise stored for a maximum of 48 hours at + 4°C (excluding blood cultures at room temperature). Neonatal samples should be sent within an hour of birth at room temperature or otherwise stored at + 4°C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

The diagnosis is based on finding the organism in pathological matter.

- **Direct examination:** microbiological examination of the pathological matter reveals a small Gram positive, intracellular bacillus, either isolated or bacteria clustered in short chains.
- **Culture:** this is performed on normal isolation medium, blood agar or selective media for multiple microbial samples. *Lm* grows in 24 hours and produces small smooth colonies with regular borders which are translucent, blue, green, iridescent and β-haemolytic on blood agar. *Lm* is catalase positive and rapidly hydrolyses esculin. It is mobile at 22°C and immobile at 37°C. It can grow at +4°C and ferments glucose.

  The main species of *Listeria* genus are distinguished by identifying their biochemical characteristics on galleries.

INDIRECT DIAGNOSIS

Serological diagnosis involves testing serum for the presence or absence of antibodies against somatic O and flagellar H antigens of serotypes 1 and 4b, those serotypes most commonly found in France in human listeriosis.

Of the serological reactions:

– ***The agglutination reaction*** is widely used in everyday practice. Common antigenic features, however, exist with Staphylococci and enterococci making interpretation difficult and requiring pre-absorption of the serum with these organisms.

– ***Measurement of antibodies against listeriolysin O (ALLO):*** the ALLO appear early after the clinical onset of infection reaching high titres lasting for several months. They are titred using the dot blot method. Western blot may be used to confirm their specificity.

– ***Listeria monocytogenes typing***

Serological phenotyping of *Listeria* strains uses the distinction between the 15 somatic O and 5 flagellar H antigens. Combinations of these antigens have been used to define 17 serovars of the *Listeria* genus: 95% of Lm strains isolated in human disease involve ½ a, ½ b and 4b serovars.

Lm typing, which is used in epidemiological monitoring of the disease, is performed using phenotypic and molecular methods in specialist laboratories.

INTERPRETATION

Serological diagnosis is useful particularly for retrospective diagnosis or if the organism cannot be isolated on direct examination, although has limitations due to the common antigenic features with common organisms. It can also not be used in new-born babies. For this reason the diagnosis is mostly bacteriological.

TREATMENT

CURATIVE TREATMENT

Lm is highly sensitive to antibiotics, such as penicillins, amino glycosides, tetracyclins and trimethoprim-sulfamethoxazole.

The usual treatment regimen is shown below:

– ***For suspected listeriosis (including pregnant women):*** amoxicillin 3 g/day for 15 to 21 days (macrolide or pristinamycin if allergic)

– ***For confirmed listeriosis:*** amoxicillin 200 mg/kg/day by infusion for 3 to 4 weeks + aminoglycoside (gentamicin 3 mg/kg/day) IV if neonatal or adult neuromeningeal disease is present. If patients are allergic to β lactams, trimethoprim-sulfamethoxazole is recommended because of its very good distribution into the CNS.

PREVENTION OF LISTERIOSIS

Treatment is above all preventative and involves:

– Educating at risk people and consumers to avoid potentially contaminated foods, cooking foods of animal origin (meat, fish) correctly, washing raw vegetables and aromatic herbs carefully and following food hygiene principles when preparing foods (cleaning and disinfecting refrigerators, observing expiry dates, etc.)

– Following strict control of industrial foods (refrigeration, controlled cooking, testing milk and animals).

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LITHIUM

DEFINITION

Lithium is a mood-regulating psychotropic agent indicated for use in the curative treatment of manic or hypomanic excitatory states and in the prevention of relapse of manic depressive psychoses and intermittent schizo-affective disorders.

Commercially available forms in France:

Lithium salts	Name of proprietary products	Galenic form	Lithium contents
Lithium gluconate	Neurolithium®	Oral vials (5 ml) containing 1 g Oral vials (10 ml) containing 2 g	4.95 mmol 9.90 mEq
Lithium carbonate	Théralithe®	250 mg immediate release tablets. 400 mg prolonged release tablets (PL)	6.80 mEq 10.80 mEq

The usual dosage is 10 to 20 mEq of lithium/day, administered as 2 or 3 daily doses with meals for standard forms (oral vials and immediate release forms) and as a single daily dose for prolonged release forms.

PHARMACOKINETICS

Bioavailability (oral)	Almost 100%
Concentration peak	Conventional form: 0.5 to 3 hours in plasma; 5 hours in erythrocytes. Prolonged release form: 2.5 to 6 hours in plasma
Defined steady state	Approximately 1 week (4 to 8 days)
Plasma protein binding and metabolism	Weak or zero
Elimination	Mostly renal. Lithium is 100% filtered by the glomerulus and 75% reabsorbed in the proximal tubule (reabsorption of sodium and lithium is competitive)
Half-life of elimination	Varies between people: from 12 to 45 hours
Clearance	10 to 30 ml/min in people with normal renal function

INDICATIONS FOR MEASUREMENT

■ PLASMA/SERUM MEASUREMENT

Regular blood lithium measurements are required to monitor treatment for the following reasons:

a) Lithium has a narrow therapeutic index (low margin of safety between the therapeutic and toxic ranges)

b) Large inter-individual variability in pharmacokinetics and relationship between dosage and blood concentrations.

The main factors which alter the pharmacokinetics of lithium are:

– Renal insufficiency, age, diarrhoea/vomiting, physical exercise and low sodium diet (reduced lithium excretion); pregnancy, cold, rest, obesity (increased lithium excretion)

– Therapeutic interactions *(cf. below)*.

c) To establish the relationship between serum concentrations and therapeutic effect and between serum concentrations and toxicity. The most common signs of overdose are nausea, tremor, thirst and balance disorders. Electroencephalographic abnormalities develop early.

Plasma/serum measurements are also used to monitor treatment of lithium salt poisoning.

■ ERYTHROCYTE MEASUREMENT

a) Prevention of toxic effects of treatment

The relationship between the toxic effects of lithium (particularly electroencephalographic abnormalities) and erythrocyte concentrations is better than the relationship between toxic effects and plasma/serum concentrations.

b) Identification of poor adherence to treatment

Plasma lithium concentrations in a patient who has only taken his/her treatment a few hours before blood sampling are within the therapeutic range whereas erythrocyte concentrations are extremely low.

c) Monitoring treatment of poisoning

After lithium salt poisoning (attempted suicide), erythrocyte measurement is of prognostic value and helps in treatment decisions (osmotic diuresis with mannitol or extra-renal removal).

■ URINARY MEASUREMENT

a) Main indication: to confirm that the patient is adhering to treatment when plasma concentrations are low despite *a priori* effective dosage.

b) Other indications:

– Measurements are used to calculate lithium clearance, which is useful to determine optimal dosage in some patients in whom therapeutic concentrations are difficult to achieve

– Monitoring treatment of lithium poisoning to assess the amount of lithium taken

– When prolonged release forms are used, urinary measurements can confirm their bioavailability in patients with accelerated bowel transit which reduces lithium absorption.

INFORMATION

■ SAMPLE

- **Serum/plasma lithium:** serum (taken into a dry plastic, polypropylene or polystyrene tube with a plastic stopper) or heparinised plasma not containing lithium (ammonium, sodium or calcium heparinate or synthetic heparinoid, anticlot® tube). Any tubes containing lithium heparinate or iodo-acetate must not be used. Avoid tubes with separator gel.

  Discard haemolysed samples.
- **Erythrocyte lithium:** heparinised whole blood without lithium.
- **Urinary lithium** *(see below).*

■ METHODS

A steady state sample should be taken for therapeutic drug monitoring, i.e. one week after starting treatment or changing dosage.

- **Blood:**
  – Immediate release form:
    - Take the sample fasting in the morning, 12 hours after the evening dose of lithium and before any further dose has been taken: trough concentration.
  – Prolonged release forms administered in the evening:
    - Take sample in the morning 12 hours after the lithium dose: intermediary concentration, or
    - Take sample in the evening 24 hours after the dose before the next dose: trough concentration.
- **Urine:**
  To calculate clearance: 24 hour or 3 hour urine collection, 3 hours after the lithium dose (in a patient who has been taking a constant dose for at least 1 week). State urine output and collection time.

  If a 3 hour urine collection is used the blood sample is taken in the middle of the urine collection period. If a 24 hour collection is used, plasma lithium measurements should be performed on at least 6 samples throughout the 24 hour period (before and 3 hours after each dose.). The plasma lithium concentration used to calculate clearance is the mean concentration calculated from a curve of concentration over time. For monitoring lithium poisoning, the whole forced diuresis urine sample should be collected. To confirm adherence to treatment a 24 hour urine sample should be collected for at least 3 consecutive days.

ESSENTIAL INFORMATION

Any request for a drug measurement must include reasons for request (testing for efficacy or toxicity), sampling time, treatment start date and/or any change in dosage, dosage information (amount administered, frequency and route of administration) and the age, height and weight of the person whenever possible.

Plasma creatinine levels.

Question for patients: are you taking any of the following drugs (risk of interference with lithium metabolism):

- **Reduced plasma/serum lithium concentration** in association with osmotic diuretics (due to an increase in lithium clearance).
- **Increased plasma lithium concentration** (risk of overdose mostly due to a fall in the lithium clearance) when associated with:
  – Any non-steroidal anti-inflammatory drugs (including the coxibs) except for salicylates; particularly indomethacin where steady state blood lithium is increased by 40 to 60% and ibuprofen can increase the blood lithium by 12 to 66%
  – Thiazide diuretics: blood lithium increased by 25 to 40%
  – Converting enzyme inhibitors: blood lithium increased on average by 36%
  – High dose neuroleptics: chlorpromazine, fluphenazine, risperidone, thioridazine
  – Methyldopa and verapamil.

SAMPLE STORAGE AND TRANSPORT

Store serum or plasma at + 4 °C. Transport at + 4°C.

For erythrocyte lithium measurement: store whole blood at + 4°C.

Store urine at: + 4°C.

ASSAY METHODS

Atomic emission flame photometry, selective electrodes (direct or indirect potentiometry), reflectometry and atomic absorption spectrophotometry.

EXPECTED VALUES

RECOMMENDED PLASMA/SERUM CONCENTRATIONS

- **Standard immediate release form administered in the evening:**
  – Minimum effective concentration: 0.5 to 0.8 mEq/l (or mmol/l) (trough concentration of morning sample, 12 hours after the evening dose).
- **Prolonged release form administered in the evening:**
  – Minimum effective concentration: 0.5 to 0.8 mmol/l (trough concentration evening sample, 24 hours post-dose)
  – Intermediary concentration: 0.8 to 1.2 mmol/l (morning sample, 12 hours post-dose).

  NB: Blood lithium concentrations approximately 0.10 mmol/l lower are generally sufficient in the elderly; similarly, patients of Asian origin usually respond to doses equivalent to lower plasma concentrations.

ERYTHROCYTE CONCENTRATIONS

At steady state (after approximately 5 half-lives, i.e. after treatment for approximately 1 week), these provide a good reflection of tissue exposure, particularly cerebral. At steady state, intra-erythrocyte lithium is related to serum lithium by the following relationship:

Li erythrocyte = (Li serum x 0.49) – 0.035.

For standard forms the therapeutic range is between 0.2 and 0.4 mmol/l. For prolonged release forms the range is between 0.3 and 0.6 mmol/l.

Signs of poisoning develop at intra-erythrocyte blood lithium concentrations of 0.60 mmol/l.

When monitoring lithium poisoning, the change in concentrations is shown on the figures below.

Figure 1: Change in plasma and erythrocyte lithium concentrations during mil acute poisoning.

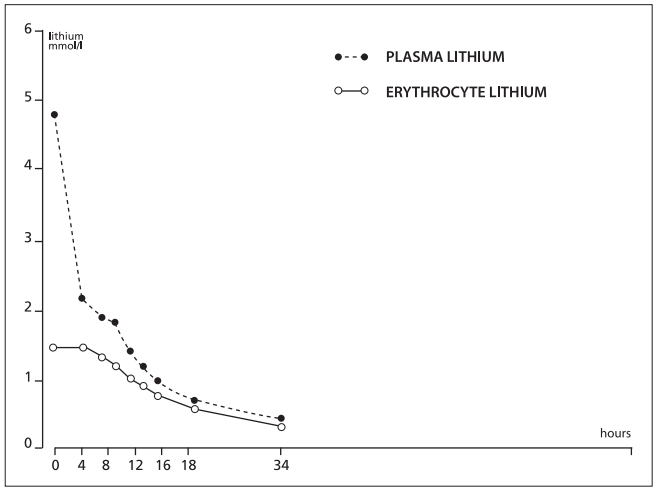
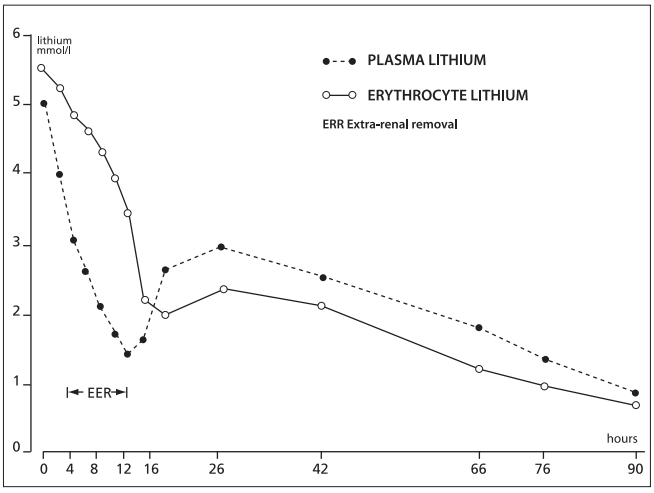


Figure 2: Change in plasma and erythrocyte lithium concentrations during severe acute poisoning.



Figures obtained from Cahier de formation BIOFORMA no. 19 December 1997, lithium chapter, pages 43 to 75.

■ URINE CONCENTRATIONS/CLEARANCE

– Clearance: 10 to 30 ml/min in people with normal renal function.

– Confirmation of adherence to treatment: after urine collections for several consecutive days with parallel measurements of lithium in plasma and urine:

- The patient is non-adherent if at least 80% of the amount of lithium prescribed is not recovered daily in urine
- The patient is adherent if the amount prescribed is found in urine and the plasma concentrations are stable
- It is likely that the patient was not taking his/her treatment before the urine tests if the amount prescribed is recovered in urine and the plasma concentrations are increasing.

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LSD

DEFINITION AND SYNONYMS

LSD *(lysergic acid diethylamide)* or LSD-25 is the 25th molecule synthesised by its discoverer and is a hallucinogenic drug discovered in 1938 by a Swiss chemist Albert Hofmann. It is synthesised from lysergic acid, which is extracted from ergot, a parasite fungus of rye and other cereals.

LSD is also commonly called "acid" and is available in different forms (tablets, capsules and liquid), which can be impregnated onto blotting paper. LSD is colourless and odourless, has a slightly bitter taste and is mostly taken orally. The amount of LSD currently contained in a dose ranges from 20 to 80 µg, which is considerably less than what was seen in the 1960-70s (100 to 200 µg).

INTRODUCTION

The mechanism of action of LSD is still poorly understood. Experimental results, however, suggest that like other hallucinogens, LSD may act on serotonin receptors, which are present particularly in two cerebral territories; the cortex and the *locus ceruleus*. The effects of LSD begin within 30 to 90 minutes after oral ingestion and may last for up to 12 hours. LSD users refer to a "trip" but also to unpleasant experiences ("bad trip"). The trip involves an initial period lasting approximately two hours, the "start" of which involves a combination of mydriasis, sweating, tachycardia and hyperthermia and a second period called the "trip" itself which lasts between five and eight hours and involves an association of visual, tactile and auditory hallucinations and finally a third period, the "come down" which involves a decline in the above effects until normality is restored, combined with asthenia.

Some LSD users develop psychotic problems after the end of the "trip" which may last for several years after they stop using LSD. "Flash-backs", episodes which may involve hallucinations but are above all characterised by visual defects, may also occur outside of and distant to taking the substance. Regular users also develop habituation making them increase the doses they take.

LSD is metabolised in the body into desmethyl-LSD, 13 and 14 hydroxy-LSD which are the major metabolites found in urine and tested for in urine tests.

INDICATIONS FOR MEASUREMENT

Testing for LSD in urine or blood, possibly combined with measurement of metabolites, is only indicated to test for drug use within three to five days before the urine sample is taken or within a few hours before the blood sample was taken.

INFORMATION

SAMPLE

Blood measurements are performed on serum or plasma after taking whole blood in lithium heparinate or into a dry tube.

Blood samples must be taken within a few hours of the LSD being taken. Urine sample: a random urine sample collected at any time in the day, preferably at the laboratory, in order to avoid risks of substitution. The patient's identity and temperature of the sample which should be between + 32 and 38°C, together with its pH (between 3 and 11), density (between 1.0010 and 1.0200) and creatinine (between 20 and 200 mg/l) should be checked according to the American recommendations published in April 2004.

QUESTIONS FOR THE PATIENT

When was the patient potentially exposed to LSD?

Is the patient taking phenothiazines?

SAMPLE STORAGE AND TRANSPORT

Samples are stable for several days in a refrigerator and for several months frozen.

ASSAY METHODS

The main methods available are firstly screening methods based on immunoenzymatic techniques and secondly classical confirmatory methods involving chromatography coupled to mass spectrometry. The former, which are only performed on urine, have the advantage of being easy to use and provide a fast result although the result is qualitative and these methods are sensitive to interference from phenothiazines which may produce false positive results Any positive result obtained with the screening method must therefore be confirmed by a confirmatory method. Chromatographic methods are specific and can also be used to quantify LSD in blood and urine.

NORMAL EXPECTED VALUES

LSD is normally absent in blood and urine

The *National Institute on Drug Abuse* (NIDA) does not provide a threshold value for LSD testing in urine.

PATHOPHYSIOLOGICAL VARIATIONS

LSD is classically found in urine for up to three to five days after being taken. Be aware of interferences from phenothiazines in screening tests.

FOR FURTHER INFORMATION

- NIDA info facts. LSD. www.drugabuse.gov

LYME'S DISEASE

DEFINITION AND SYNONYM

Lyme's disease is caused by the spirochete, *Borrelia burgdorferi*, and is prevalent in the northern hemisphere (occasional suspected cases in Australia and South America). It is an anthropozoonosis transmitted by bites from female ticks belonging to the *Ixodes genus*, several species of which exist: *I. ricinus* in Western Europe, *I. persucaltus* in Eastern Europe and Asia and *I pacificus* and *I. scapularis* in America. There is only one species of *Borrelia* in America: *B. burgdorferi ss; B. garinii* and *B. afzelii* are found in Asia and Eastern Europe and *B. burgdorferi ss, B. garinii, B. afzelii, B. spielmanii, B. valisiana* and *B. lusitaniae* in Western Europe.

INTRODUCTION

EPIDEMIOLOGY

This is a worldwide disease found particularly in cold and temperate regions of the northern hemisphere. The average incidence of the disease in France is estimated to be 9 cases per year per 100,000 people. Lyme's disease is a zoonosis transmitted by ticks, which differ in species depending on the geographical region concerned. It is found particularly in wooded areas, in spring and at the end of summer. The ticks act as parasites for a large variety of hosts: small (mostly rodents) or larger (deer, sheep and cattle) mammals, reptiles and birds. Human beings and domestic animals are accidental hosts. The risk of human infection depends on the tick density and their attachment time.

LIFE CYCLE

Ixodes ricinus has a 3 stage lifecycle: the larva, nymph and adult, all 3 of which can transmit the disease. Adult females lay eggs in a hole in sheltered ground. These then rupture and release larvae. The larvae wait for 2 to 3 weeks to seek out a host and gorge themselves on its blood in a single feed. The larva then transforms into the nymph which in turn seeks a host 2 to 3 weeks later. It feeds once from the host and then detaches. The nymph then mutates 3 to 5 months later into an adult tick, only the female of which feeds on the blood of a new host (human being or animal).

SYMPTOMS

Following an incubation period of 3 to 30 days, the disease progresses in 3 phases:

- **Primary phase:** This is erythema migrans (EM), which is a painless erythematous macule or papule that spreads in diameter to form a "bulls eye" rash on the lower limbs. The lesion is characteristic of the disease and is the predominant clinical form in France. Its presence confirms the diagnosis, although EM is only seen in 65 to 75% of cases. It is occasionally accompanied by systemic signs, such as fever, pain and asthenia.
- **Secondary phase:** This only develops in the absence of antibiotic treatment or if the primary phase has not been recognised. It involves a variable fever and assorted neurological, rheumatological, cardiac or cutaneous features.

Neurological features (neuroborrelioses) are the most common in Europe. These may include mostly sensory nerve root involvement, peripheral motor involvement, disease of the cranial nerves such as uni- or bilateral peripheral facial paralysis or central or meningeal disease.

The rheumatological features include early arthralgia followed by arthritis affecting the large joints (particularly the knee).

The following are seen more rarely:

Cardiac features due mostly to atrio-ventricular conduction disorders which may progress to block. Pericarditis or myocarditis occurs occasionally.

Cutaneous features, such as lymphocytoma of the ears, nipples or genital organs, the diagnosis of which is histological (skin biopsy).

- **Tertiary phase:** This involves development of chronic problems.

Neurological features, such as encephalomyelitis which represents isolated spinal cord or cerebral damage.

Rheumatological features involving chronic arthritis affecting the large joints, particularly the knee occurring months or even years after infection.

The skin features are atypical apart from the characteristic lesions of Pick-Herxeihmer disease, also known as chronic atrophying acrodermatitis (CAA). This is an inflammatory skin lesion limited at the outset which becomes atrophic over a few years. Benign cutaneous lymphocytoma (BCL) is another specific form of skin disease characterised by a lympho-histiocyte infiltrate.

SEARCH INDICATIONS

Diagnosis of Lyme's disease in a person with compatible symptoms and a history of tick bites, or someone who has stayed in an area liable to contain infected ticks (undergrowth).

A differential diagnosis from other bacterial and viral diseases.

The Consensus Conference on Borrelioses (13-12-2006) in France stated that serology testing was not indicated in the following situations:

– EM (Typical erythema migrans)

– Systematic serological testing of treated patients

– Asymptomatic people

– Systematic screening of exposed people

– Tick bite without clinical features.

INFORMATION

SAMPLE

CSF, skin biopsy, joint fluid and/or synovial biopsy for direct diagnosis.

Serum, CSF and possibly joint fluid for serological diagnosis.

For measurement of the intrathecal IgG synthesis index: 1 ml of CSF and 1 ml of paired serum taken within 24 hours before or after the CSF.

■ QUESTIONS FOR THE PATIENT

History of tick bite?

Clinical features?

Exposed patients (forestry occupation, hunter, rambler, etc)?

Current antibiotic treatment?

■ SAMPLE STORAGE AND TRANSPORT

Serum and joint fluid should be stored and transported at + 4°C; CSF must be transported frozen at – 20°C.

DIAGNOSTIC METHODS

The diagnosis of Lyme's disease is mostly clinical if characteristic signs such as EM have not gone by unnoticed. Diagnostic difficulties also, however arise, because of the variety of clinical forms of the illness. For this reason, laboratory diagnosis provides valuable assistance. In practice, diagnosis is mostly serological as culture is difficult to perform and molecular biology methods are reserved for a few specialist laboratories.

Antibodies (Ab) are detected in blood or CSF, usually by the ELISA method with confirmation using Western blot (WB), if the ELISA is positive or equivocal (WB is not indicated if the ELISA is negative).

The minimum clinical performance, recommended by EUCALB is a specificity of ≥ 90% by ELISA and IIF and a "cut-off" established on 100 samples from healthy donors. The CE mark is not sufficient to guarantee the quality of commercial kits: specificity must be assessed on the local population and sensitivity on confirmed cases of borreliosis. Beware of differences in sensitivity between ELISA kits. Overall, the sensitivity of serology in serum is 50% at the erythema migrans stage, 70% at the neuroborreliosis stage and almost 100% in chronic atrophying acrodermatitis or Lyme's arthritis.

Immunoblotting or the Western blot (WB) is a qualitative test which identifies Ab specificity. In-house and commercially available tests exist using native or recombinant antigens (Ag) and vary in interpretation depending on the type and number of immuno-reactive Ag. Because of a lack of standardisation, each laboratory must validate its own test; the minimum standard required is a specificity of 95%.

■ DIRECT DIAGNOSIS

– **Direct culture diagnosis** requires specific media (BSK Barbour-Stoenner-Kelly or Modified MKP Kelly Pettenkofer).

Sensitivity is 50% in EM or CAA (on a skin sample), but is too low in CSF or synovial fluid. Following culture, bacteria are examined on dark background or fluorescence microscopy. Bacterial growth however, is slow and culture is not used commonly in diagnosis.

– **Direct diagnosis by PCR**

As few bacteria may be present in some tissues, the sensitivity of PCR varies. It is useful, however, in Lyme's arthritis, on a synovial biopsy or fluid.

	Sensitivity (%)
Skin	68
Plasma	26
Synovial fluid	73
CSF	19

■ INDIRECT DIAGNOSIS: SEROLOGY

This is the most widely used diagnostic method. Recommendations exist for interpretation (Consensus Conference, December 2006).

Sensitivity varies greatly between kits in the primary stage (20 to 70%) and depending on how early the sample is taken from the development of EM, which itself is pathognomonic. Because of this, it is not recommended in this situation. Sensitivity is over 80% in the secondary stage and test is used as an exclusion criterion for the tertiary stage when sensitivity rises to 100% for IgG (conversely IgM is absent).

IgM develop after 3 to 5 weeks and IgG after 6 to 8 weeks. With early treatment, IgM and IgG may fall or even never be detected. Once it has developed, IgM can persist long after early treatment.

Serology testing is performed in two stages: a screening test followed by a confirmation test, only if the screening test is positive.

1st generation screening tests used complete cell antigens, as with IIF, and then the 2nd generation ELISA tests were enriched with purified antigens in order to minimise cross-reactions. Recombinant Ag which is not present in large amounts in culture (such as VlSE protein) was then added to the 3rd generation tests, increasing their sensitivity and specificity.

Confirmation tests (according to the CC): WesternBlot or immunoblot

The reagents for these are poorly standardised and interpretation is based on the EUCALB criteria to determine the most useful proteins. For IgM: p41 OspC and VlSE; for IgG: VlSE, p23, p83/P100, p18 p58, p39 and p30 (OspA) which offer supporting evidence of infection several months previously. 2, 3 or 4 of these bands must be positive to confirm the diagnosis.

Serology is difficult to interpret because of the relatively high prevalence of Ab in the general population (3 to 5%, and up to 30% in butchers and hunters). The antibody titre can therefore be used to partially distinguish between a serological "scar" and active infection.

Interpretation is based on distinguishing the isotype: the presence of IgM suggests early stage infection as IgM is still present at the start of the secondary phase but absent in the tertiary phase. IgG develop at the end of the primary phase and are present at high titres in the tertiary phase.

Cross-reactions are described particularly with syphilis, but also with other spirochetes (leptospira in autoimmune disease), or EBV or herpes virus infection, etc. for IgM.

An isolated positive serology is never therefore an indication for treatment.

Diagnosis of Lyme's neuroborrelliosis by calculating the intrathecal specific IgG antibody synthesis index.

Synthesis of antibodies is activated in CSF in primary-secondary Lyme's disease as soon as the initial symptoms of neuroborreliosis are present. High levels of anti-*Borrelia* IgG are produced in the intrathecal compartment when features of chronic neuroborreliosis are present. Isolated detection of anti-*Borrélia* antibodies in CSF however is insufficient to make a diagnosis of neuroborreliosis as the specific immunoglobulins may originate from serum, due to transudation, as the blood-brain barrier is patent with any

meningeal inflammation. In order to distinguish between passive diffusion and *in-situ* formation of specific IgG in the CSF, an anti-*Borrelia* IgG intrathecal synthesis index is calculated using the Reiber concept: anti-*Borrelia* IgG is measured in CSF and blood and is expressed as a ratio of the concentrations to total IgG in each compartment.

Index = CSF titre x total serum IgG / serum titre x total CSF IgG.

In some conditions, however - oligoclonal production of immunoglobulins in the CSF – the anti-*Borrelia* IgG ratio must be compared to a ratio taking account of albumen concentrations in each compartment.

According to the December 2006 Consensus Conference on the diagnosis and treatment of Lyme's disease and EUCALB, calculation of the intrathecal anti-*Borrelia* IgG synthesis index is the best diagnostic approach for neurological forms of Lyme's disease. As the number of bacteria present in the CSF is too low, testing by PCR does not provide a diagnosis of neuroborreliosis.

RECOMMENDATIONS FOR THE LABORATORY DIAGNOSIS DEPENDING ON THE DIFFERENT CLINICAL FORMS.

From the 16th Consensus Conference in Anti-Infection Therapy (December 2006).

Clinical forms	Indications and results of essential diagnostic investigations	Optional investigations (2nd line in suggestive clinical context and 1st line investigations negative)
Erythema migrans	- NO investigation	- NONE
Early neuroborreliosis	- Lymphocyte cell reaction in the CSF and/or raised cerebrospinal fluid protein - Serology + in CSF occasionally delayed in blood - Intrathecal specific IgG synthesis	- CSF culture and PCR - Seroconversion or rise in serum IgG titre
Borrelia lymphocytoma	- Histological appearance of the lymphocytoma - Positive serology (blood)	- Skin sample, culture and PCR
Cardiac disease	- Positive serology (blood)	- On specialist advice
Arthritis	- Positive serology (blood): IgG titre usually raised - Inflamed joint fluid	- Synovial fluid and/or tissue culture and PCR
Chronic neuroborreliosis	- Intrathecal production of specific IgG	- CSF culture and PCR
Chronic atrophying acrodermatitis	- Suggestive histological appearances - High titre positive serology (IgG)	- Skin sample culture and PCR
Ocular forms	- Positive serology - Confirmation by specialist advice	- On specialist advice

TREATMENT

The 1st line treatment in adults is amoxicillin *per* os: 1 g x 3/d or doxycycline 100 mg x 2/d for 14 to 21 days. 2nd line treatment is cefuroxime-axetil, 500 mg x 2/d, for 14 to 21 days. Azithromycin, 500 mg x 1/d for 10 days can be used for patients who are allergic or have contra-indications to these 1st line treatments. The later treatment is started the less effective it is and the greater the risk of complications. Specific treatment methods for children and in certain clinical situations are described in the 2006 Consensus Conference report.

Systematic antibiotic prophylaxis is not recommended after a tick bite, although is prescribed in patients at high risk (multiple bites, long attachment periods and high tick infestation rates). Oral doxycycline is used as 200 mg in a single dose or oral amoxicillin: 3 g/d for 10 to 14 days.

Monitoring after treatment

lMonitoring relies mostly on resolution of clinical signs. Serologically, the time taken for Ab titres to fall increases with higher initial levels.

Generally, IgM falls significantly 3 to 6 months after clinical signs have resolved (IIF often remains positive for longer than ELISA), although IgM may persist for years the same year as IgG. Serological follow up is only useful for late forms to assess the effectiveness of treatment, as long term complications may develop.

Prophylaxis

This involves informing the public and health professionals in at risk areas and wearing clothing cover, particularly over the arms and legs (light coloured clothing makes ticks easier to identify). Insect repellents are recommended together with vigilance when returning from tick infested area.

How to remove a tick?

1- Remove the tick as soon as possible.

2- Do not apply any substances (ether, etc.) which the tick could regurgitate.

3- Grasp the tick as close to the skin as possible (fine tweezers).

4- Avoid direct contact between fingers and the tick or regurgitated material.

5- Always disinfect the bite with alcohol after removing the tick.

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- XVIe Conférence de Consensus en Thérapeutique antiinfectieuse: Borréliose de LYME; 13 December 2006 (Short text): www.infectiologie.com
- European Union Concerted Action on Lyme Borreliosis (EUCALB) (*http://meduni09.edis.at/eucalb/cms/index.php*)

LYSOZYME

DEFINITION

Lysozyme is a bacteriolytic hydrolase enzyme produced by granular and monocyte cell lines and secreted into many biological fluids (serum, saliva, colostrum, nasal mucus, tears, urine, milk, etc.).

Synonyms: muramidase and mucopeptide N-acetylmuramohydrolase.

INTRODUCTION

Serum lysozyme concentrations rather crudely reflect the granulocyte and monocyte cell pool in the body. Lysozyme is found in very small amounts in urine as it is filtered by the glomerulous and is almost entirely reabsorbed by the renal tubule.

INDICATIONS FOR MEASUREMENT

Lysozyme measurements may be requested to differentiate myelomonocytic from lymphocytic syndromes, neutropaenias of central, from peripheral origin, and to monitor some diseases involving macrophage activation such as sarcoidosis.

Lysozyme measurement can be used to assess the bacteriolytic activity of many biological fluids such as tears, nasal secretions, saliva and breast milk.

INFORMATION

■ SAMPLE

The blood sample is taken into a dry tube.

■ QUESTIONS FOR THE PATIENT

Do you have chronic disease (tuberculosis, sarcoidosis, Crohn's disease or Hodgkin's disease) or a haematological disease (AML, CMML, CLL or lymphoma)?

Do you have renal disease which could influence urine lysozyme concentration (proximal tubulopathy)?

■ SAMPLE STORAGE AND TRANSPORT

Serum can be stored for a few days and transported at + 4°C.

ASSAY METHODS

Lysozyme is measured by turbidimetry. A suspension of killed *Micrococcus lysodeikticus* bacteria is lysed by the lysozyme contained in the test fluid.

Radial immunodiffusion (RID) with a specific antibody is also performed.

Protein electrophoresis (which distinguishes lactotransferrin from lysozyme) is used in preference for lysozyme measurement in tears.

NORMAL EXPECTED VALUES

Biological fluid	Turbidimetry concentration
Serum	< 15 mg/l
Urine	< 2 mg/24h

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Small differences are found between serum concentrations in men and women (difference less than 0.5 mg/l).

■ PATHOLOGICAL VARIATIONS

Increases in serum lysozyme are seen mostly in any malignant or non malignant granulocyte or monocyte cell proliferation. The highest values are seen in acute myelomonocytic and monoblastic leukaemia; lesser increases are seen in chronic myelomonocytic leukaemia and in tubulopathies. Urine concentrations often parallel those in serum.

A fall in serum lysozyme is seen in bone marrow aplasia.

Lysozyme measurement helps to distinguish between central and peripheral neutropaenia. It is low in central neutropaenia.

Concentrations are raised in granulomatous diseases (sarcoidosis, tuberculosis, Crohn's disease and Hodgkin's disease) as a result of macrophage activation. Angiotensin converting enzyme and beta-2 microglobulin are raised in sarcoidosis and associated with hypercalcaemia. Lysozyme has been proposed to monitor disease activity and the therapeutic response to corticosteroids, although angiotensin converting enzyme monitoring appears to be a better indicator.

Intrathecal lysozyme synthesis in HIV-infected patients may be a marker of a cell reaction to an opportunistic pathogen. Intrathecal lysozyme measurement is therefore proposed to diagnose tuberculus meningitis.

Urinary lysozyme concentrations are increased in proximal convoluted tubule disease (diabetic nephropathy).

Finally, a genetic lysozyme mutation exists and is associated with hereditary non-neurological systemic amyloidosis (Ostertag's disease).

FOR FURTHER INFORMATION

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MAGNESIUM

DEFINITION

Magnesium (Mg) is the most abundant intracellular cation in human beings after potassium. The body contains approximately 1 mole, located mostly in bone (65%, as an easily mobilisable reservoir), in cells (34%) and in extracellular fluids (only 1). After ingestion in food, magnesium is absorbed in the intestine. It circulates into the plasma in three forms: a free ionised form (65%), a protein-bound form (35%) and a complexed form (5%). It is then removed in faeces (2/3) and urine (1/3) in the form of phosphates (90% is reabsorbed in the tubule).

INTRODUCTION

Magnesium is involved in all of the body's energy reactions to activate adenosine triphosphate (ATP) molecules. It takes part in the synthesis of carbohydrates, fats, proteins and nucleic acids, in the calcification of soft tissues, bone formation processes, neuromuscular excitability and muscle contractions, including that of vascular smooth muscle, suggesting that it may be involved in hypertension.

INDICATIONS FOR MEASUREMENT

Total Mg can be measured in serum, plasma, erythrocytes or urine. These measurements can generally be used to estimate the body's magnesium status.

SERUM OR PLASMA ALUMINIUM

This test is mostly requested to test for deficiency (which is relatively common in industrialised countries) and may worsen various diseases, particularly cardiovascular, respiratory (asthma) and others (diabetes and eclampsia). Hypomagnesaemia is often however associated with hypocalcaemia and hypokalaemia and as such it is difficult to attribute individual responsibility of any of these deficiencies to the resulting clinical picture. This may include muscle overirritability resulting in tetany or spasmophilia (if the blood Mg concentration is below 0.5 mmol/l), cardiovascular disease (dysrhythmia, heart failure and hypertension), bowel disorders and psychiatric features, dizziness and anxiety).

Magnesium measurement may also be requested to test for hypermagnesaemia, which clinically presents as reduced neuro-muscular tone with abolition of tendon reflexes at concentrations of > 3 mmol/l and muscle paralysis, bradycardia and hypotension at concentrations of 5 mmol/l, progressing to death at concentrations of 6 to 7.5 mmol/l.

ERYTHROCYTE MAGNESIUM

This may be a more sensitive measurement that serum or plasma magnesium to test for latent deficiency. Cases of reduced erythrocyte magnesium concentrations associated with normal serum concentrations have been described in the literature in some cases of chronic fatigue or hypertension.

URINARY MAGNESIUM

This is useful in the context of preventing renal stones; magnesium inhibits the growth of calcium phosphate crystals and reduces urinary oxalate excretion, thereby limiting the formation of calcium oxalate crystals. In magnesium deficiency it can also be used to identify a renal cause.

INFORMATION

SAMPLE

Serum or plasma magnesium

Serum (dry tube) or plasma (heparinised tube); tubes containing EDTA, oxalate or citrate must not be used (these anticoagulants can form complexes with magnesium) unless the measurements are performed by atomic absorption spectrophotometry.

The sample should preferably be taken from the patient fasting in the morning between 0600 and 0800 hours (post-prandial variations may occur although this is debated; the circadian rhythm is greater for ionised magnesium than for total magnesium: minimum 9 hours, maximum 15 hours).

A tourniquet should not be kept in place for more than one minute (approximately 35% of magnesium is protein bound and therefore altered by venous stasis).

Haemolysed samples should be discarded (red cells contain almost three times more magnesium than serum); excess bilirubin or lipids in serum/plasma may also interfere with measurement (increasing or reducing magnesium concentration).

Centrifuge and separate the plasma or serum promptly.

Erythrocyte magnesium

Whole blood collected into a heparinised or EDTA tube.

Urine magnesium

Measured on a 24 hour urine sample collected into a plastic tube without preservative. State urine output.

Do not use haematuric urine (artefactual hypermagnesuria).

QUESTIONS FOR THE PATIENT

Are you taking any of the following drugs?

Drugs which may reduce magnesium concentration in blood:

– Laxatives

– Long term loop diuretics: furosemide, bumetanide

– Ciclosporin, tacrolimus

– Cisplatin

– Aminoglycosides.

Drugs which may increase magnesium concentrations in blood:

– magnesium supplements

– lithium

– Some diuretics which act on tubular Mg reabsorption: amiloride, hydrochlorothiazide.

SAMPLE STORAGE AND TRANSPORT

Serum or plasma magnesium: Store serum or plasma at + 4°C. Transport at + 4°C.

- **Erythrocyte magnesium:** Store and transport whole blood at room temperature or at + 4°C.
- **Urinary magnesium:** Store and transport at +4°C.

ASSAY METHOD

Atomic absorption spectrophotometry (AAS): reference method.

Colourimetry.

Enzymatic methods.

NORMAL EXPECTED VALUES

These vary slightly depending on the method used. For reference:

- **Total serum or plasma magnesium:**
  - Newborn < 7 days: 0.61 to 0.86 mmol/l
  - Infants (7 days - 3 months): 0.65 to 1.02 mmol/l
  - Children and adults: 0.75 to 0.96 mmol/l
- **Erythrocyte magnesium:** 1.65 to 2.50 mmol/l
- **Urinary magnesium:** 3.00 to 7.00 mmol/24 hours
- **Conversion factor:** 1 mmol/l = 0.041 mg/l
  1 mg/l = 24.3 mmol/l

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Age-related variations particularly in infants *(cf. above)*.

Mg is increased in pregnancy.

Mg is reduced in smokers.

Variations depending on dietary intake: magnesium rich products include green vegetables, legumes, cereals and some mineral waters.

PATHOLOGICAL VARIATIONS

Main causes of variations in serum/plasma and/or erythrocyte magnesium concentrations

Falls (< 0.75 mmol/l)	Rises (> 1.1 mmol/l)
• Defective intestinal absorption: diarrhoea, malabsorption, pancreatic insufficiency, laxatives…	• Excess intake: mostly intravenous, rarely oral.
• Inadequate intake: severe malnutrition, prolonged parenteral nutrition without magnesium, chronic alcoholism (even moderate)…	• Acute or chronic renal insufficiency, haemodialysis patients
• Excessive urine excretion due to reduced tubular reabsorption (chronic interstitial nephropathy, tubular acidosis, drugs)	• Metabolic causes: diabetic keto-acidosis, metabolic alkalosis, excess hormones acting on tubular reabsorption: parathyroid hormone, insulin, calcitonin …
•Endocrinopathies: hypoparathyroidism, hyperthyroidism, hyperaldosteronism, Schwartz-Bartter syndrome, diabetes (with severe polyuria).	• Cell lysis: rhabdomyolysis, haemolytic anaemia
• Skin losses (burns).	• Endocrine causes: hypothyroidism, Addison's disease, phaeochromocytoma
• Association with other ion losses: potassium phosphate depletions, spasmophilia…	• Drugs: *cf. above*.

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MALARIA

DEFINITION

Malaria is a protozoonosis caused by a protozoon belonging to the *Plasmodium* genus, which is transmitted by bites from the female Anopheles mosquito.

Malaria is still one of the main scourges of hot, humid, tropical areas and is responsible for a large number of deaths.

Synonym: malaria.

INTRODUCTION

EPIDEMIOLOGY

According to the World Health Organisation (WHO), malaria affects 300 to 500 million people throughout the world annually and causes 1 to 2 million deaths (mostly children), 90% of which occur in Africa. Increasing populations, travel, climate change, development of resistance to antimalarials and insecticides, all explain why the disease is continuing to increase. Approximately 7000 imported cases are estimated to occur in France each year.

Malaria is caused by 4 species of *Plasmodium* which have different geographical distributions: *Plasmodium falciparum* in tropical areas, *Plasmodium vivax* in South-East Asia and South America, *Plasmodium* ovale in Central Africa and *Plasmodium malariae* in tropical regions.

LIFE-CYCLE

The 3 components of the cycle are the Plasmodium, the mosquito vector and human beings (the only reservoir of the parasite). The 3 stages of the cycle are the sexual cycle in the Anopheles, the human stage characterised by its asexual hepatic cycle and its erythrocyte cycle.

The sexual cycle in the female Anopheles: Requires fresh water where the female lays its eggs (approximately 50 every 3 days). The larvae change into nymphs and then adult insects (or imagos). The adult lifespan varies between 1 week (males) and 3 weeks (females) after mating. Fertilised females can only lay eggs again after a meal of blood. They bite mostly at night and inoculate the sporozoites via saliva.

The asexual hepatic cycle in human beings: The hundreds of parasites injected by the mosquito into human beings, or sporozoites, reach the liver cells where they begin to multiply. After the schizont stage, they leave the liver as merozoites reaching the red blood cells.

The asexual erythrocyte cycle in human beings: The merozoites change into trophozoites, which grow and in turn, change into schizontes laden with haemozoin. The ripe schizonts also called "malarial rosettes" rupture releasing merozoites, which in turn infect human red blood cells and create new erythrocyte cycles. Red blood cells rupture, which occurs every 48 or 72 hours and is accompanied by episodes of malarial fever.

After several endoerythrocyte cycles, the male and female gametocytes produced from the transformation of some trophozoites, develop and are potentially ingested by the Anopheles in a bite. The ingested gametocytes pass into the mosquito's stomach, resulting in the formation of an egg (ookinete), after the female gamete has been fertilised. The sporozoites released from rupture of the oocyst reach the mosquito's salivary glands in 9 to 15 days.

SYMPTOMS

- **Invasion phase:** The primary invasion episode develops after an incubation period of between 7 and 21 days and occasionally several months. This is characterised by more or less regular fever often accompanied by headaches, myalgia, abdominal pain, nausea and vomiting, which initially more resembles a gastro-intestinal infection.
- **Uncomplicated acute malaria:** This consists of a succession of 3 episodes of rigors, fever and sweating, which recur in a regular rhythm. Each episode lasts for approximately 1 hour. The frequency of attacks varies depending on the plasmodium species responsible and is every 2 days for tertiary fever (*Pl vivax* and *Pl ovale*) or every 3 days for quaternary fever (*Pl malariae*).
- **Severe and cerebral malaria:** Neuromalaria is the fatal form of the disease and requires emergency intravenous treatment. It represents 6 to 10% of acute *Plasmodium falciparum* malaria, and more specifically affects children or non-immune adults. Clinical features may develop gradually or suddenly and are a combination of fever of 39 - 40°C, tachycardia, dyspnoea, jaundice, hepatosplenomegaly, frequently renal failure and serious neurological signs including severe headache, seizures and disordered consciousness which may progress to profound coma. Pulmonary oedema is a relatively common but serious complication, responsible for 80% of deaths.
- **Blackwater fever (haemoglobinuria biliary fever):** This is an anaphylactic immune reaction triggered by quinine in a person who has a past history of acute *Plasmodium falciparum* malaria treated with quinine or other antimalarial drugs, such as mefloquine or halofantrin. It involves a sudden onset fever of around 40°C, asthenia, low back pain and then jaundice, vomiting, fall in blood pressure and oliguria with "porto red" urine, due to extensive haemoglobinuria. It requires emergency treatment in specialist care units with renal replacement therapy and exchange transfusion.
- **Malaria and pregnancy:** Immune defences are reduced in pregnancy and the frequency and severity of acute malarial attacks increases affecting both indigenous women and female travellers. The severity of the malaria also depends on the species of plasmodium responsible.
- **Childhood malaria:** Congenital malaria is rare but extremely serious and malaria is an important cause of deaths in children. They are protected up to the age of approximately 3 months by maternal antibodies in endemic areas, but, attacks of malaria are common until adolescence. The symptoms are identical to those in adults with frequent gastro-intestinal problems mimicking gastroenteritis, seizures and even ENT disorders. Cerebral and active visceral malaria are common with *Plasmodium falciparum*.

SEARCH INDICATIONS

Fever in a patient returning from an endemic area. Thick and thin blood films must be performed if the least doubt is present, together with testing for *Plasmodium* antigen. This should include travellers who have taken their anti-malarial chemoprophylaxis. An initial examination must be repeated after 24 to 48 hours if the fever persists, in a primary care or hospital laboratory and particularly during prolonged hospitalisation (orthopaedics, intensive care, etc.).

INFORMATION

SAMPLE

Venous blood collected into a tube with EDTA anticoagulant.

Capillary blood obtained from a finger prick. A minimum of 2 drops are needed for a film and thick smear.

The test is often performed on an emergency basis at the patient's bedside when fever begins or at the peak of a spiked fever.

ESSENTIAL INFORMATION

Clinical symptoms, such as fever, rigors, sweating, headache, gastro-intestinal problems, periodicity of febrile episodes, synchronous or asynchronous?

History of travel, stay in an endemic area within 5 years? Establish the precise place (country) and date of stay?

History of recent transfusion(s)?

Proximity to an international airport?

Chemoprophylaxis?

SAMPLE STORAGE AND TRANSPORT

The sample should be transported urgently to the laboratory.

Serum should be stored at + 4°C.

LABORATORY DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

Direct testing for parasite on a blood film or thick smear:

The conventional technique involves demonstrating haematozoites on a blood film or thick smear stained with May-Grünwald-Giemsa. The species can be diagnosed from the film by distinguishing the different intra-erythrocyte stages of *Plasmodium* and estimating the parasitaemia. The thick film concentrates parasites and therefore increases sensitivity (the limit of detection is in the region of 10 to 20 parasites/mm^3 versus 150 to 200 parasites/mm^3 in a thin film). Species diagnosis, however, is more difficult. The 2 methods are complementary and must be combined.

Other methods:

– Direct identification of the parasite using the QBC malaria test® method, based on centrifugation in a capillary tube and staining with acridine orange to increase the sensitivity of diagnosis.

– Molecular biology: Hybridisation, PCR (*nested-PCR* or *real-time PCR*) are very sensitive, highly specific methods and are useful for low parasitaemias but are not suitable for emergency diagnosis.

– Testing for specific antigens: Rapid immunochromatographic methods (5 minutes) allow *Plasmodium* antigens to be detected. Two examples:

The Binax Now® Malaria test

This demonstrates the HRP-2 or *Histidine Rich Protein* secreted by haematozoites located on the surface of infested red blood cells. This offers high sensitivity for *P. falciparum (96% compared to PCR)*, less for *P. vivax (87%)*, and is inadequate for *P. malariae and P. ovale (67%)*.

HRP-2 antigenaemia may persist for 15 to 30 days (even if the film is negative or if only gametocytes are present) after the malarial attack. Detection therefore provides a retrospective diagnosis.

In practice, it is quick and easy to use and no specific equipment is required. False positives do not occur (rheumatoid factor does not interfere); if the film is negative, a positive rapid test may indicate pauci-parasitaemia, which needs the film to be re-read together with a thick film, hence the merits of combining these two tests. False negatives for HRP-2 are rare with *P. falciparum* (slightly more for the other species, *see above*).

The OptiMAL-IT® test

This tests both pfHRP-2 and pLDH. pLDH is an enzyme secreted by all human plasmodia during their intra-erythrocyte development. It disappears from blood at the same time as the parasites, becoming negative on treatment, unlike HRP-2. pLDH is 92.6% sensitive for *Plasmodium falciparum*.

INDIRECT DIAGNOSIS

Many serological methods are available. These include indirect immunofluorescence, electrosyneresis and ELISA. Antibodies develop 15 days after the first infestation, reaching a peak after 4 to 8 weeks and thereafter falling slowly. Antibodies disappear after treatment, only after approximately a year. For this reason, serology is of no use in emergency diagnosis, although it is useful as a retrospective diagnostic method, in diagnosing active visceral malaria, screening for potentially infected donors or in epidemiology.

NON-SPECIFIC DIAGNOSIS

A blood count shows often late onset haemolytic anaemia, leukocytosis which is more pronounced early on, and early severe thrombocytopaenia, particularly during acute P. falciparum malaria, but also with P. vivax and P. ovale. Hypocholesterolaemia, hypertriglyceridaemia and raised LDH may occur in the acute phase.

TREATMENT

CURATIVE TREATMENT

– The treatment for malaria due to *P. ovale*, *malaria* or *vivax*, is chloroquine.

– IV quinine must be used initially for *P. falciparum* malaria if any vomiting is present. Patients should be assessed for clinical and laboratory signs of severe disease. If these are present, the patient should be hospitalised immediately and treated with IV quinine. If not, the patient can be treated on an outpatient basis, only if the following 10 criteria are met: adult patients, reliable diagnosis, no risk factors for poor adherence, no associated risk factor (isolation, age > 60 years old, underlying disease, splenomegaly, pregnancy, etc.),

proximity to a hospital, immediate availability of the antimalarial prescribed, follow-up possible on D3 and D7, platelet count > 50 000/mm^3, Hb > 10 g/dl, serum creatinine < 150 µmol/l, parasitaemia < 2%. Outpatient treatment with atovaquone + proguanil or artemether + lumefantrine; or quinine or mefloquine second line may be given if all of these criteria are met.

Monitoring with thick and thin blood films must be performed on D3 (the parasitaemia must be below 25% of its initial value), D7 (parasitaemia must be negative) and D28 (recommended because of asymptomatic carriers including Europeans). Daily parasitaemia tests are of no merit.

The WHO criteria for early treatment failure are fever and persistent parasitaemia on D3.

■ PROPHYLAXIS

This involves chemoprophylaxis and simple protective measures, such as the use of domestic insecticides, contact repellents, mosquito nets at night and wearing long clothing impregnated with repellents. Chemoprophylaxis should be appropriate for the resistance regions (information is updated annually).

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MALASSEZIA FURFUR

DEFINITION

Malassezia furfur is a lipophilic, keratinophilic yeast and is a skin and hair follicle saprophyte, particularly in the sebaceous glands. It is mostly responsible for a benign cosmopolitan superficial fungal infection, *pityriasis versicolor.* The major concerns of affected patients are its unaesthetic nature (a rash of brown or achromic blemishes over the upper thorax) and its tendency to recur.

There are many taxonomic controversies about yeasts belonging to the *Malassezia* genus. Since 1961, the *Malassezia* genus has contained two species: *M. furfur*, including both the round forms of *pityriasis versicolor* (formerly called *Pityrosporum orbiculare*) and the oval forms of *pityriasis capitis* or seborrhoeic dermatitis (*Pityrosporum ovale*) and *Malassezia pachydermatis* (formerly *Pityrosporum canis*), the only non-lipid dependent species isolated particularly from healthy or inflamed dog ears.

In 1995, the genus was broadened to 7 species including *M. furfur, M. pachydermatis, M. sympodialis, M. globosa and M. restricta*. These small yeasts (4 to 8 microns) are characterised by repeated unipolar budding giving the budding mother cell a "stirrup" or "small bottle" appearance.

Synonyms: Pityrosporum orbiculare and Pityrosporum ovale (former names).

INTRODUCTION

In 95 to 99% of cases the presence of yeasts belonging to the *Malassezia* genus on the skin represents healthy carriage, including in infants. When the person's underlying situation changes the yeasts proliferate and become pathogenic. Several predisposing factors have been identified, particularly sebaceous oversecretion heavy sweating, use of body oils, weather factors (heat and moisture) and hormonal factors (cortisol excess, oral contraception and pregnancy). A genetic predisposition also exists (family forms are seen).

M. furfur is responsible for *pityriasis versicolor*, a skin disorder preferentially affecting adolescents and young adults of either sex (incidence peak between 20 and 25 years old). Human-to-human transmission is unlikely: possible transmission from beach sand, a relatively commonly held belief in the general public has no basis.

Clinically the basic lesion is a rounded macule with clearly demarcated edges, 2 to 10 mm in diameter. It may be coloured (brown) or achromic; it is not itchy and scales finely on scraping (flake sign). The lesion begins around a hair follicle opening and extends excentrically. It is asymptomatic. The preferred affected sites are the upper thorax, neck and shoulders although any part of the skin can be affected excluding the palms and plantar surfaces of the feet.

Other clinical forms are described, particularly folliculitis or seborrhoeic dermatitis, fine dry scaling of the scalp usually extending to the sebhorreic areas of the face, chest and back as greasy, yellow, adherent scales. Septicaemia has also been reported in premature infants and patients receiving fat rich parenteral nutrition. *M. furfur* is also believed probably to be responsible for urinary infections and vaginitis.

Pathophysiologically the pathogenic action of the yeast may occur as a result of its ability to activate the alternative complement pathway and induce lymphoblast transformation and from a chemotactic effect on macrophages. The achromic skin form blocks the synthesis of melanin and its transfer to keratinocytes, probably due to secretion of a tyrosinase inhibitory factor.

INDICATIONS FOR MEASUREMENT

Lesions suggestive of *Pityriasis versicolor*, seborrhoeic dermatitis, folliculitis for suspected Malassezia septicaemia in a suggestive context *(cf. above)*.

INFORMATION

SAMPLE

Scotch-test (or transparent adhesive cellophane test). A transparent single side adhesive piece of scotch tape is applied to the skin lesions and then applied to a pre-cleaned degreased slide.

Scales are obtained after scratching skin lesions with a curette or vaccine needle.

QUESTIONS FOR THE PATIENT

Are you being or have you been treated with any of the following medicines: azole derivatives, ciclopiroxolamine or terbinafine (antifungal agents active on *M. furfur*), or with oral contraceptives or corticosteroid therapy (predisposing factors)?

SAMPLE STORAGE AND TRANSPORT

Store and transport at room temperature.

DIAGNOSTIC METHODS

Direct microscopic examination of skin scales (lightened with the potassium hydroxide) or Scotch tape applied to lesions between slide and cover slip, possibly after staining (with phenyolic methylene blue).

It may be cultured on Sabouraud medium supplemented with olive oil (1 ml/10 ml) or Dixon medium. This is only rarely used in everyday practice.

Examination of lesions under a Wood's light shows pale yellow-green fluorescence.

INTERPRETATION

The presence of *M. furfur* is suggested from direct examination of skin scales or Scotch tape applied to the lesions showing bunches of round thick walled yeasts and short non-septated filaments.

Yeasts belonging to the *Malassezia* genus do not grow in the synthetic media used for auxanograms and zymograms for carbonated substrates and cannot be distinguished by classical yeast identification methods. When it is cultured on enriched

medium, *Malassezia* colonies usually grow in a few days, optimum growth being between + 32 and 35°C (although *M. furfur* can grow up to temperatures of 41-42°C). All species hydrolyse urea and their precise diagnosis is based on studying their lipid assimilation (Tweens, Cremophor EL®).

M. furfur and probably *M. globosa*, are responsible for skin diseases. *M. furfur* and *M. pachydermatis* can cause septicaemia. *M. pachydermatis* is implicated in canine otitis.

TREATMENT

Local treatment is sufficient in localised forms of the disease; concomitant systemic treatment is useful in diffuse and/or recurrent follicular forms. Different local antifungal agents can be used (imidazoles, ciclopiroxolamine, terbinafine) as 1 or 2 applications per day for 2 to 4 weeks. The systemic treatment which may be associated with local treatment is ketoconazole (200 to 400 mg/d for 10 days). Single dose ketoconazole foaming gel may be useful to prevent relapses.

FOR FURTHER INFORMATION

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■ Guého E., *Malassezia*. In: Annales du contrôle national de qualité n° 4. Agence du médicament, Saint Denis, novembre 1995: 34-6.

MALE SUBFERTILITY–CHROMOSOME Y MICRODELETIONS

DEFINITION

Subfertility is defined as the inability to conceive a child after 2 years of unprotected sexual intercourse. Problems affect approximately 20% of couples of childbearing age. Y deletion is a genetic disorder responsible for 5 to 20% of cases of severe secretory, azoospermia or oligozoospermia in men.

INTRODUCTION

The development of medically assisted pregnancy programmes has brought new knowledge about the mechanisms involved in male subfertility. The fertilising capacity of sperm is assessed using clinical (observation of the testes), radiological (investigation of the vas deferens and epididymis) and laboratory investigations (investigation of endocrine control and sperm quality). Sperm investigations in particular include semen analysis, which specifically establishes the concentration, mobility and morphology of spermatozoa.

The set of investigations performed, reveals a wide range of causes for male subfertility, although the cause remains unknown in 30% of cases. Male subfertility can be caused by anatomical, cytogenetic, genetic or environmental effects.

Abnormalities of the autosomes mostly involve Robertsonnian translocations, reciprocal translocations and inversions. Translocations affecting the Y chromosome and an autosome are also seen and the impact of these on subfertility depends on whether or not the euchromatid region of the Y chromosome is involved.

Cytogenetic abnormalities involving the gonosomes are seen more often in subfertile men. For example, Klinefelter's syndrome affects 1 out of 500 men in the general population, although it is more common in subfertile men.

Genetic factors have recently emerged as being essential for male fertility, although may have deletions or mutations. Several genes are involved in male subfertility. The persistent Mullerian products syndrome is an autosomal recessive genetic disorder associated with mutations of the gene coding for antimullerian hormone (AMH). Kallmann's syndrome is slightly more common with an incidence of 1 in 10000 men and is the most common cause of gonadotropic hormone deficiency in men. Genetic causes can therefore produce functional anatomical abnormalities resulting in obstructive or excretory azoospermia.

Obstructive azoospermia is a relatively common cause of male subfertility affecting approximately 1 out of 2000 births. It may be due to mutations in the CFTR *(Cystic Fibrosis Transmembrane-conductance Regulator)* gene involved in cystic fibrosis. The CFTR gene is located on 7q31.2 and contains 27 exons spread out over 250 kb. More than 700 mutations have been described including the delta F508 mutation which makes up more than 70% of the mutated alleles. CFTR gene mutations may have severe or modest effects depending on its effects on CFTR protein synthesis or function. At least one CFTR gene mutation is found in 50% of patients with bilateral vas deferens agenesis (BVDA). 20% of cases are compound heterozygotes with a serious mutation and a non-serious mutation. The mutations most commonly seen in the Caucasian population are delta F508 and R711H. There is a 5T variant called IVS8-5T, located at the junction of the intron 8 and exon 9 of the CFTR gene. In the presence of the 5T variant, the RNA synthesised does not contain exon 9 on CFTR gene in 63 to 94% of cases on splicing, which causes a reduced number of functional CFTR proteins. In medically assisted pregnancy techniques, the female partner must be screened, as there is a high likelihood of a couple transmitting cystic fibrosis when the woman is a female carrier of a mutation (1/25 risk in the general population). Approximately 1 out of 30 couples consulting for vas deferens agenesis are at risk of transmitting the abnormality to each pregnancy.

Secretory azoospermia constitutes 7% of male subfertility. Recent studies have demonstrated a correlation between interstitial deletions or "microdeletions" in chromosome Y and male subfertility due to secretory azoospermia.

The microdeletions are located in the 5 and 6 intervals of the long arm of chromosome Y, called Yq. This region which is located precisely at Yq11.23 is called the AZF *(AZoospermia Factor)*. This male subfertility factor plays a major role in maintaining and regulating normal spermatogenesis. The AZF factor is subdivided into three functional regions: AZFa, AZFb and AZFc. Development of microdeletions in any of these regions appears to be responsible for variable subfertility phenotypes. Microdeletions however may be found in one or more AZF regions.

AZFa region deletions are associated with a complete absence of germ cells *(Sertoli Cell Only syndrome)* and small testicular volume, suggesting a prepubertal abnormality when spermatogonal stem cells are produced. The genes believed to be involved in this syndrome are DFFRY, DBY and UTY.

AZFb region microdeletions cause azoospermia or severe oligozoospermia. This phenotype is caused by germ cell maturation arrest in the spermatocyte stage, suggesting interruption of spermatogenesis at puberty during or before the myosis stage. The genes present in this region are CDY, XKRY, SMCY and RBMY.

Testicular histology in patients with AZFc region deletions reveals variable spermatogenesis, ranging from the *Sertoli Cell Only* phenotype to a few spermatocytes being present.

INDICATIONS FOR MEASUREMENT

The first stage of diagnosis in a person suspected of being subfertile is semen analysis. If severe oligozoospermia is present the initial investigations to perform are plasma FSH measurement and the blood karyotype. If the karyotype is normal or a large deletion is suspected, molecular analysis of the Y chromosome should be considered for all patients with spermatozoa concentrations of less than 5 millions/ml. Yq screening is recommended in obstructive azoospermia if histological abnormalities are seen in the testicular biopsy.

INFORMATION

SAMPLE

5 ml of whole EDTA blood stored and transported at room temperature.

The clinical information sheet, medical request form and consultation certification must be attached in France.

ASSAY METHODS

Deletions are detected from peripheral blood lymphocyte DNA using the PCR technique. The primers used are specific Y chromosome sequences (*Sequence Tagged Sites*, STSs). The molecular biology method used to test for Y chromosome microdeletions follows the European EMQN *(European Molecular Genetics Quality Network)* quality control recommendations. In this protocol the primers are used sequentially. The first series of primers is used for routine patient screening (two STSs are tested for each AZF region). A second series of primers is used to precisely define the deletion breakage point. The two PCR reactions are used to analyse three AZF regions and two control SRY and ZFY fragments. The STSs markers used to test for Y chromosome microdeletions are sY84 and sY86 (AZFa); sY127 and sY134 (AZFb); sY254 and sY255 (AZFc).

FOR FURTHER INFORMATION

- lst.inserm.fr

MANDELIC ACID - PHENYLGLYOXYLIC ACID

DEFINITION

Mandelic acid (MA) and phenylglycoxylic acid (PGA) are the main metabolites of ethylbenzene and styrene (ethenylbenzene) which are mainly used as solvents in the rubber and plastics industries. Their use is strictly regulated by French occupational health and safety legislation: mean Threshold Limit Value for styrene = 50 ppm; mean Threshold Limit Value for ethylbenzene = 100 ppm).

INTRODUCTION

Ethylbenzene and styrene can be absorbed orally or via the skin and lungs in industrial settings. Both tend to build up in the liver, kidneys and fatty tissues. Styrene and ethylbenzene are mainly broken down in the liver by cytochrome P450 monooxygenases, giving rise to mandelic acid (MA) and phenylglycoxylic acid (PGA) which are then excreted in the urine. MA is eliminated in a two-phase process, the first phase having a half-life of 4 hours and the second one of 18 hours. The half-life of PGA is longer: 7 hours. Virtually all is cleared within 4 days.

INDICATIONS FOR MEASUREMENT

■ STYRENE POISONING

MA and PGA are non-specific indicators of exposure to styrene. The concentrations of MA and PGA in the urine collected at the end of the working day mainly reflect that day's exposure but also, to a lesser extent, exposure over the preceding two days. Ideally, both metabolites should be assayed in parallel because this gives comprehensive information about this metabolic pathway. The results of assays of MA + PGA and of MA alone correlate with exposure levels and also with neurological effects.

■ ETHYLBENZENE POISONING

The urine MA concentration over the last 4 hours of shift at the end of the working week reflects exposure that day and is proportional to the atmospheric concentration.

INFORMATION

■ SAMPLE

100 ml of urine in an unused recipient made of polyethylene or polystyrene to be produced at the end of the working shift.

■ ESSENTIAL INFORMATION

When interpreting the results of urine MA and PGA assays, stock should be taken of alcohol consumption (which decreases the rate of excretion) as well as current medication. The possibility of simultaneous exposure to both ethylbenzene and styrene or exposure to other solvents (such as acetone, toluene, benzene and xylenes) should also be taken into account, because competitive inhibition can reduce the rate of metabolite formation and therefore delay their excretion in the urine.

■ STORAGE AND TRANSPORT

MA is stable for 15 days at +4°C but PGA should be assayed within 48 hours of sampling because it breaks down fast.

ASSAY METHODS

Gas phase chromatography.

High performance liquid chromatography with or without diode array or ultraviolet detection.

REFERENCE VALUES

Reference values for the general population:

– Mandelic acid in the urine < 2.4 mg/g creatinine

– Phenylglycoxylic acid in the urine < 1.3 mg/g creatinine.

■ EXPOSURE TO ETHYLBENZENE:

French guideline and Quebec reference threshold (IRSST): Urinary mandelic acid < 1.5 g/g creatinine at the end of a shift at the end of the working week.

■ EXPOSURE TO STYRENE:

– Urinary mandelic acid:

– French guidelines:

800 mg/g creatinine at the end of the shift

300 mg/g creatinine at the beginning of the shift

– Urinary phenylglycoxylic acid:

– French guidelines:

240 mg/g creatinine at the end of the shift

100 mg/g creatinine at the beginning of the shift.

FOR FURTHER INFORMATION

■ Institut National de Recherche et de Sécurité (INRS). www.inrs.fr

■ *Styrène*, Fiche toxicologique N° 2 de l'INRS (Institut National. de Recherche et de Sécurité). www.inrs.fr

MANGANESE

DEFINITION

Manganese (Mn) is a very hard, steel grey coloured metal which is widespread on the surface of the earth's crust. Plants contain large amounts (seeds, nuts and tea). Manganese has an essential biological role in the human body, due particularly to its involvement in many enzyme mechanisms. Whilst it does not have great acute toxicity, manganese can cause irreversible neurological disorders in chronic poisoning, which may occur in workers involved in extracting and transporting the mineral, in the iron industry, in soldering procedures, manufacturing dry batteries, in the chemical industry (oxidising agent) and when using Mn salts as dyes (in glassware and ceramics).

METABOLISM

The main absorption route for smoke and dust is through the lungs. Gastro-intestinal absorption only plays a secondary role (< 5%).

Organic manganese compounds can be absorbed through the skin. Manganese is widely distributed throughout the body, highest concentrations being found in the liver, kidney, central grey nuclei and endocrine glands. Mn is found mostly within erythrocytes in the blood (at concentrations 20 times higher than in serum). More than 90% of manganese is excreted in bile and then faeces and a very small amount in urine (< 1.5%), sweat and the skin. Elimination is biphasic with half-lives of 4 to 40 days.

MECHANISM OF ACTION

Manganese has a complex effect on cerebral and neuronal activity, where the metal appears to be both necessary and toxic, as the brain is sensitive to both deficiency and overload.

Manganese forms an integral part of some metalloenzymes: arginase involved in urea metabolism, active pyruvate carboxylase in gluco-neogenesis and superoxide dismutase in "oxidative stress".

Its toxic effect is believed to be due to blockade of synaptic transmission at the neuromuscular junction: Mn is believed to reduce the amount of neurotransmitter released in the pre-synaptic space. This action may be both peripheral and central. Manganese is believed to reduce dopamine concentrations in the hypothalamic region. A further proposed neurotoxic effect of manganese is stimulation of oxygen radicals. Finally, manganese has been reported to inhibit adenylate cyclase and stimulate cAMP phosphodiesterase, causing a fall in cAMP in nerve cells.

SYMPTOMS OF ACUTE POISONING

Gastric burning with bloody vomiting and glottal oedema: poisoning is generally accidental by swallowing permanganate.

Chemical inhalation pneumonia in exposed workers: fever, cough, viscous expectoration and occasionally haemoptysis.

SYMPTOMS OF CHRONIC POISONING

Erythematous ulcerative stomatitis, rhinitis with epistaxis and obstructive lung disease.

Neuronal lesions seen in Mn mine workers after exposure, ranging between a few months and 10 or 20 years.

Emotional lability.

Memory disorders and reduced mental faculties.

Fatigue and increased sleep requirement.

Resting tremor, increasing with movements.

Spastic walk with hypertonic spread legs: the patient "walks on tiptoes".

Impotence or, conversely, increased sexual appetite.

Manic-depressive psychosis.

The neuronal symptoms of chronic manganism generally do not resolve and the best that can be hoped for is to arrest their progression by removing the person from exposure.

INDICATION FOR MEASUREMENT

Blood manganese measurements have been proposed for laboratory monitoring of people who have been exposed. Measurement in whole blood is preferred to serum because of the high intra-erythrocyte Mn concentrations. A correlation appears to exist between whole blood measurement and body manganese load.

In theory this test should provide information about whether a group of workers is gradually accumulating manganese over time. It has not been shown to be an individual predictive marker.

Measurement of urinary manganese on a sample taken at "the end of the week at the end of shift" can be useful to confirm recent exposure and correlates (in a group of workers) with the extent of exposure when exposure is high. Values return to normal a few days after exposure ceases.

INFORMATION

SAMPLE

5 ml of whole heparinised blood (whole blood manganese); 2 ml of serum (serum manganese); 10 ml of a non-acidified random urine sample (urinary manganese).

Whole blood, serum or plasma: the sample should preferably be taken at the end of the shift at the end of the week.

Urine: sample taken at the end of the shift at the end of the week.

SAMPLE STORAGE AND TRANSPORT

Whole blood can be stored and transported to the laboratory at room temperature.

Serum plasma or urine samples should be stored and transported to the laboratory preferably between + 2 and 8°C.

ASSAY METHODS

Electrothermal atomisation (graphite furnace) atomic absorption spectrophotometry with Zeeman correction.

REFERENCE VALUES

Reference values in the general population:

– Blood manganese < 15.00 µg/l

– Serum manganese < 4.00 µg/l

– Urinary manganese < 2.00 µg/g of creatinine.

In exposed people, reference German and Swiss values in blood: 20.00 µg/l at end of shift after several shifts.

FOR FURTHER INFORMATION

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■ Guide BIOTOX 2002, INRS.

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MATURE B CELL LYMPHOPROLIFERATIONS (typing)

DEFINITION

Mature B cell proliferations are:

– Proliferations which predominantly present as leukaemia, such as chronic B cell lymphoid blood diseases, the leading one of which is chronic lymphoid leukaemia (CLL-B).

– Monoclonal B cell diseases presenting as lymph node or extra-lymph node tumours, the B lymphomas.

Most are small cell lymphomas which spread into blood (called the leukaemic phase with or without lymphocytosis) present at the time of diagnosis.

The current classification of mature B lymphocyte neoplasias is the WHO *(World Health Organisation)* classification produced in 2001 in which the different entities are recognised by their morphological appearance and their immunophenotypic and genetic features.

Mature B lymphocyte neoplasias	Frequency (if > 5% of all lymphomas)
– chronic lymphoid leukaemia/lymphocytic lymphoma	6.7%
– prolymphocytic B leukaemia	
– lymphoplasmocytic leukaemia/ Waldenström's macroglobulinaemia	
– mantel cell lymphoma	6%
– follicular lymphoma	22%
– lymph node marginal zone lymphoma	
– extranodal marginal zone lymphoma (MALT lymphoma)	7.6%
– splenic marginal zone lymphoma (+ villous lymphocytes)	
– tricholeukocyte leukaemia	
– plasmocytic myeloma / plasmocytoma	
– diffuse large cell B lymphoma • mediastinal large B cell lymphoma • intravascular B cell lymphoma • serosal lymphoma	30.6%
– Burkitt's lymphoma/leukaemia	

INTRODUCTION

■ MATURE B CELL PROLIFERATION WITH PREDOMINANTLY LEUKAEMIC PRESENTATION

■ Chronic lymphoid leukaemia B (CLL-B)

CLL-B is the most common of the adult leukaemias in industrialised countries apart from the Asian continent in which it is rare. It has an incidence of 2.7 per 10^5 people in the United States and Europe.

Cytological appearances: accumulation of a monomorphic population of small lymphocytes in peripheral blood (lymphocytosis of more than 5 x 10^9/l for several months) with a high nucleo-cytoplasmic ratio; the cytoplasm is sparse and pale or slightly basophilic with a regular contour; the nucleus is round and consists of blocks of dark chromatin separated by clear narrow spaces. The nucleolus is not visible. A few cells with a regular or notched nucleus may occasionally be seen; damaged cells and naked nuclei (Gumprecht shadows) are commonly seen particularly when the lymphocytosis is high. The lymphocyte population is sometimes more heterogeneous with larger cells present which have a smaller nucleo-cytoplasmic ratio, an eccentric less dense nucleus containing a characteristic large nucleolus of the prolymphocyte. These cells do not exceed 10% in number.

Clinical appearance: the disease is discovered fortuitously in most cases by a full blood count showing lymphocytosis in an adult in apparently good health (*sex ratio*: 2 men/1 woman). A third of patients are under 60 years old at the time of diagnosis. The blood count is occasionally requested and therefore the diagnosis made after finding lymphadenopathy or splenomegaly on a routine clinical examination.

Clinical course: a significant proportion of patients remain with stable blood disease for several years, although the usual course is a gradual increase in lymphocyte tumour mass, which varies greatly in rate between patients. Prognostic indicators have been researched in an attempt to move the uncertainty about its progress, on which the indications for treatment depend. Prognosis was firstly determined on simple clinical and haematological grounds (lymphadenopathy, splenomegaly, lymphocytosis, haemoglobin and platelet count) which were incorporated into the Rai (1975) and Binet (1981) classifications. The prognostic indicators used now are genomic: degree of mutations of genes coding for the variable parts of immunoglobulin heavy chains (IgVH) and karyotype abnormalities (17p, 11 q and 13 q deletions).

The clinical course of CLL is characterised by complications, the most common of which are episodes of infection due to immunodeficiency or bone marrow failure, autoimmune cytopaenias (particularly affecting the red blood cells and also platelets). One serious risk is transformation into large cell lymphoma (Richter syndrome).

Alongside CLL-B is lymphocytic lymphoma; these two diseases are considered to be different clinical presentations of the same disease. The distinction between lymphocytic lymphoma and CLL-B is based on the lymphocytosis, which is below 10 x 10^9/l in the lymphoma at the time of diagnosis.

■ Prolymphocytic B leukaemia (PLL-B)

PLL-B makes up less than 1% of mature B cell lymphoid blood diseases.

Cytological appearances: the lymphocytosis is usually severe (approximately 100 x 10^9/l). B prolymphocytes are found in blood (at least 55% of lymphoid cells), bone marrow and the spleen. These are large cells with a nucleus containing relatively dense chromatin, a large nucleolus and a low nucleo-cytoplasmic ratio.

Clinical features: in more than 80% of cases patients present with palpable splenomegaly and little or no lymphadenopathy.

Clinical course: PLL-B is usually aggressive.

■ Tricholeukocyte leukaemia (TL)

TL represents 2% of all leukaemias.

Cytological appearances: the lymphoid tumour cells, tricholeukocytes, may be sparse on the blood film; these are large cells with an often excentric oval or rounded nucleus, a large slightly basophilic cytoplasm with fine cytoplasmic projections. The blood count shows pancytopaenia, occasionally only neutropaenia, monocytopaenia, thrombocytopaenia or, occasionally macrocytic, anaemia.

Clinical features: TL occurs more often in men (8 out of 10 cases) over 50 years old and it may be discovered from a routine blood count or as a result of asthenia. TL may more rarely be discovered from infections causing prolonged fever or bleeding secondary to thrombocytopaenia or from clinical signs of anaemia. In 75% of cases patients have splenomegaly without superficial lymphadenopathy.

Clinical course: There is believed to be an increased risk of secondary cancer: multiple myeloma or large granular lymphocyte leukaemia.

■ MATURE B CELL LYMPHOMAS (LYMPH NODE AND EXTRA-LYMPH NODE) WITH HAEMATOLOGICAL PRESENTATION

These lymphomas frequently spread into the blood, producing lymphocytosis.

The cellular proliferation involves small or medium sized lymphoid cells with an irregular nucleus.

■ Follicular lymphoma

Cytological appearances: it is rare for this lymphoma to have spread into blood at diagnosis, which is therefore based on a lymph node biopsy. The tumour is formed from small (centrocytes) and/or large (centroblasts) centrofollicular B lymphoid cells.

Clinical features: follicular lymphoma is an indolent lymphoma generally occurring in adults between 55 and 60 years old with a moderate male predominance. It usually presents as a result of disseminated superficial lymphadenopathy and rarely with skin or gastro-intestinal disease. The patients are generally clinically well.

Clinical course: a major risk of progression is transformation into diffuse large B cell lymphoma.

■ Mantel cell lymphoma

Cytological appearances: this lymphoma commonly spreads into the blood causing lymphocytosis. The proliferation involves small or medium sized lymphoid cells with irregular nuclei.

Clinical features: this lymphoma often occurs in people over 60 years old and is usually generalised from the outset with disseminated lymphadenopathy, frequently splenomegaly and systemic signs.

Clinical course: this is an aggressive chronic lymphoproliferative syndrome which is progressively resistant to chemotherapy and has a median survival time of less than 3 years.

■ Marginal zone lymphomas

Classical marginal zone lymphoma

Cytological appearances: spread into the blood is common, although often modest without lymphocytosis. The absence of cytological features makes this difficult to distinguish between other diseases: lymphocytic: lymphoma /CLL, follicular lymphoma and mantel cell lymphoma.

Clinical features: patients usually present with clinical or ultrasound evidence of splenomegaly.

Clinical course: it remains indolent for long periods of time.

Villous lymphocyte splenic lymphoma (VL SL)

Cytological appearances: in three quarters of cases the blood count shows a chronic absolute lymphocytosis of more than 4 x 10^9/l and the presence of lymphoid cells (villous cells) with a dense chromatin and polar villi in variable percentages (more than 20% according to some authors). Anaemia and thrombocytopaenia may be present. Nodular disease is present in the white pulp and sometimes in the red pulp of the spleen.

Clinical features: this blood disease occurs in people over 60 years old and is characterised by pronounced splenomegaly without lymphadenopathy.

Clinical course: it has a relatively long survival (median 5 to 10 years) although without prolonged complete remission.

Lymph node marginal zone lymphoma and MALT (mucosal associated lymphoid tissue) lymphoma

Spread to the blood is rare or even extremely rare in the latter at the time of diagnosis.

INDICATIONS

Testing for mature B cell proliferation is usually performed for persistent lymphocytosis, whether or not accompanied by lymphadenopathy and/or splenomegaly.

INFORMATION

■ SAMPLE

The sample is taken into an EDTA tube. When transported, 10 ml of whole EDTA blood should be sent within 24 hours after sampling together with the result of the full blood count performed on the day of venipuncture.

■ QUESTIONS FOR THE PATIENT

Do you have a high white cell count? If yes, for how long?

Do you have palpable lymph nodes?

Do you feel heaviness in your flanks, on the left?

Are you tired?

ASSAY METHODS

Mature B lymphoid proliferations are identified by a panel of monoclonal antibodies which identify normal mature B (CD19, CD20) and pathological (CD5, CD23, FMC7, CD10…) populations. The general principle of lymphocyte labelling is the same for all monoclonal antibodies tested: whole blood is incubated with a monoclonal antibody and the red cells are lysed. The leukocyte suspensions are analysed in a flow cytometer after creating a region of interest around the lymphoid population.

INTERPRETATION

The phenotypes of chronic B lymphoid blood diseases (from H. Merle-Béral) are:

marker	CLL	PLL	HCL	VLSL	MCL	FL	MZL
Intensity Ig surface	weak	strong	strong	medium	strong	medium / strong	medium / strong
CD19	+	+	+	+	+	+	+
CD5	+	+/-	-	+/-	+	-	-
CD23	+	-	-	+/-	-	+/-	-
CD10	-	+/-	-	-	-	+/-	-
CD22	-	+	+	+	+	+	+
CD79b	-	+	+	+	+	+	+
CD25	+/-	-	+	-	-	+/-	+/-
CD11c	+/-	-	+	+	-	+/-	+/-
CD103	-	-	+	-	-	-	-

CLL: chronic lymphoid leukaemia
PLL: prolymphocytic leukaemia
HCL: tricholeukocyte leukaemia
VLSL: villous lymphocyte splenic lymphoma
MCL: mantel cell lymphoma
FL: follicular lymphoma
MZL: marginal zone lymphoma

In CLL, the Matutes score can be established depending on whether the immunological markers are positive or negative by allocating points and adding the points together using the following rule:

marker	points	
	1	0
Ig surface intensity	weak	Moderate / strong
CD5	+	-
CD23	+	-
FMC7	-	+
CD79b / CD22	Weak / -	Moderate / strong

CLL is defined by a Matutes score of 4 or above. A score of 3 generally represents "atypical CLL". Scores of less than 3 characterise the other chronic B cell lymphoproliferative syndromes. A score of less than 3 formally excludes the diagnosis of CLL.

Some phenotypic markers (such as CD38) may be of prognostic relevance, although results for these markers do not all agree.

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MEASLES

DEFINITION

The measles virus belongs to the *Paramyxoviridae* family and *Morbillivirus* genus. It is antigenically stable and only one serotype is known. It is a single chain, negative polarity, RNA virus measuring between 120 and 250 nm in diameter with a helicoidal capsid surrounded by an envelope. Despite vaccination coverage in France, measles still affects unvaccinated children and adolescents and usually results in a benign infection with a rash. It may on the other hand be severe with many neurological complications in developing countries, where it is still the leading cause of death in children between 2 and 5 years old.

Synonyms: measles virus.

INTRODUCTION

EPIDEMIOLOGY

The measles virus is responsible for epidemics which occur every 2 to 5 years in non-immunised populations, particularly in children. Since a vaccine has been available the incidence of the disease has changed and the infection occurs later, affecting adolescents and occasionally young adults in isolated outbreaks. It is endemic with peaks in winter and spring.

PATHOPHYSIOLOGY AND SYMPTOMS

After aerial transmission, the virus multiplies locally in rhino-pharyngeal epithelial cells and in the lymphoid organs.

A primary viraemia which occurs around day 5 after infection is responsible for the spread of the virus to reticuloendothelial cells. Destruction of lymphocyte-monocytes by the virus results in leukopaenia. The second viraemia causes the infection to generalise to all tissues and organs, particularly the skin and mucosal membranes. Infection induces transient immunosuppression, the mechanism of which is poorly understood.

- **In the common form:** After an incubation period of approximately 10 days, measles presents with oculo-naso-bronchial catarrh and fever. The characteristic rash develops a few days later as an enanthema on the interal surface of the cheeks (Köplick's spots) and maculo-papular facial exanthema which generalises very rapidly. Patients usually recover over a few days.
- **Complications:** Respiratory (measles pneumonia) and particularly neurological.

Of the neurological complications:

– *Acute post-infectious encephalitis* is the commonest complication. This occurs fairly rapidly after the rash and is due to an autoimmune process directed against cerebral tissue. It is associated with seizures and disordered consciousness.

– *Acute progressive encephalitis* occurs later, after primary infection and is seen in immunosuppressed children.

– *Sub-acute sclerosing panencephalitis (SSPE)*, is a rare complication (1 per 1 million cases) and is a degenerative encephalitis which develops a few years after the measles and which is invariably fatal. It does not appear to have an associated immunodeficiency and its mechanism is poorly understood.

Respiratory complications are mostly due to bacterial superinfections causing otitis or laryngitis, related to the transient immunosuppression, particularly in deprived populations. Bronchopulmonary or pulmonary complications occur more rarely.

SEARCH INDICATIONS

Investigation of the cause of acute or progressive encephalitis or meningoencephalitis.

Atypical rash in an unvaccinated child or adolescent.

Differential diagnosis with rubella or Parvovirus B19 infection.

In epidemiological studies to establish immunity in a vaccinated population or the immune status of at risk people.

INFORMATION

SAMPLE

Nasopharyngeal or bronchial secretions or urine for direct detection and culture of the virus.

Serum (1 ml) for serological diagnosis.

CSF for neurological complications.

QUESTIONS FOR THE PATIENT

History of contact with an infected person?

Vaccination?

Immune status?

Clinical symptoms?

SAMPLE STORAGEAND TRANSPORT

Nasopharyngeal or bronchial samples are stored and transported at + 4°C.

Swab samples require viral transport media.

Serum should be stored and transported at + 4°C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

- **Direct detection of viral antigens:** This is performed by immunofluorescence using monoclonal antibodies in pathological specimens (respiratory secretions, urine centrifugation pellet, etc.). Antigens can be detected at the time of the rash and for up to 6 days afterwards.
- **Cell culture:** This is difficult to perform. It is however useful epidemiologically and provides an unequivocal diagnosis of measles. The virus is identified using specific monoclonal antibodies directly on a culture cell smear before the cytopathogenic effect develops.
- **Molecular biology:** RT-PCR is used on CSF in acute measles encephalitis and also epidemiologically to identify the genome of circulating strains of the virus.

INDIRECT DIAGNOSIS

IgM develops in serum at the time of the rash and lasts for a month afterwards. IgG can be detected a few days after the IgM and remain throughout life.

The serology is very useful in the diagnosis of common measles virus infection when IgM or seroconversion is found, or from a significant rise in IgG titre in 2 samples taken at 15 days apart. It is also very useful, however, in early neurological complications when IgM can be detected. High titres of IgG are found in serum and CSF in SSPE.

The most widely used methods are immunoenzymatic (ELISA) as these can distinguish between IgG and IgM and can quantify IgG.

Other methods including immunofluorescence, the complement fixation reaction and haemagglutination inhibition are available.

INTERPRETATION

- **Direct detection of antigens by immunofluorescence** can be used on samples taken at the time of the rash as the virus is present in the upper respiratory tract and in urine in the acute phase of the disease. It is used particularly for respiratory and neurological complications and in atypical forms of the disease.
- **Cell culture methods and RT-PCR** can be used to monitor the types of strains circulating for epidemiological purposes.
- **Serology** is useful in common measles. Serology can only be interpreted when the person has not recently been vaccinated. If they have, testing for specific IgG should be requested for an immune control.

  Laboratory results must always be interpreted in light of all of the clinical and epidemiological findings.

TREATMENT

There is no specific curative treatment for measles.

On the other hand, prevention of infection and complications is achieved by administration of a live attenuated combined vaccine intended for children from the age of 12 months onwards, with a second injection between 3 and 6 years old. The possibility of eradicating measles which would only occur if it were worldwide and had been intended by 2007 by the WHO is now questioned.

FOR FURTHER INFORMATION

- Gouarin S, *le virus de la rougeole*, Encycl. Med. Chir., Elsevier Paris, 2003.
- Société française de microbiologie, *Virus de la rougeole*, référentiel en virologie médicale; chapter 21: p 91-92.

MEPROBAMATE

DEFINITION

Meprobamate is a psychotropic medicinal product, the head of the carbamate class. It is still currently the only commercially available representative of this class, together with felbamate which has been used since 1995 as an anti-epileptic drug.

Meprobamate has anxiolytic, sedative and myorelaxant actions. It acts by inhibiting thalamic, hypothalamic and cortical synaptic circuits and reduces rhinencephalic post discharges. It is marketed as 250 or 400 mg tablets and is indicated for use in the treatment of mild to severe anxiety states and in the treatment of painful reflex contractures. It is also available in powder form for intramuscular injections (400 mg/5 ml vial) for the treatment of acute anxiety or agitation states, pre-delirium and *delirium tremens*, as premedication for endoscopy and minor procedures (pre and post-operative station) and in the treatment of painful reflex contractures. It is often used in practice as an anxiolytic or sedative-hypnotic and is preferred to the benzodiazepines in people with a past history of medical drug abuse.

In France it is responsible for approximately 5% of the cases of psychotropic agent poisoning and often raises severe problems because of its cardiovascular complications.

PHARMACOKINETICS

Plasma peak (Tmax)	1 to 3 hours **NB:** intra-gastric masses may form containing undissolved tablets producing a late absorption effect.
Protein binding	20%.
Metabolism	Hepatic through hydroxylation (10%) and glucuronide conjugation (65%).
Plasma half-life of elimination	6 to 16 hours.
Elimination	Approximately 90% in urine (10% in the unchanged form); 10% in faeces.

INDICATIONS FOR MEASUREMENT

– As an aid to acute meprobamate poisoning in a suicide attempt: colourimetric screening methods are available for qualitative purposes and quantitative methods to determine the extent of poisoning and guide treatments.

Clinical symptoms of overdose are drowsiness with inebriation and muscle hypotonia, coma, usually flaccid, respiratory failure and circulatory insufficiency. In severe poisoning, meprobamate has direct cardiac toxicity through a negative inotropic effect.

NB: The formation of gastric masses results in a triphasic progression of symptoms with coma, short duration wakening (< 12 hours) and then recurrence of coma after bowel transit has returned and the substance is reabsorbed.

– Suspected chronic poisoning because of side effects or signs of toxicity. The major undesirable effects are daytime drowsiness particularly when treatment is started, neurological disorders (headaches, dizziness, etc.), general allergic reactions (bronchospasm, anuria) and agranulocytosis (rare).

INFORMATION

■ SAMPLE

Serum or plasma taken into EDTA or heparin; avoid tubes with separator gel.

Take the sample immediately before the next dose (trough concentration) for suspected chronic poisoning or when signs or overdose develop for acute poisoning.

■ QUESTIONS FOR THE PATIENT

What is the context of the measurement (suspected chronic overdose, acute unintentional poisoning or attempted suicide)? If possible; amount taken, date and time of dose(s) and other substances taken at the same time? Alcohol and other central nervous system depressants (benzodiazepines, sedative antidepressants, antihistaminergic agents, morphine derivatives, neuroleptics, etc.) increase the central depression.

■ SAMPLE STORAGE AND TRANSPORT

Store separated plasma/serum for up to a few days at + 4°C and for several months at – 20°C.

Transport at – 20°C if assay is to be deferred.

ASSAY METHOD

Screening methods in various biological fluids (plasma, serum, gastric fluid, urine) are colorimetric methods.

Quantitative assay: gas phase chromatography coupled to mass spectrometry or FID detection.

NORMAL EXPECTED VALUES

Usual therapeutic range: 10 to 20 mg/l.

In acute poisoning, plasma/serum concentrations correlate well with severity. The conventional toxic threshold is 40 mg/l. Patients usually remain awake at concentrations of up to 50 mg/l; between 60 and 120 mg/l they are in a light coma. The risk of profound coma emerges when values exceed 100 mg/l and cardiovascular complications when over 150 mg/l.

In the absence of a specific antidote the treatment of acute poisoning is with removal methods (gastric lavage, administration of activated charcoal), management of cardiovascular disorders (vascular filling, dobutamine) and extra-renal removal: extra-renal removal is indicated in patients in a very severe clinical state with plasma/serum meprobamate concentrations of ≥ 200 mg/l.

FOR FURTHER INFORMATION

■ Dictionnaire Vidal®.

■ Delahaye A., Baud F.J., Lamiable D., Capolaghi B., *Carbamates anxiolytiques*, Encycl Med Biol, Elsevier, Paris, 2003.

■ Lavit M., Saivin S., Houin G., *Le méprobamate en toxicologie d'urgence*, Rev Fr Lab 2000 ; 322: 61-3.

MERCURY

DEFINITION

Mercury (Hg) is grouped within the heavy metals and has no physiological role. It is a liquid metal at room temperature with a density of 13.6 and is the only known metal to be liquid at 0°C. Mercury sulphate or cinnabar is most widely found in raw form.

The main sources of exposure are firstly from mercury metallurgies and processing factories in producing countries and secondly the use of mercury in the manufacture of high precision scientific instruments (thermometers, manometers, etc.), in the electrical industry (mercury vapour lamps, current rectifiers and interrupters, industrial control instruments and fluorescent tubes), in caustic soda and chlorine production factories which use a mercury cell and in the preparation of dental amalgams. Calomel (HgCl) was used many years ago in pharmacy as an anti-syphilis agent. Mercuric chloride ($HGCl_2$) or mercuric iodides (HgI_2) are external antiseptics. Mercuric nitrate ($Hg\ (NO_3)_2$) is used in the hat industry to produce felts from combining hairs. Mercury ammonium is also used in whitening skin creams. Hg fulminate ($Hg\ (CNO)_2$) is used in the manufacture of explosive detonators. Red mercury oxide (HgO) is used in the manufacture of protective paints for hulls of ships. Organic mercury compounds are used in agriculture as insecticides, fungicides and bactericides, in the paper and paints industry and in some cosmetics and disinfectants (throat preparations, creams and soaps).

INTRODUCTION

Occupationally, the main route by which metallic mercury enters the body is through the respiratory tract after inhaling vapours. Mercury evaporates at room temperature (80% of inhaled mercury is absorbed in the alveoli). Subcutaneous absorption is an accidental route of entry for metallic mercury, the main victims of which are nurses (skin injury from a broken thermometer). Although to a limited extent, metallic mercury and some organic compounds may be absorbed through the skin (poisoning from creams and soaps). 2 to 10% of an oral dose is absorbed. The three routes of entry, pulmonary, cutaneous and oral may all occur for organic compounds. After entering the body, metallic mercury (Hg°) is rapidly oxidised into the mercuric ion (Hg^{++}) which binds to blood and tissue proteins. The mercuric ion however is exchangeable, explaining the effectiveness of chelating agents in removing inorganic mercury from the body, except for that which has already bound to the brain.

In blood, mercury derived from inorganic mercury salts is equally distributed between plasma proteins and red blood cells, whereas mercury derived from organo-mercurials binds particularly to red blood cells, onto the haemoglobin SH groups.

Following intravenous, subcutaneous and oral administration of inorganic compounds, mercury accumulates preferentially in the kidney, in the proximal convoluted tubule and loop of Henlé. In the same way as cadmium, mercury is believed to induce synthesis of a protective transport protein provided that its binding capacity is not exceeded. Inhalation of mercury vapours, however, results in 10 times higher concentrations of metallic mercury in the brain, which is then preferentially found in the grey matter Purkinje cells. Inorganic mercury is excreted by the colon and kidneys and a small percentage by the skin, integument and in saliva. The use of mercury amalgam in dental care may cause transient rises in urinary mercury excretion which do not represent any danger to the patient.

MECHANISM OF ACTION

The mercuric ion binds to SH groups and therefore interferes with the activity of many enzymes and co-enzymes which contain this group: in the same way as lead, mercury inhibits delta aminolevulinic acid dehydratase and interferes with the haem synthetic pathway. Mercury also inhibits protein synthesis in neuronal cells. Methyl mercury has been shown to have a catalytic effect on membrane phospholipids causing membrane lysis. Similarly, methyl mercury has antimitotic action, inhibiting micro-tubule polymerisation and interfering with spindle formation during mitosis.

SYMPTOMS

■ ACUTE POISONING

This is very rare industrially, from inhaling mercury vapours in poorly ventilated spaces. It usually occurs as a result of ingesting large amounts of mercurial compounds either accidentally or in attempted suicide with mercuric chloride ($HgCl_2$) which is better absorbed by the gastro-intestinal tract than metallic mercury.

■ **General symptoms:**

Acute gastro-enteritis, stomatitis and ulcerative, haemorrhagic colitis, vomiting and salivation.

Anuria with uraemia due to renal tubular necrosis

Acute pulmonary oedema from inhaling high concentrations.

■ **Local symptoms:**

Dermatitis from mercury fulminate.

Irritation from HgCl2, Hg (NO) 2 and HgI2.

Skin allergies are seen in dentists.

■ CHRONIC POISONING

The features of chronic mercury poisoning begin insidiously:

– Gingivitis and stomatitis (bleeding and mercury line on the gums)

– Central nervous system damage with tremor of the fingers, eyelids, tongue and lips

– Disorders of character, personality and psychomotor performance

– Peripheral nervous system damage, such as sensory motor polyneuritis

– Renal damage: classical nephrotic syndrome

– Deterioration in general health, diarrhoea and cachexia being common.

INDICATIONS FOR MEASUREMENT

Measurement of inorganic blood mercury at the end of the week, at the end of the work shift, is a good indicator of mercury exposure in the previous week and correlates well with atmospheric concentrations. This is also useful in accidental acute exposure.

Measurement of total urinary inorganic mercury can be used to assess longer-standing exposure (over 3 months) to inorganic mercury and correlates well with the extent of exposure. This is the parameter which correlates best with effects on health. No physiological, psychological or biochemical abnormalities are usually seen if urinary concentrations are less than 50 µg/g of creatinine.

Overall, for long standing constant exposure, measurement of urinary mercury is preferable, ideally after exposure for 6 months as urine concentrations reach a steady state between 10 days and 6 months; blood mercury measurement is preferable for fluctuating exposure and for laboratory monitoring of methyl mercury, which is removed mostly in faeces.

INFORMATION

■ SAMPLE

5 ml of whole EDTA or heparinised blood, preferably taken at the end of week and at the end of the shift.

20 ml of an unacidified, random urine sample without mercurial antiseptic (avoid sample contamination). A sample taken at the beginning of the shift is preferable to one at the end of shift in the context of environmental contamination.

■ QUESTIONS FOR THE PATIENT

Ask about consumption of fish and shellfish, which are rich in organic mercury that interferes with measurement of total mercury.

Also ask about dental amalgams which may contain mercury that interferes with interpretation, particularly in low level exposure. To overcome the various interferences from organic mercury, speciation measurements may be performed which separate organic from inorganic forms.

■ SAMPLE STORAGE AND TRANSPORT

Whole blood samples can be stored and transported to the laboratory at room temperature.

Urine samples can be stored and transported to the laboratory at + 2 and + 8°C.

ASSAY METHOD

Electrothermal atomisation atomic absorption spectrophotometry (graphite furnace) with Zeeman correction.

Mass spectrometry coupled with plasma torch (ICP-MS).

REFERENCE VALUES

– Whole blood inorganic mercury:

- In the general population < 5 µg/l
- In exposed people < 15 µg/l at the end of shift, end of week (laboratory index of exposure).

– Total urinary inorganic mercury:

- In the general population < 5 µg/g of creatinine
- In exposed people (before the beginning of the shift; French guideline value) < 50 µg/g of creatinine

Biological exposure indices (ACGIH BEI): 35 µg/g of creatinine before shift.

FOR FURTHER INFORMATION

■ Toxicologie industrielle et intoxications professionnelles, Mercure, Lauwerys. R., 3rd edition, Masson; p170-181.

■ Guide BIOTOX 2002, *Mercure organique et inorganique*, INRS.

METHADONE

DEFINITION

Methadone is a synthetic opioid and an opiate receptor agonist which acts principally on mu receptors. It has analgesic and antitussive properties and is a sedative after accumulating, following repeated use. It has a low pro-euphoric action, although high induced dependency. It is used therapeutically as substitution treatment for major opiate drug dependency as part of the overall medical, social and psychological management (in the same way as buprenorphine). It is marketed as a syrup containing the racemic mixture of methadone isomers, the levo isomer being the most active and the most toxic. Administration of methadone under medical control is initially combined with systematic urine testing to confirm that illegal drugs have not been taken and regular toxicology follow-up. For equivalent efficacy, buprenorphine has the advantage of causing a far less severe withdrawal syndrome, whereas long-term use of methadone produces prolonged morphine type dependency. Methadone is still used however, as the syrup form avoids other misuse.

The dispensing conditions are strictly regulated in France: secure prescription limited to 7 days in specialist centres for drug addicts, then possibly under the supervision of a primary care doctor. The treatment is started at low dose, 20 to 30 mg/day, and then increased gradually to 40-60 mg over 1 to 2 weeks. Maintenance treatment is then reached after increasing the dose by 10 mg/week at around 60 to 100 mg/day, occasionally to 200 mg/day in highly dependent people.

PHARMACOKINETICS

Plasma peak (Tmax)	4 hours
Plasma protein binding	60 to 90% to albumin and to other tissue proteins explaining its cumulative effects, slow rate of elimination and high tissue concentrations.
Plasma ½ life	8 to 30 hours after single dose increasing up to 40 hours during long term treatment; large inter-individual variation.
Metabolism	Main metabolite: inactive; EDDP (2 ethylidene-1,5 dimethyl-3,3-diphenylpyrrolidine) and EMDP (2-ethyl-5-methyl-3,3 diphenylpyrroline), Methadone, EDDP and EMDP are then partially hydrolysed and glucuronide conjugated. Small amounts of highly active metabolites (alpha1-methadol and alpha 1-1-normethadol) are also formed.
Elimination	Mostly in urine (methadone, EDDP and EMDP).

INDICATIONS FOR MEASUREMENT

– ***Methadone poisoning,*** , the main causes of which are taking excessive amount of methadone (low therapeutic margin), taking methadone after a period without using it (reduced habituation), accidental ingestion by a child (syrup form) or association with other central nervous system depressants (heroin, benzodiazepines, alcohol, etc.). The clinical picture of poisoning is stupor, myosis, bradypnoea and then respiratory depression, pulmonary oedema, drowsiness followed by coma with hypotension bradycardia and apnoea which may be fatal.

– ***Monitoring substitution treatment*** by urine analysis (screening), 1 to twice per week: testing for methadone associated with possible testing for other narcotics (cocaine, cannabis, LSD, amphetamines, etc.).

INFORMATION

SAMPLE

Urine screening: random urine sample (40 ml sample).

Measurements in serum for treatment monitoring (steady state after treatment for 5 days). Samples taken at peak (4 hours post-dose) and trough (immediately before the next dose), or testing for poisoning or confirmation of a positive urine screening test.

QUESTIONS FOR THE PATIENT

Any request for drug measurement must include the reasons for requesting (testing for efficacy or toxicity), sampling time, date of starting treatment and/or any change in dosage, dosage information (amount administered, frequency and route of administration), together with age, height and weight of a subject, wherever possible.

SAMPLE STORAGE AND TRANSPORT

Store urine at +4°C for up to two to three days and then at –20°C for 1 year. Serum can be stored for 48 h at +4°C. Transport frozen at – 20°C.

ASSAY METHOD

Urine screening: Immunochemistry and immunoezymatic (positivity threshold 0.3 mg/l by the EMIT method).

Specific confirmatory methods in blood and urine: Gas phase chromatography linked to mass spectrometry or a nitrogen phosphorous detector, HPLC coupled to mass spectrometry, etc.

NORMAL EXPECTED VALUES

Urine screening: negative if methadone is not taken, positive if the substitution treatment is being taken. Methadone can be detected in urine for 72 hours post-dose.

Therapeutic concentrations in serum in substitution treatment:

The target therapeutic range for methadone is between 400 and 500 µg/l, in a blood sample taken immediately before the drug is administered, at steady state. This concentration range generally suppresses withdrawal signs.

– A trough methadone concentration of less than 200 µg/l is generally associated with ineffective treatment.

– A trough methadone concentration of greater than 700 µg/l requires increased clinical monitoring because of the risk of overdose.

– For reference, the ratio between trough EDDP and methadone concentrations is generally in the region of 0.05 to 0.15.

– Peak concentrations (4 hour post-dose): 600 to 1000 µg/l (signs of toxicity may develop above this).

Methadone can be detected in serum for 24 to 48 hours post-dose.

INTERPRETATION

Individual methadone sensitivity varies between people. Toxic concentrations described in serum are > 1000 µg/l. Post mortem serum methadone concentrations of 0 to 3000 µg/l (mean 1200 µg/l from 21 cases) and EDDP of 0 to 0.4 µg/ml (mean 0.1 µg/ml in 10 cases) have been described in the literature.

The management of poisoning involves treatment of respiratory depression and naloxone (specific antidote).

FOR FURTHER INFORMATION

■ Pepin G., Cheze M., *Opiacés et produits de substitution*, Encycl Med Biol, Elsevier, Paris 2003.

■ Pepin G., Dubourvieux N., Cheze M., *Opiacés et opioïdes : usages, effets, dépistage, dosage*, Revue française des laboratoires 2000; 322 : 41-5.

■ Pepin G., *Opiacés et opioïdes*. In : Toxicologie et pharmacologie médicolégales, coordinateur P. Kintz, Collection Option/Bio, Elsevier Ed, Paris, 1998: 373-9.

■ Dictionnaire Vidal®.

METHOTREXATE

DEFINITION

Methotrexate is a cytostatic anti-neoplastic agent belonging to the anti-folate group. It is marketed in the form of 2.5 mg tablets (Methotrexate Bellon®, Novatrex®), 5, 25, 50 or 500 mg injectable solution (Ledertrexate®, Methotrexate Bellon®) or freeze-dried powder for parenteral use containing 1 g (Ledertrexate®).

Methotrexate is indicated for use in the treatment of placental choriocarcinoma, breast and ovarian adenocarcinoma, small cell lung carcinoma and upper respiratory, gastro-intestinal carcinomas, bladder cancers and at high dose in the treatment of childhood acute lymphoblastic leukaemia, non-Hodgkin's lymphoma and osteosarcoma.

The usual oral dose is 10 to 15 mg/m² as a once weekly dose, although doses vary depending on the protocol and must be adjusted according to clinical response and haematological tolerability.

Parenterally (subcutaneous, intra-venous, intra-muscular and intra-arterial), methotrexate is prescribed at very different doses of between 15 mg/m² and 3 g/m²/d up to 12 g/m² as a weekly course in osteosarcoma. High doses (≥ 1 g/m²) are only administered in a hospital setting in association with sequential administration of folic acid and under forced alkaline diuresis cover. Administration of moderate doses (100 mg to 1 g/m²) requires a minimum of oral hydration; it is recommended that folinic acid be added 6 and 24 hours after the methotrexate to reduce its toxic effects.

Methotrexate toxicity is mostly haematopoietic (thrombocytopaenia, neutropaenia, more rarely anaemia, agranulocytosis or pancytopaenia), renal (increased serum creatinine) and hepatic (increased transaminases).

It is essential that the full blood count is checked and that patients are tested for possible renal or hepatic insufficiency before each dose of methotrexate.

Synonyms: names of proprietary products: Methotrexate Bellon®, Novatrex® and Metoject®.

PHARMACOKINETICS

Bioavailability (oral)	Variable absorption, generally good at doses of < 30 mg/m², incomplete at doses of > 80 mg/m²
Plasma peak (Tmax)	• After oral administration: 1 to 2 hours after administration of low doses, 4 hours after administration of high doses • After IV, IM injection: approximately 30 minutes
Time to steady state	One week for daily administration; never for weekly administrations (or less frequent)
Protein binding	50 to 60% to albumin
Metabolism	• After oral administration: approximately 35% of methotrexate is metabolised • After IV administration: less than 10%
Plasma half-life	2 hours
Elimination	Urinary: 60 to 90%; biliary < 20%

INDICATIONS FOR MEASUREMENT

Therapeutic drug monitoring of methotrexate is recommended because of the large inter-individual variability in the pharmacokinetics of the drug and plasma or serum concentrations may vary by a factor of 1 to 7 after the same dose in different patients. Monitoring is particularly useful when it is used in association with cyclosporin (reciprocal reduction in the clearance of both drugs) and sulphonamide antibiotics (displacement of plasma protein binding) in people with reduced hepatic or renal function and in the elderly. Therapeutic drug monitoring can be used to confirm that the dosage is effective without reaching the toxic range. It also allows doses to be better adjusted.

In the treatment of childhood ALL and osteosarcoma, a relationship has been demonstrated between the methotrexate exposure and the effectiveness of treatment. A randomised study in children suffering from type B ALL showed a significant increase in complete remission rate in the group which underwent therapeutic drug monitoring.

Finally, repeated trough plasma/serum methotrexate concentrations are useful to determine the dose and duration of folinic acid rescue treatment.

INFORMATION

■ SAMPLE

Serum or EDTA or heparinised plasma; potassium oxalate or sodium fluorate may be used.

Avoid tubes with separator gel.

Take the sample at steady state after treatment for approximately 1 week (for daily administration).

The sampling time varies depending on the protocol and treatment used; for reference, a sample may be taken 24 hours after the end of the infusion.

■ QUESTIONS FOR THE PATIENT

Are you taking any other medical treatment?

Association with the following medical treatment may cause an increase in toxic effects (particularly haematological) of methotrexate, in parallel to a rise in plasma serum concentrations:

– Probenecid (contra-indicated association), penicillins: due to reduction of renal tubular MTX secretion

– Trimethoprim (contra-indicated association): by displacement of plasma protein binding and reduced renal excretion

– Salicylates (contra-indicated association), non-steroidal anti-inflammatory drugs, sulphonamide antibiotics: due to displacement of plasma protein binding

– Phenylbutazone (contra-indicated association), cyclosporin: by reduced renal MTX clearance.

Any request for drug measurement must include the reasons for the request (testing for efficacy or toxicity), the sampling time, treatment start date and any change in dosage, dosage information (amount administered, frequency and route of administration), together with the age, height and weight of the person, wherever possible.

SAMPLE STORAGE AND TRANSPORT

Centrifuge and separate the sample promptly.

Separated plasma/serum is stored at + 4°C. Transport at + 4°C, preferably away from light.

ASSAY METHOD

Immunological methods: EMIT, FPIA and RIA.

High performance liquid chromatography (HPLC).

NORMAL EXPECTED VALUES

Conversion factor: 1 mg/l = 0.454 µmol/l.

Toxicity thresholds have been established and vary depending on the indication and treatment protocol used. As an indication, in the treatment of childhood ALL, a plasma/serum methotrexate steady state concentration (after administration of a bolus followed by a 24 hour infusion) > 16, or even 20 µmol/l, appears to be associated with a reduced risk or relapse.

In addition, the dosage and duration of folinic acid treatment are adjusted according to trough plasma/serum methotrexate concentrations.

Dosage of folinic acid in mg/d depending on trough plasma/serum methotrexate (MTX) concentrations (from Marquet P, Elsevier 2004). Folinic acid is administered as 4 divided daily doses.

Time (hours) MTX (mol/l)	24 h	48 h	72 h	> 72 h
> 10^{-5}	4 x 50	4 x 200	4 x 200	4 x 200
5.10^{-6} to 10^{-5}	4 x 20	4 x 100	4 x 200	4 x 200
10^{-6} to 5.10^{-6}	4 x 20	4 x 50	4 x 100	4 x 200
5.10^{-7} to 10^{-6}	4 x 20	4 x 20	4 x 50	4 x 100
10^{-6} to 5.10^{-7}	4 x 20	4 x 20	4 x 20	4 x 50
5.10^{-8} to 10^{-7}	4 x 20	4 x 20	4 x 20	4 x 20
< 5.10^{-8}	0	0	0	0

*Time = time since last dose of methotrexate.

FOR FURTHER INFORMATION

- Dictionnaire Vidal®.
- Marquet P., Carpentier M., Milano G., Thyss A., Favre R., *Suivi thérapeutique du méthotrexate. In: Suivi thérapeutique pharmacologique pour l'adaptation de posologie des médicaments*, Collection Option/Bio, Ed Elsevier, Paris, 2004: 223-35.

METHYL ALCOHOL

DEFINITION

Methyl alcohol (methanol) is used in industry as a solvent, particularly in products intended for cleaning (windscreen washer fluid) or degreasing, as a thinner for paints and varnish and at household level, it is used as an antifreeze and degreaser. Methanol is a component of methylated spirit and is widely employed in organic synthesis.

The methanol molecule is only slightly toxic, but its principal metabolite, formic acid, is severely toxic to the eyes and nervous system and can be fatal. In most cases, poisoning occurs accidentally, for example due to inadequate awareness of toxicity and home production of alcoholic beverages, etc.

Synonyms: methanol (methanolaemia), "wood spirits" (so named because once obtained from the distillation of wood).

PATHOPHYSIOLOGY

After ingestion, methanol is rapidly absorbed by the gastrointestinal tract, with a plasma peak obtained in 30 to 90 minutes. Absorption can also occur through the skin and lungs and may produce poisoning in children or in workers exposed to the product. Approximately 95% of methanol is metabolised in the liver into formaldehyde by the action of alcohol dehydrogenase. The formaldehyde is itself rapidly converted to formic acid. A small proportion (2% to 5%) of methanol is eliminated unchanged through urine and the lungs.

The half-life for the elimination of methanol is from 2 to 3 hours for low plasma concentrations, rising rapidly to some 30 hours if the concentration is high. It also rises as far as 30 to 52 hours during treatment of methanol poisoning by ethanol, which acts by competing with alcohol dehydrogenase has higher affinity for ethanol, which inhibits the metabolisation of methanol and thus its toxicity).

From the clinical point of view, acute methanol poisoning manifests itself within 2 hours of ingestion by inebriation, dizziness and digestive problems, followed within 12 to 24 hours by ocular effects, neurological problems (optical neuritis and encephalopathy) and coma, with metabolic acidosis and convulsions. Cases of blindness have been reported following ingestion of 4 ml of the pure product; death can occur after the absorption of approximately 15 ml of a 40% solution of methanol.

From the pathophysiological viewpoint, the acute toxicity of methanol is essentially that of its principal metabolite, formic acid, whose accumulation is the cause of the metabolic acidosis. The mechanism of ocular toxicity remains poorly understood.

INDICATIONS FOR MEASUREMENT

Search and confirmation of acute methanol poisoning, prompted by questioning the patient and their contacts when faced with ocular problems, and/or metabolic acidosis with an increase in the anion gap.

Monitoring the treatment of methanol poisoning with ethanol.

INFORMATION

SAMPLE

Collect in a dry tube (serum) or a tube with EDTA, heparin or sodium fluoride anticoagulant (plasma). Do not use a tube containing a gel phase separator.

QUESTIONS FOR THE PATIENT

Try to find out the circumstances of poisoning: Product ingested, time elapsed since ingestion, workplace contact, etc.

SAMPLE STORAGE AND TRANSPORT

Store sample at +4° C for a few hours only then freeze to -20° C.

Transport the sample at -20° C.

ASSAY METHODS

Gas phase chromatography (GPC).

NORMAL EXPECTED VALUES

In the absence of intoxication, methanolaemia is below the detection threshold of the technique used.

Conversion factor: 1 mg/l = 32 x mmol/l

INTERPRETATION

A diagnosis of acute methanol poisoning is confirmed by measuring plasma methanol and (if appropriate) formic acid, by GPC or enzymatic methods better correlated with the toxicity of the product. The toxicity of methanol is greater than that of an equal concentration of ethanol, due to its metabolism. The first stage of poisoning, characterised by slight inebriation, may be deceptive due to a false resemblance to ethanol intoxication.

Treatment is symptomatic (correction of acidosis by bicarbonates) and uses an antidote: ethanol (see above) or 4-methyl pyrazole (4MP or fomepizole), another powerful alcohol dehydrogenase inhibitor, administered intravenously or orally. Joint treatment with folates (cofactor, in the form of tetrahydrofolates, of the conversion of folic acid) is useful.

Recourse to haemodialysis is common, due to the fact that after administration of the antidote, methanol metabolism is blocked and renal elimination is weak. Haemodialysis increases the excretion of methanol and of the toxic metabolites formed. It is generally initiated if the plasma methanol concentration exceeds 0.5 g/l and is maintained at least until it falls below 0.25 g/l.

FOR FURTHER INFORMATION

■ Latrech B., Tournoud C., *Produits domestiques*, Encycl Med Biol, Elsevier, Paris 2003.

■ Lamiable D., Hoizey G., Marty H., Vistelle R., *Intoxication aiguë au méthanol*, Revue Française des laboratoires, 2000; 323: 31-34.

MICROALBUMIN

DEFINITION

Microalbuminuria is defined as a urinary albumin excretion of between 30 and 300 mg/24h for a normal urine output, or between 20 and 200 µg/minute in an early morning urine sample. It therefore refers to measurement of albumin in urine at a concentration which is not detected by conventional chemical assay methods or screening methods for proteinuria (test strip).

An increase in microalbuminuria reflects an intermediary stage between normal albumin excretion and overt pathological proteinuria. It is pathological if permanent, i.e. found to be positive (> 20 µg/min or > 30 mg/24 hours) in at least two tests out of three performed over a period of 6 months.

Microalbuminuria was initially described as a predictive marker for progression of diabetic nephropathy in type 1 diabetes and is also a marker for endothelial dysfunction. Nowadays, it is increasingly being considered to be an independent marker of cardiovascular risk in the general population.

Synonym: pauci-albuminuria.

PATHOPHYSIOLOGY

Physiological urinary albumin excretion depends on:

– Size: 69 kDa, approximately the same as the size of the glomerular endothelial pores

– Its charge: albumin is a polyanion, which has lower clearance than neutral or cationic molecules because of the negative charge on the glomerular membrane

– Haemodynamic conditions: blood pressure, renal plasma flow

– Tubular reabsorption: albumin is normally 95% reabsorbed in the proximal tubule and is then catabolised.

In practice, very little albumin passes out physiologically in the urine. Raised urinary albumin secretion reflects an abnormality of the glomerular wall and/or intra-glomerular pressures, due to deterioration in glomerular permeability as a result of raised systemic blood pressure, reduced pre-glomerular resistance in chronic hyperglycaemia or a rise in post-glomerular resistance in response to angiotensin II.

The finding of microalbuminuria in a type 1 diabetic patient is the first laboratory indicator of micro-angiopathic renal damage with a risk of progression to renal failure if not treated. Early nephropathy is characterised by increased glomerular infiltration and permanent microalbuminuria; overt proteinuria then develops with a fall in glomerular filtration and systemic hypertension.

As in the general population, in type 2 diabetics, microalbuminuria is above all an indicator of increased cardiovascular risk, and a risk albeit less, of progressing to renal failure.

It is particularly important to detect any increase in albuminuria early at a subclinical stage of renal damage as we now have effective treatments to slow progression of diabetic nephropathy and reduce cardiovascular risk in these patients.

INDICATIONS FOR MEASUREMENT

Screening and monitoring patients at risk of developing nephropathy. Those at risk of renal damage are diabetic patients (types 1 and 2), hypertensives, people with monoclonal dysglobulinaemia, systemic disease (systemic lupus erythematosus, amyloidosis and sarcoidosis) or heart failure. It is recommended that microalbuminuria be measured annually as part of the monitoring of diabetic patients.

INFORMATION

SAMPLE

A 24 hour urine collection is preferable but not mandatory, in view of the difficulties in obtaining this type of sample. Alternatives are a collection of a first morning sample (= overnight urine) or random sample (combined measurement of albumin and creatinine in a urine sample). The use of the albumin/creatinine ratio removes the need for a 24 hour urine collection and corrects for some "physiological variations" such as increased microalbuminuria after physical activity.

Ideally, the measurement should be performed during a stable metabolic period. Not during menstruation in women, in the absence of severe dyslipidaemia, acute intercurrent disease, urinary or gynaecological infection, acute decompensation of diabetes or cardiac decompensation. These situations can artificially increase microalbuminuria ("false positives").

QUESTIONS FOR THE PATIENT

Are you diabetic?

Are you taking medicines? Drugs which reduce microalbuminuria include the converting enzyme inhibitors and angiotensin II receptor antagonists.

SAMPLE STORAGE AND TRANSPORT

Store for up to 1 week at between + 2 and + 8°C and for several months at –20°C.

Transport at + 4°C, preferably in a glass container (which avoids adsorption of albumin onto the plastic).

ASSAY METHOD

Immunological assays: Nephelometry, turbidimetry, immuno-enzymology and radioimmunoassay.

NORMAL EXPECTED VALUES

Usual values in the general population:

< 15 µg/min.

Usual threshold value:

20 µg/min or < 30 mg/24 hours.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Large intra-individual variability in microalbuminuria is seen (25 to 60%, depending on the collection method used); for this reason three measurements are recommended over a period of between 1 and 6 months before confirming that microalbuminuria is permanent.

Microalbuminuria is also increased in healthy people by smoking, alcohol, physical activity and a protein-rich diet.

PATHOLOGICAL VARIATIONS

The term microalbuminuria refers to a urine albumin excretion rate of between 20 and 200 µg/min or between 30 and 300 mg/24 hours. Values above these levels reflect macroalbuminuria or clinical proteinuria.

- **In diabetes:** Microalbuminuria is present in 20 to 40% of diabetic patients. 30% to 40% of type 1 diabetics and approximately 5% of type 2 diabetics (who are far greater in number) progress to chronic end stage renal failure. Nephropathy generally develops in these patients after their diabetes has been present for 10 to 20 years.

  In type 1 diabetes, microalbuminuria mostly reflects early subclinical glomerulopathy. As the disease progresses it becomes a marker of interstitial fibrosis. Its presence is associated with a 10 fold increase in cardiovascular risk.

  In patients with type 2 diabetes, a rise in microalbuminuria is associated with classical cardiovascular risk factors (obesity, hypertension and hyperlipidaemia), which is more common, develops earlier and is more serious than in non-diabetic patients. Above all it predicts excessive coronary mortality (three fold increased risk of death over 10 years) and to a lesser extent a risk of progressing to chronic renal failure.

- **In hypertensive patients:** The prevalence of microalbuminuria in hypertensive patients is between 5 and 25% depending on the study, although it is not possible to say whether this is an early manifestation or the cause of early nephropathy. The extent of microalbuminuria correlates with blood pressure and left ventricular hypertrophy, which itself is an indicator of increased cardiovascular mortality.

- **Other diseases:** In the general population, microalbuminuria is increasingly considered to be an independent cardiovascular risk marker.

  A fall in microalbuminuria between results obtained 8 hours after admission and day 5 after admission in patients hospitalised in intensive care with multi-organ damage predicts a good outcome.

  Microalbuminuria is the first laboratory sign of renal damage due to circulating immune complex deposition in the glomerulus in patients with auto-immune disease such as systemic lupus erythematosus.

  In pregnant women, a rise in microalbuminuria is a sign of pre-eclampsia.

- **Treatment of early nephropathy:** Patients found to have permanent microalbuminuria can be treated with converting enzyme inhibitors or angiotensin II receptor antagonists. Studies with these substances have mostly been conducted in diabetic patients and show that they have a specific nephroprotective effect, reduce microalbuminuria and lower blood pressure. Their long term effects are still to be established.

FOR FURTHER INFORMATION

- Giraudeaux V., Zerbani A., *Microalbuminurie (paucialbuminurie)*, Cahier de formation Biochimie, tome IV, Bioforma, Paris, 1999: 125-31.
- Fonfrede M., *Microalbuminurie*, Encycl Med Biol, Elsevier, Paris 2003.

MICROSPORIDIOSIS

DEFINITION

The microsporidia are eukaryotic protozoa which belong to the Microspora phylum, the taxonomy of which has changed many times. More than 140 genera are currently identified, seven of which affect human beings: *Enterocytozoon, Encephalitozoon, Microsporidium, Nosema, Pleistophora, Trachipleistophora,* and *Vittaforma*. The two most common species in human disease are *Enterocytozoon bieneusi* and *Encephalitozoon intestinalis*. They are small unicellular parasites with a nucleus and nuclear membrane but no mitochondria; their growth is therefore strictly intracellular.

The main part of the microsporidia is the spore. This is what passes the infection between cells and from one host to another and the diagnosis is usually made from its presence in various biological samples.

Synonyms: Enterocytozoon bieneusi and Encephalitozoon intestinalis.

INTRODUCTION

EPIDEMIOLOGY – SYMPTOMS

Microsporidia spores are widespread throughout nature; they are highly resistant and remain infectious for several months. A large number of genera infest invertebrates (for example, *Nosema bombycis* is a microsporidium responsible for pebrine, a silkworm disease) and vertebrates, although the mode of infestation remains poorly understood. The microsporidia cause various infections in human beings (depending on the species, target organ and patient's immune status) the commonest sites being intestinal. A microsporidium was first identified in human disease in 1985: I. Desportes-Livage described a case of chronic diarrhoea due to *Enterocytozoon bieneusi* found in intestinal biopsies of a patient suffering from AIDS. A second microsporidium, *Encephalitozoon intestinalis*, was then described in 1992. This was also responsible for extra-intestinal disease (cholangitis, peritonitis and disseminated infections). The microsporidioses mostly affect immunosuppressed patients, particularly AIDS patients at an advanced stage of immunosuppression and to a lesser extent transplant or cancer patients. *E. bieneusi* is found in the intestinal epithelium (apical enterocyte pole), causing destruction of the mucosa and villous atrophy; *E. intestinalis* infects the intestinal epithelium and the crypts, and may extend to the rectum, liver, maxillary sinuses, kidneys and urinary tract (it can be found in urine).

Clinically, the intestinal microsporidia cause progressive diarrhoea which becomes chronic. The diarrhoea is watery without mucus or blood and with 4 to 10 bowel movements per day. It is worsened by food and accompanied by sugar and fat malabsorption and occasionally by nausea and vomiting.

The prevalence of intestinal microsporidia in diarrhoea of HIV infected patients is between 6 and 30%; their incidence has fallen greatly since the use of antiretroviral treatments. They are found throughout the whole world and studies also describe the parasites being found in diarrhoea in immunocompetent travellers.

SEARCH INDICATIONS

Main indication: The development of diarrhoea in a patient with severe immunosuppression. Mostly in patients with HIV infection and a lymphocyte count of less than 100 CD4/mm^3; to a lesser extent in other immunosuppressed patients, such as cancer and transplant patients.

Secondary indications: Unexplained diarrhoea in an immunocompetent patient, extra-intestinal disease in an immunosuppressed patient (cholangitis, cholecystitis, bronchitis, sinusitis, rhinitis, keratoconjunctivitis and disseminated infection).

INFORMATION

SAMPLE

Testing for intestinal microsporidiosis is performed on a stool sample.

Testing for extra-intestinal microsporidiosis may be performed in various samples, such as urine, pleural fluid, sputum, nasal secretions, duodenal aspiration fluid and cerebrospinal fluid.

Intestinal biopsies are used above all for histology.

QUESTIONS FOR THE PATIENT

Are you HIV seropositive?

Are you taking antiretroviral treatment and/or anti-diarrhoeal treatment? Albendazole is effective on diarrhoea caused by *E. intestinalis*; fumagillin and nitazoxanide are effective on diarrhoea caused by *E. bieneusi*.

SAMPLE STORAGE AND TRANSPORT

Store and transport at + 4°C.

DIAGNOSTIC METHODS

Direct parasitological examination of stools identifying parasite spores:

– After using the trichrome stain (Weber technique and variants).

– After staining with chemofluorescent agents (Uvitex 2B is the most widely used).

Both methods are often combined. PCR confirmation of microsporidiosis is recommended in equivocal or positive results.

Molecular biology: PCR identification of DNA using generic primers (confirmation of the diagnosis of microsporidiosis) or specific primers (for species diagnosis). Nowadays, gene amplification is replacing electron microscopy which was previously used to confirm the diagnosis and identify the species in question.

INTERPRETATION

The diagnosis is based on finding the parasite on direct examination.

– After staining by the Weber technique, the microsporidia spores are round or ovoid, measuring 1.5 to 2 microns in diameter, pink satin stained with fuchsia, with a colourless vacuole and often containing a dark red point at one pole of the parasite, on a green or blue background depending on the counter stain used.

Differential morphological diagnosis with other enteropathogenic parasites described in a similar clinical context *(from Himy R and Molet B, Annales CNQ n° 15)*

	Microsporidia	Cryptosporidia	Cyclospora	Yeasts
Size *(microns)*	1 to 3.5	5 to 7	8 to 10	2 to 10
Colour of parasite (Weber)	pink to red	very pale pink	dark red	pink, dark violet to blue
Shape	ovoid	rounded	spherical	round, oval or rectangular
Content	specific excentric colourless vacuole	Naked sporocysts, slightly darker	rough granulations	granular with vacuoles
Casing	moderately visible	poorly visible, clear surrounding halo	distinct	very distinct, thick

– On Uvitex 2B staining, the spores appear ovoid with blue fluorescence on a black background (fluorescence microscopy). This method is not specific (it also stains yeasts, bacteria and even small residual particles). It is, however, more sensitive than the Weber technique.

The microsporidium species is identified by gene amplification techniques.

FOR FURTHER INFORMATION

- Miegeville M., *Microsporidia*, Encycl Med Biol, Elsevier, Paris, 2003.
- Deluol A.M., *Microsporidioses*, Feuillets de Biologie 1997; XXXVIII (214): 43-6.
- Himy R., Molet B., *Microsporidia*. In : Annales du contrôle national de qualité n° 15. Agence du médicament, Saint Denis, 1998: 23-5.

MOLYBDENUM

DEFINITION AND SYNONYMS

Molybdenum is a transition element with an atomic weight of 95.94, which has oxidation states between + 3 and + 6. It is present in the crust of the earth in the form of sulphides MoS_2 (molybdenite) or lead molybdate MoO_4Pb (wulfenite). Molybdenum is extracted from molybdenite.

Molybdenum is used in the manufacture of special steels (reactor turbines, industrial tools and gun barrels, etc.) and in the production of tungsten and non-ferrous alloys. Its oxides, hydroxides and salts have various industrial applications. It has been described as having a moderate anti-cariogenic effect in medicine.

All foods contain molybdenum. The richest in content are split peas, beans and cereals (pastas and bakery products). Molybdenum is a co-factor in several metallo-enzymes: sulphite oxidase, xanthine oxidase, aldehyde oxidase, nitrate reductase and formate dehydrogenase and is considered to be one of the least toxic metals.

INTRODUCTION

The human body contains approximately 10 mg of molybdenum, mostly contained in the liver, kidneys, adipose tissue, adrenal glands and bone. Daily dietary intake is between 50 and 120 µg, 30 to 40% is provided from cereals. Some authors recommend systematic supplementation for premature infants.

Molybdenum absorption depends on the solubility of its compound. Water soluble hexavalent compounds are well absorbed through the gastro-intestinal (rapidly and fully) and respiratory tract. High concentrations of copper and tungsten inhibit absorption. In the blood, molybdenum is present mostly in the form of molybdate ions and is carried to the liver by erythrocytes. In the form of hexavalent ions, molybdenum is not bound to any plasma proteins, whereas the pentavalent ions bind to α_2-macroglobulin. Molybdenum is stored in the liver and kidney. Other tissues may store it when intake is high (adrenal glands, gonads and bones). It is mostly removed by the kidneys (rapid excretion: 70% of the ingested dose over three hours, 90% in 24 hours) and in faeces. Large amounts of copper and sulphates in the diet increase urinary molybdenum excretion. A small proportion is excreted in sweat.

INDICATIONS FOR MEASUREMENT

Molybdenum deficiencies are seen mostly in patients with gastro-intestinal disease (Crohn's disease and ulcerative colitis). The risk of deficiency may be increased in these patients by total parenteral nutrition, as blood molybdenum concentrations are reduced in parenteral nutrition patients. Conversely, concentrations are increased in some, particularly hepatobiliary, diseases.

Molybdenum poisoning is rare because of its low toxicity and almost exclusively involves occupational poisoning. Acute poisoning is extremely rare. Chronic poisoning, which is also rare, causes lung disorders (pneumoconiosis, restrictive lung disease and radiological opacifications), laboratory abnormalities in workers exposed to molybdenum dust who develop joint pains associated with high caeruloplasmin concentrations and allergic skin reactions. Molybdenum excess is believed to predispose to copper deficiency by reducing copper absorption. Urine molybdenum measurements are preferred to blood measurements for laboratory monitoring of exposed people. Urinary concentrations appear to correlate well with extent of exposure.

INFORMATION

SAMPLE

Fresh blood is taken by venepuncture from the antecubital fossa following usual laboratory recommendations. A 5 ml EDTA tube is required for measurement. For urine samples, an end of shift sample is recommended.

QUESTIONS FOR THE PATIENT

Is the patient suffering from gastro-intestinal disease?

Is the patient exposed to molybdenum at work? If yes, in what form?

Does the patient have joint pain or skin problems?

SAMPLE STORAGE AND TRANSPORT

Whole blood at room temperature or at + 4°C.

Urine at + 4°C.

ASSAY METHOD

Several methods are offered for molybdenum measurement (colorimetric, neutron activation, polarography and plasma torch). In practice, the reference method is atomic absorption spectrometry: the method is sensitive and can be used without sample pre-treatment when molybdenum concentrations are in the region of 5 to 20 µg/l. For measurements in biological fluid it is recommended that the spiked additions method be used. The limit of detection is in the region of 0.1 µg/l. Urine measurements must be expressed at a ratio to urine creatinine.

NORMAL EXPECTED VALUES

Usual values in the general population in urine are ≤ 150 µg/g of creatinine.

No reference values are defined in France for plasma.

PATHOPHYSIOLOGICAL VARIATIONS

Molybdenum deficiency is exceptionally rare in human beings, the occasional cases described being due to exclusive prolonged parenteral nutrition.

Concentrations of molybdenum below the values usually found may be seen in some inflammatory gastro-intestinal diseases, (Crohn's disease, ulcerative colitis) or gastro-intestinal resection.

FOR FURTHER INFORMATION

- Fiche Biotox : *Molybdène*. www.inrs.fr

MUMPS

DEFINITION

Mumps virus is classically responsible for a highly contagious and usually benign disease in children. The classical clinical presentation is with parotitis (more rarely orchitis or pancreatitis), although it can also cause meningeal disease (meningitis and encephalitis). These clinical features have greatly reduced in France with the generalisation of the MMR (measles-mumps-rubella) vaccination campaign since 1989.

Mumps virus belongs to the *Paramyxoviridae* virus family, the *Paramyxovirinae* sub-family and the *Rubulavirus* genus. Only one serotype exists.

The virus measures 200 to 400 nm in diameter and has a single chain, negative polarity RNA. It is enveloped and has a symmetrical helocoidal nucleocapsid.

Synonym: Mumps virus.

INTRODUCTION

EPIDEMIOLOGY

Before vaccination was available, mumps infection was endemic with a few epidemic outbreaks affecting communities of children and young adults.

The incidence of the disease has fallen markedly since the introduction of systematic vaccination. Human-to-human transmission occurs through the respiratory tract from pharyngeal secretions.

PATHOPHYSIOLOGY AND SYMPTOMS

After entering the body, the virus replicates in respiratory tract epithelial cells (incubation period) and then reaches the lymph nodes and meninges through the blood stream. The virus multiplies in these target organs, causing a secondary viraemia. It is excreted in saliva and urine.

The average age of contracting mumps is between 2 and 9 years old. The person is contagious for a period between the 3rd day before clinical signs appear until the 4th day after they have appeared.

The infection is asymptomatic in 20% of cases and half of mumps infections are associated with non-specific or respiratory clinical signs.

The classical form of the disease is parotitis, and represents 30 to 40% of cases of mumps.

A very wide range of clinical features exist and serious complications can occur, particularly in adults.

The classical form of the infection

The average incubation period is in the region of 16 to 21 days.

The invasive period which corresponds to primary viraemia, is an association of a fever spike with general malaise, asthenia and anorexia, lasting approximately 3 days.

The established disease phase is dominated by bilateral and more rarely unilateral parotitis (present in 90% of symptomatic cases) accompanied by a moderate fever. The disease resolves in around approximately ten days, without complications.

Parotitis however is not a constant finding and other clinical features may present before, during, after or in the absence of parotitis, such as extra-salivary gland or neuromeningeal involvement.

Of the extra-salivary gland involvement:

– ***Mumps orchitis*** develops in pubertal boys in 20% of cases. The risk of testicular atrophy is low but existent (< 10%). The orchitis usually follows a benign course over around ten days. Sterility may however occur in bilateral disease.

– ***Ovaritis*** is rarer (5% of cases; presenting in pubertal girls).

– ***Pancreatitis*** is seen in less than 10% of cases.

– ***Thyroiditis, prostatitis, mastitis, myocarditis and renal disease*** are very rare complications.

Of the neuromeningeal involvement:

– ***Lymphocytic meningitis*** is the commonest complication of a mumps infection in unvaccinated patients. It presents in isolation or may precede the parotitis and resolves without complications.

– ***Meningoencephalitis*** is rare and more serious.

SEARCH INDICATIONS

The diagnosis of acute lymphocytic meningitis in a young patient, in order to identify the virus responsible. The mumps virus is one of the main viruses responsible for acute lymphocytic meningitis after Enterovirus.

Aetiological diagnosis of a rare complication.

To define the immune status of a person after vaccination.

INFORMATION

SAMPLE

Pharyngeal swab, salivary, CSF or a urine sample (for direct testing for the virus).

Serum (1 ml) for serological diagnosis.

QUESTIONS FOR THE PATIENT

Age?

History of contacts?

Clinical signs?

Vaccination?.

SAMPLE STORAGE AND TRANSPORT

Samples taken for direct testing require a transport medium and should be transported at +4°C to the laboratory.

CSF must be transported as soon as possible to the laboratory, failing which, it should be frozen.

The serum should be stored and transported at + 4°C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

- **Direct testing for viral antigens** by immunofluorescence (IF) is performed on a cell smear, usually taken from rhinopharyngeal samples in view of the poor sensitivity.
- **Cell culture** is performed from salivary, throat, urine or CSF samples. This may be complemented by IF detection of viral antigens on a culture cell smear.
- **Gene amplification:** RT-PCR methods on CSF are reserved for neuromeningeal disease.

INDIRECT DIAGNOSIS

IgM develops very early (from the 2nd or 3rd day after the onset of clinical signs) and persists for a few weeks to a few months. IgG develops in the acute phase of the disease reaching high titres and persisting throughout life. Intrathecal production of IgG and IgM antibodies occurs in mumps meningitis.

The methods available are:

Complement fixation reactions (CFR): This method has poor sensitivity particularly in young children.

The haemagglutination inhibition reaction: This also lacks sensitivity.

The immunoenzymatic method: This is very easy to perform and is the most widely used. It is currently replacing the above two methods. It is the most sensitive method and can distinguish between IgG and IgM classes of antibodies. On the other hand, cross antigenic reactions occur, particularly with *parainfluenza* virus.

NON-SPECIFIC DIAGNOSIS

Raised serum amylase (an almost constant finding at the start of the disease).

Moderate blood lymphocytosis.

INTERPRETATION

In classical forms of disease, when a contact history is present and particularly with characteristic parotitis, the diagnosis of mumps is purely clinical.

Laboratory diagnosis is essential in atypical forms and in meningeal disease. This is mostly ***serological*** and involves detecting the presence of specific IgM in serum when clinical signs first appear, or finding a significant rise in IgG titres in 2 different samples. In mumps meningitis, IgM and IgG may be found in cerebrospinal fluid. Molecular biology is useful in the diagnosis of mumps infections with meningeal complications.

TREATMENT

The treatment of mumps is symptomatic.

Prevention relies on effective vaccination. The vaccine available is a live attenuated vaccine which has been used since 1999, the RIT 4385 strain. Combined measles, mumps, rubella vaccination is recommended for all children from 12 months old with a second dose at between 3 and 6 years old.

FOR FURTHER INFORMATION

- Gouarin S., *Virus des oreillons*, Encycl Med Biol (Elsevier Paris) 2003.
- Guerin N., *Actualités sur les vaccins Rougeole, Rubéole et oreillons*, Rev Fr Lab October 2000; 326: 41 to 47.

MYCOBACTERIA

DEFINITION

The genus *Mycobacterium* belongs to the order Actinomycetales and constitutes the only genus of the family *Mycobacteriacae*. Mycobacteria are straight or slightly-curved bacilli from 1 to 10 μm long and 0.2 to 0.6 μ wide; they are nonmotile, and do not produce spores, conidia, bifurcated hyphae or capsules. They do not take Gram colouration but are acid-alcohol fast.

The classification of species within the *Mycobacterium* genus has evolved greatly, notably due to the appearance of molecular biology techniques. The Runyon classification system defines 4 groups among the mycobacteria, on the basis of culture characteristics (growth time on solid media) and morphological features (pigmentation of colonies). Normal practice distinguishes between tuberculous mycobacteria, which are without exception pathogenic and have their reservoirs in humans or certain mammals *(Mycobacterium tuberculosis complex)* and non-tuberculous mycobacteria, still referred to as atypical, which are present in the environment and are not necessarily pathogenic. The latter include M. *avium*.

Synonyms: Tuberculosis bacillus/Koch bacillus (KB).

INTRODUCTION

TUBERCULOSIS BACILLI

Tuberculosis is a worldwide affliction which particularly occurs in developing nations. Each year, 8 million people develop tuberculosis and 2 million die as a result. The incidence of the disease has diminished greatly in developed countries, although the rate of reduction has fallen since 1986, largely as a result of the pandemic of HIV infection. In addition, multiresistance phenomena are increasing around the world. Tuberculous mycobacteria are invariably pathogenic. The main species included in *Mycobacterium tuberculosis complex* are *M. tuberculosis, M. bovis, and M. africanum*, which are responsible for human tuberculosis.

Symptoms

Tuberculosis is a chronic necrotising infection which is usually spread through the air. The primary infection is generally asymptomatic, then the bacilli remain in a latent condition. In 5 to 10% of cases, however, the bacilli multiply again and lead to an extension of the infection, most often to the lungs. Pulmonary signs are then an early, persistent, productive cough with bouts of coughing. The general signs are combined persistent mild fever (38°C) accompanied by profuse night sweats, anorexia and asthenia. Acute pulmonary forms may occur, as can complications such cavitation and an evolution towards pleurisy. Other observed localisations involve the kidneys, lymph nodes, urogenital system, articulations, pericardium, peritoneum, etc. Immunodeficient subjects, children and the elderly may develop serious generalised forms of tuberculosis or tubercular meningitis.

NON-TUBERCULOUS MYCOBACTERIA (NTM)

These are mainly ubiquitous micro-organisms found in water or soil. They are present in the commensal state but can become opportunistic and pathogenic in susceptible subjects with immunodeficiency (AIDS, cancer and immunosuppressive treatments), chronic obstructive bronchitis or tuberculosis. The most frequently pathogenic species are *M. xenopi, M. avium-intracellulare* and *M. kansasii*.

Other species may be incriminated, including *M. malmoense, M. szulgaï, M. asiaticum, M. simiae*, etc

Symptoms

There are various clinical features: NTM infections are mainly respiratory, with clinical symptoms closely resembling those of active tuberculosis. Other observed clinical forms include:

Systemic forms in subjects with an acquired cellular immunodeficiency (AIDS, cancer or anticancer chemotherapy) or, more rarely, a congenital immunodeficiency. The systemic form in AIDS occurs in the terminal stage of the illness in patients with $CD4^+$ T-cells < 100/μl and is most often due to *M. avium-intracellulare*.

Adenitis is seen in young children and is often localised in the cervix. The species most frequently found are above all *M. avium-intracellulare*, but also *M. kansasii, M. malmoense, M. scrofulaceum, and M. szulgaï*.

Cutaneous and subcutaneous conditions: The species most frequently isolated in France is *M. marinum*. Contamination occurs when swimming pool or aquarium water comes into contact with an existing wound, which becomes infected and eventually forms a granuloma. Two other species are incriminated as causing similar conditions and these are *M. ulcerans* in tropical regions and *M. haemophilum*, which is responsible for certain disseminated cutaneous lesions, chiefly in immunodeficient subjects. Subcutaneous abscesses forming in infected wounds or bites are caused by fast-growing mycobacteria, mainly *M. fortuitum, M. peregrinum, M. abcessus*, and *M. chelonae*.

Post-traumatic or post-operative osteoarticular conditions are caused by *M. avium-intracellulare, M. kansasii, M. xenopi, M. scrofulaceum, M. fortuitum, M. peregrinum* or *M. terrae*.

SEARCH INDICATIONS

– Diagnosis of tuberculous disease when suggested by clinical signs in non-vaccinated children, the elderly, immigrants from countries with a high incidence of endemic tuberculosis, immunodeficient subjects and medical personnel in frequent contact with tuberculosis patients.

– Diagnosis of infection by non-tuberculous mycobacteria in the presence of varied lesions in a patient with deficient cellular immunity.

– Diagnosis of mycobacteriosis in connection with epidemiological investigations.

– Monitoring efficacy of treatment.

INFORMATION

SAMPLES

Samples are the same for tyberculous and non-tuberculous mycobacteria and depend on the pathology and its localisation.

Pulmonary samples

– Sputum should be collected after a coughing effort from a subject who has previously washed out their mouth with water. A volume of at least 2 ml is required. Samples should if possible be collected on three consecutive days.

– Gastric tract: Used for elderly patients and children who produce little or no sputum.

– Samples collected under fibroscopy by bronchial aspiration, endobronchial brushing or from bronchoalveolar lavage fluid.

Other samples

– Urine: All urine produced during the morning is collected and concentrated by centrifuging. The concentrate is subjected to decontamination. Urine is collected on 3 consecutive days.

– Cerebrospinal fluid must be collected under sterile conditions.

– Effusion fluids, such as pleural, peritoneal and articular fluids.

– Pus from abscesses.

– Wicks, swabs and compresses.

– Haemocultures and myelocultures in EDTA tubes (10 ml).

– Stools, in particular, during intestinal infections in immunodeficient subjects.

– Biopsies from lymph nodes, lungs, endometrium and other areas.

- Quantiferon® test in 3 specific tubes (to be requested from the laboratory performing the test). Agitate the tubes vigorously before incubating for 24 hours at 37°C. Centrifuge before delivering them at +4°C.

SAMPLE STORAGE AND TRANSPORT

The sample, with an obligatory description of its precise nature, must be transported to the laboratory at +4°C to avoid the proliferation of other bacteria.

DIAGNOSTIC METHODS

Direct examination

This is done after disinfection, concentration and possible liquefaction of the contaminated pathological material. The two specific stains which are used are auramine for screening, followed by Ziehl Neelsen for confirmation. This low-sensitivity, non-specific test only detects the presence or absence of acid- and alcohol-resistant bacilli. A positive result nevertheless allows rapid isolation of the patient and commencement of treatment.

Culture

This is the reference component in diagnosis and is essential for identification and for the antibiotic sensitivity testing. The combination of a Middlebrook liquid medium and a traditional agar medium (some strains will only grow on one of these) is superior to the Löwenstein-Jensen and Coletsos media which were previously used. These liquid media can be used with automatic equipment and provide a faster response (approximately one week).

Identification

– The conventional method is based on the time taken for obtaining cultures and the optimum culture temperature, the micro- and macroscopic aspects (including staining) and biochemical characteristics. The method is time-consuming, laborious and subjective.

– The marketing of amplification and molecular hybridisation tests has allowed progress to be made, in terms of diagnosis. They can identify a mycobacterium in 2 days from among the fifteen or so most common mycobacteria.

Others tests allow identification of the species within the tuberculous complex.

– Sequencing compares the profile obtained with profiles from a database.

– Molecular typing techniques (RFLP, MIRU-VNTR and spoligotyping) are restricted to the study of nosocomial or laboratory-generated infections and epidemiological surveillance.

The antibiogram

An antibiogram is systematically produced for mycobacteria in the tuberculosis complex. The reference method is the method of proportions in a Löwenstein-Jensen medium, described by Canetti, Rist and Grosset in 1963, which consists of defining the proportion of mutants which are resistant to a given antibiotic. The method has been adapted for liquid media.

For non-tuberculous mycobacteria, the antibiotics tested differ according to whether the mycobacterium is fast- or slow-growing, but the methodology, in liquid or solid media, remains the same.

Searching for genes resistant to Rifampicin and Isoniazid (INH)

This PCR gene amplification technique detects the mutation in the rpoB gene which is responsible for 96% of cases of resistance to Rifampicin and in the katG gene implicated in resistance to INH. The searches take 36 hours on the basis of positive cultures and can be performed directly on the pathological material.

Searching for mycobacterium DNA or RNA

This can be used for both complexes i.e. *tuberculosis* and *avium*. Techniques using PCR and NASBA provide a result in less than 2 days but their sensitivity is imperfect and their specificity limited. It must be carried out in addition to culture.

Serodiagnosis

This is performed using the ELISA method, which detects IgM and IgG antibodies directed against the A60 antigen, but it lacks sensitivity and specificity. The diagnostic value of serodiagnosis is uncertain.

Blood test for interferon production

Diagnosis of the latent form was until now based on the intra-dermal reaction (IDR), but this may be incompatible with BCG and is difficult in practice.

Two *in-vitro* tests are now available to explore the cellular immune response. The tests explore the ability of the patient's lymphocytes to secrete interferon γ after stimulation by BK-specific ESAT-16, CFP-10 and TB7.7 proteins. They include a negative control with no antigen and a positive control with mitogen to validate the result.

Their use in France is at present limited to the following conditions:

– Investigation of a case in a subject aged more than 15 years at least 3 months after the contact.

– Employment of health and surveillance workers for work in an at-risk activity.

– Aid to diagnosis of extra-pulmonary forms of tuberculosis.

– Before starting treatment by anti-TNFα.

A positive result indicates the presence of a current infection by a mycobacterium in the *tuberculosis* group. Other mycobacteria do not give positive results in these tests, with the exception of *M. Kansasii, M. marinum* and *M. szulgai*. At the present time, the tests do not determine the active or latent nature of the infection and provide no indication of the date of infection.

Summary

Bacteriological diagnosis of mycobacterium infections are based on obtaining a culture. Direct searches by PCR or NASBA are complementary techniques for use in particular situations.

Technical progress has produced improvements on several points:

– Culture in liquid media has reduced the time needed for obtaining cultures and an antibiogram.

– Molecular biology makes it possible, in 2 days, to identify the main mycobacteria and find the main mutations responsible for resistance to the two major anti-tuberculosis products.

– Blood tests which, when used instead of IDR, provide a more precise and practical diagnosis of latent tuberculosis.

FOR FURTHER INFORMATION

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MYCOPHENOLATE MOFETIL

DEFINITION

Mycophenolate mofetil (MMF) is an immunosuppressant used in organ transplantation. It is an ester of mycophenolic acid (MPA), for which it is a prodrug. After oral or intravenous administration, MMF is rapidly and completely converted to MPA, its active metabolite, which can be assayed in the blood.

MMF is given in combination with cyclosporin and corticosteroids to prevent acute organ rejection in patients who have received renal, cardiac or hepatic allotransplants. In most of these patients it has replaced azathioprine, which is now used less frequently.

It is marketed as 250 mg capsules, 500 mg tablets and powder to be made up for oral administration at a concentration of 1g/5ml, as well as in powder form to make up for infusion at a dose of 500 mg (parenteral administration).

It is used:

– In renal transplant recipients: 1 g twice daily

– In hepatic transplant recipients: 1 g twice daily by infusion for the first four days after transplantation, then orally at a dose of 1.5 g twice daily

– In heart transplant recipients: 1.5 g twice daily.

The main side effects related to combined administration of MMF with cyclosporin and corticosteroids are diarrhoea/vomiting, leucopaenia and generalised infection.

In 2009, Roche laboratories informed prescribers of the occurrence of cases of erythroblastopaenia in patients receiving MMF in combination with other drugs including other immunosuppressants.

NB: A new form of mycophenolate has been developed (mycophenolate sodium) and marketed as film-coated tablets resistant to gastric digestion. The effectiveness and safety profiles of mycophenolate mofetil and mycophenolate sodium seem to be similar. Their bio-availability in the form of MPA is also comparable. However, individual variability in exposure when one of these products is replaced by the other is not known and the two are not bio-equivalent. The time to peak serum/plasma concentration (Tmax) of MPA derived from mycophenolate sodium is significantly longer than that from MMF, and the residual concentration is higher. Further studies are needed to define how its use as therapy should be monitored.

PHARMACOKINETICS

Bioavailability (oral administration)	After gastric absorption, MMF is rapidly and completely converted to MPA, the active metabolite, by intestinal and hepatic esterases. Absolute bioavailability: 94%
Time to peak plasma concentration (Tmax)	Tmax of the MPA form is approximately 1 hour
Time to steady state	4 to 5 days
Protein binding	95 to 97 % albumin bound
Metabolism	Conjugation in the liver and conversion to inactive MPA phenolic glucuronate (MPAg). There is an enterohepatic cycle which is responsible for a second increase in plasma MPA concentration 6 to 12 hours after administration
Clearance	93% in urine (< 1% as MPA, about 93% as MPAg), 6% in stool
Clearance half-life	11 to 18 hours

INDICATIONS FOR MEASUREMENT

Because of the considerable intra and inter-individual variability in plasma concentrations of MPA, it can be useful to follow drug concentrations to check the level of immunosuppression, so as to forestall graft rejection. It is particularly indicated in patients with altered renal function and in the elderly.

Indeed, a statistically significant relationship has been demonstrated between the risk of acute rejection of renal transplants and plasma concentrations of MPA.

The correlation with the risk of rejection is even more marked if the AUC (area under the curve) is calculated for MPA from 0 to 12 hours. The AUC can be derived using 3 samples taken at 20 minutes, one hour and 3 hours after administration of the drug. By contrast, the correlation between residual concentration of MPA and the 0-12 h AUC for MPA is poor.

In the present state of knowledge, the risk of development of side-effects does not seem to be related either to the residual serum/plasma concentration of MPA, or to the 0-12 h AUC.

INFORMATION

■ SAMPLE

Serum, or plasma in EDTA or heparin tube. Do not use tubes with gel separator.

Collect when steady state has been achieved after 4 to 5 days of treatment, to measure the MPA residual concentration (C0), just before the next dose is taken, or take three specimens to calculate the area under the curve: 20 min, 1 h and 3 h after administration.

■ QUESTIONS FOR THE PATIENT

It is mandatory that any request for a drug assay should incorporate: the reasons for the request (examination for effectiveness or toxicity), the time of sampling, the date of commencement of treatment and/or of any change that might have been made in the drug regime, information on that regime (dose magnitude, dose frequency, route of administration) as well as the age, height and weight of the subject when that is available.

Are you taking any other medication? The following drug interactions need to be taken into account when interpreting residual serum/plasma concentrations of MPA:

– Cholestyramine: reduction of about 40% in plasma concentrations (and AUC) of MPA

– Tacrolimus: possible increase of the residual concentration by 15 to 20% (not shown in all studies); increase of about 30% in plasma AUC of MPA.

Some drugs interfere with other pharmacokinetic parameters of MPA. As examples, the main ones are indicated below:

– Aciclovir: increase in plasma MPAg concentrations (about 8%)

– Iron: decrease in Cmax and in AUC of MPA

– Cyclosporin: decrease in the plasma MPA AUC by 19 to 38%

– Ganciclovir: increase in plasma concentrations of MPAg

– Non-steroidal anti-inflammatories (except niflumic acid): increase in Cmax of MPA

– Metronidazole: reduction in MPA and MPAg AUC.

Renal function (elevated creatinine)?

■ SAMPLE STORAGE AND TRANSPORT

Store and transport the specimen at + 4° C.

ASSAY METHOD

Immunoenzymology (EMIT): measurement of MPA and MPA-acylglucuronate (minor active metabolite);

HPLC or liquid chromatography coupled to mass spectrometry: enables measurement of MPA and MPAg separately.

NORMAL EXPECTED VALUES

Normal expected values for the residual concentration of MPA are between 1.5 and 4.5 mg/l. In the period immediately following the transplant procedure these concentrations can warn of the risk of acute rejection of the transplanted organ.

FOR FURTHER INFORMATION

■ Dictionnaire Vidal®.

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MYCOPLASMA PNEUMONIAE

DEFINITION

Mycoplasmas belong to the Class of Mollicutes (Order Mycoplasmatales, Genus Mycoplasma). They are Gram-negative polymorphous bacteria without a veritable cell wall and are smaller than conventional bacteria (0.2 – 2 µm) i.e. 0.15 to 0.5 µm and their shape is spherical or ovoid, with a single, triple-layer membrane.

EPIDEMIOLOGY

Infections arise in the endemic state, with small epidemic outbreaks at intervals of 4 to 7 years (1992 and 1999 in Europe). Contagiousness is moderate. Their incidence, however, is poorly understood because of the normally benign nature of the illness and the difficulties of laboratory diagnosis.

Mycoplasma pneumoniae seems to be transmitted through droplets of secretions from the respiratory system. Transfer is made more likely by close contact (family, schools, institutions, etc.). The illness particularly affects school children, adolescents and young adults. The incubation period is currently estimated to be two to three weeks. Although *Mycoplasma pneumoniae* seems to be particularly prevalent during the autumn and early winter in temperate regions, infections are reported throughout the year.

SYMPTOMS

M. pneumoniae causes acute respiratory infections, most frequently in children from 5 years of age and in young adults. Infections may be atypical pneumonias (20 to 30% of community pneumopathies), with a favourable prognosis and sometimes combined with other indicative manifestations (ENT, cutaneous, haematological, neurological, etc.) or, more frequently, with simple tracheobronchitis. The possible role of *M. pneumoniae* in asthma remains controversial.

The majority of *Mycoplasma pneumoniae* infections are benign, and recovery takes place in 2 to 4 weeks without treatment. The use of certain appropriate antibiotics seems to reduce the fever. Very rarely, the disease may be fatal.

Extra-pulmonary manifestations, either isolated or combined with pneumopathy, may appear. These take the form of subcutaneous or mucosal eruptions (erosive multi-orifice ectodermosis, morbilliform erythema, Steven-Johnson syndrome), reactive arthritis, cold-agglutinin haemolytic anaemia, etc.

INDICATION FOR BIOLOGICAL DIAGNOSIS

Possibility of contact with people infected by *Mycoplasma pneumoniae*.

Clinical signs of atypical pneumopathy in a patient (aged between 5 and 40), particularly in the autumn time.

INFORMATION

SAMPLE

Respiratory samples: samples from the throat, nasopharyngeal aspirations or bronchoalveolar lavage.

Blood samples: collected in a dry tube (serum).

SAMPLE STORAGE AND TRANSPORT

Samples can be stored at +4°C for a maximum of 48 hours, or at -70°C for longer periods. Since mycoplasmas are highly sensitive to dehydration (drying out), appropriate transport media must be used, such as saccharose-phosphate medium (2 SP) enriched with 5% serum of calf foetus, with no antibiotic.

DIAGNOSTIC METHODS AND INTERPRETATION

DIRECT DIAGNOSIS

Cultures are rarely used, due to their time-consuming (typically 2 to 3 weeks) and difficult nature. The mycoplasma can be revealed in throat samples and nasopharyngeal aspirations in children, or in bronchoalveolar lavage samples for severe forms. Culture must take place without delay, under anaerobic conditions. Growth is slow (2 to 6 weeks) and results in the appearance of small colonies of a granular appearance.

PCR is an excellent alternative method, bearing in mind the difficulties of culture (amplification of 16S ribosomic RNA).

INDIRECT DIAGNOSIS

The laboratory diagnosis of a *M. pneumoniae* infection is mainly based on serology.

The complement fixation reaction detects antibodies directed against a glycolipid antigen. Another technique, based on the agglutination of sensitised particles of latex, is more sensitive than the complement fixation reaction.

Seroconversion, or an elevation of titre by 4 times, or a titre > 1/64 in IgG, are generally significant. Serology is not very sensitive, however, and cross-reactions are reported in the course of neurological or pancreatic damage.

ELISA methods allow the separation of IgG and IgM. The presence of IgM antibodies are commonly seen in children and adolescents but are more rarely observed in adults.

TREATMENT

Mycoplasmas are naturally resistant to certain families of antibiotics (betalactamines, Rifampicin and Polymyxine). The potentially-active antibiotics are tetracyclines, fluoroquinolones, macrolides and related compounds. Very rare cases of resistance to macrolides have been reported. In severe forms of illness, the intravenous administration of erythromycin is employed.

FOR FURTHER INFORMATION

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- Baum S.T., *Mycoplasma infection : Immunologic and Molecular Biologis Diagnostic Techniques*, Manual of Clinical Laboratory Immunology Sixth Edition, 2002: 516-21.

MYOGLOBIN

DEFINITION

Myoglobin is a metalloprotein formed from a globin chain and a haeminic section. It is found in the cellular cytoplasm of skeletal muscles and of the myocardium, performing the functions of oxygen transport and storage in these tissues.

PATHOPHYSIOLOGY

Any lysis of cardiac or skeletal muscle cells results in myoglobin being released into the bloodstream. Its low molecular weight (17 kDa) and high intramyocytic concentration explain its value as a marker for myocardial illness. Although not specific to cardiac muscle, myoglobin is a sensitive marker for diagnosing myocardial infarction in the first 6 hours. The dynamics of its appearance in the serum are rapid (2 to 4 hours after the onset of pain) and serum concentration reaches a maximum at between 6 and 12 hours. Its main interest lies in its negative predictive value, where the absence of an increase in two consecutive samples taken 1 to 3 hours apart, excludes a diagnosis of infarction. It has been superseded, however, by the availability of new markers, namely the troponins, while the use of copeptin as a marker, is an even more recent development.

Because of its rapid elimination through the kidneys, it is not a retrospective diagnostic marker. In the absence of complications, a normal value may be observed 24 hours after the infarction.

INDICATIONS FOR MEASUREMENT

Emergency diagnosis of myocardial infarction, during which, it is one of the first biological markers to show a rising level in the blood. At the present time, its measurement in such cases is combined with (or replaced by) that of troponin, which is a much more specific marker for myocardial ischaemia. In general, if clinical signs and the electrocardiogram (ECG) are typical of myocardial infarction, the biological markers are not indispensable; they are mainly of value in an atypical scenario, particularly when the ECG is not helpful.

Looking for an early relapse of myocardial infarction. Myoglobin is valuable in this situation, due to its rapid dynamics (plasma half-life ranging from 1 to 3 hours).

Monitoring the therapeutic efficacy of treatment by thrombolytic medication or angioplasty.

INFORMATION

SAMPLE

Serum or plasma collected in heparin or EDTA. Haemolysis or hyperlipidaemia may interfere with measurement.

When dealing with a patient presenting chest pain, two consecutive samples taken 1 to 3 hours apart, targeting a myoglobin serum concentration below the threshold level are required, in order to exclude a diagnosis of myocardial infarction.

QUESTIONS FOR THE PATIENT

In a context of diagnosing acute myocardial infarction, note the time of appearance and duration of chest pains.

SAMPLE STORAGE AND TRANSPORT

Centrifuge within 2 hours of collection.

Serum or plasma can be stored for 8 hours at room temperature, 48 hours at +4°C and several months at -20°C.

ASSAY METHOD

Quantitative tests: Immunoturbidimetric, immuno-nephelometric or ELISA-type immunoenzymatic methods.

NORMAL EXPECTED VALUES

In subjects with no muscular or cardiac disorders, the serum concentration of myoglobin is < 90 µg/l.

PATHOLOGICAL VARIATIONS

A serum concentration in excess of 90 µg/l is a sign of muscular lesions.

Diagnosis of myocardial infarction

Serum concentration rises after 2 hours, reaches a maximum in 6 to 12 hours and returns to normal within 24 to 36 hours after the onset of pain. The sensitivity for diagnosing myocardial infarction 2 hours after pain starts is 73%, reaching 100% in 6 hours; specificity, on the other hand, is low (55 to 85% in different studies). Serum concentration increases in various other circumstances:

– In cardiac pathology, such as angina pectoris, pericarditis and cardiac surgery, etc.

– Other situations, such as intense physical exercise, intramuscular injection, myopathy, rhabdomyolysis, crush syndrome, pulmonary embolism, renal failure and burns, etc.

In practice, measurement of myoglobin is combined with that of troponin. A study of 817 patients demonstrated that a normal level of myoglobin and troponin on admission to A&E and 90 minutes thereafter had a negative predictive value of 99.6% to exclude a diagnosis of myocardial infarction.

Prognostic interest during myocardial infarction

Patients with ST segment elevation treated by IV thrombolysis have a mortality risk at 30 days, three times higher, if they had an elevated serum concentration of myoglobin on admission. In patients with no ST segment elevation, a high myoglobin level is also associated with a higher mortality risk, independently of other biological, clinical or electrical markers.

Marker for monitoring the efficacy of treatment for myocardial infarction (thrombolytic treatment or cardiac angioplasty): Less than 2 hours after myocardial reperfusion, the serum myoglobin concentration reaches a significantly elevated level, and then returns to normal. In general, if reperfusion is more rapid and of better quality, serum myoglobin rises more rapidly during treatment. The ratio of

serum myoglobin levels measured at T0 and T60 (or T90 or T120 minutes) T60/T0, or the gradient of the rise in concentration (e.g. ((T60 – T0)/T60)), is used by certain groups as a marker for reperfusion after thrombolytic treatment or coronary angioplasty. According to certain authors, reperfusion is considered to be effective if:

(myoglobin T60 min – T0)/myoglobin T60 ≥ 4 or if myoglobin T120 min/myoglobin T0 ≥ 4.6.

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NEISSERIA MENINGITIDIS

DEFINITION

Neisseria meningitidis (Nm) is the agent responsible for cerebrospinal meningitis and meningococcal septicaemia It is an intracellular Gram negative diplococcus belonging to the *Neisseriacae* family and *Neisseria* genus. Meningococcus is a strictly human saprophytic bacterium of the rhinopharynx, which is very poorly resistant to the external environment. Pharyngeal carriers are common and carrier status varies between a few days and several months. The disease is transmitted aerially, directly between human beings (through salivary droplets). Meningococcal infections can be endemic or sporadic with epidemic outbreaks due to the emergence of a virulent strain. Nm is the main organism responsible for childhood and infant meningitis. Cerebrospinal meningitis varies seasonally, and is more common in the winter and spring.

Synonym: Meningococcus.

INTRODUCTION

EPIDEMIOLOGY

Invasive meningococcal infection is a sudden onset disease characterised by bacterial meningitis or septicaemia. Approximately 500,000 cases occur throughout the world annually and have a mortality rate of 5 to 10%, despite correct diagnosis and appropriate early therapy. **Meningococcal infection affects all ages although infants and adolescents are particularly sensitive**.

Of the 12 serogroups described, the most widely found throughout the world are A, B and C, with a predominance of the A serogroup in Africa and Asia, and the B serogroup in Europe and particularly in France. The C serogroup is found in the United States of America and as sporadic cases in Europe. The proportion of serogroups W135 and Y are constantly increasing in Africa and both Northern and Southern America. In recent years, serogroup Y has emerged in the United States and epidemics of serogroup W135 meningococcal infections have occurred in Mecca pilgrims in 2000 and 2001.

News about epidemics of invasive meningococcal infections can be viewed on the WHO website at: http://www.who.int/csr/don.

PATHOPHYSIOLOGY

The Meningococcus enters through the respiratory tract and binds to the pharyngeal wall via pili. Under the influence of various events (such as viral infection), it disseminates through the body via the blood stream and causes meningococcal disease, pneumonia or arthritis. The virulence of the bacterium is increased because of the presence of the capsule.

SYMPTOMS

- **Cerebrospinal meningitis:** This is an association of meningeal and infectious features. Meningeal involvement causes fever, headache, neck stiffness, vomiting, photophobia and seizures in children.
- **Meningococcal septicaemia:** This may occur in isolation or precede or complicate cerebrospinal meningitis. It is due to spread of Meningococci into the systemic circulation and untreated, is an association of infection and progressive purpura. It is usually complicated by DIC. A hyperacute form of the disease may occur and has a very poor prognosis. This is known as the Waterhouse-Friderichsen syndrome and is a combination of meningeal infection with septicaemia and *purpura fulminans* progressing rapidly to shock and death.
- **Other symptoms:** Sore throat, bronchopulmonary disease, pericarditis, osteomyelitis and arthritis.

SEARCH INDICATIONS

Diagnosis of infant or childhood cerebrospinal meningitis.

Diagnosis of invasive forms of the disease, particularly in children and adolescents.

Differential diagnosis from other bacterial or viral meningitis.

INFORMATION

SAMPLES

– ***Cerebrospinal fluid*** collected by lumbar puncture into 3 sterile tubes.

– ***Blood culture*** recommended for meningococcal infections.

– ***Nasopharynx*** the sample should be taken from the posterior wall of the rhinopharynx.

– ***Samples from skin lesions*** in the event of purpura.

QUESTIONS FOR THE PATIENT

Age?

Have you returned from an endemic area?

Clinical symptoms?

Current or previous antibiotic treatment (reported meningitis)?

SAMPLE STORAGE AND TRANSPORT

Neisseria meningitidis is a fragile bacterium which is particularly sensitive to changes in temperature. The sample should therefore be transported promptly to the laboratory, avoiding cold temperatures.

DIAGNOSTIC METHODS

DIRECT EXAMINATION

- **Macroscopic:** The CSF is cloudy or even purulent in cerebrospinal meningitis. On a stained smear of the CSF centrifugation pellet the Meningococcus is seen as coffee ground groups of Gram negative diplococci.
- **Microscopic:** Red blood cell and white blood cell count in CSF.

CULTURE

Generous amounts of CSF are innoculated systematically onto rich media: Mueller Hinton, polyvitex chocolate agar, and incubated at 37°C in a CO2 enriched atmosphere. Colonies grow over 24 hours on chocolate agar + polyvitex and these are round, white or greyish in colour with regular outlines, mucous-like if the strain is encapsulated. The Meningococcus can also be grown on selective media from various other samples, such a and pharyngeal, joint and bronchopulmonary.

IDENTIFICATION

This is based on morphological, biochemical and antigenic features. Strain serogroups are identified immunologically from the meningococcal capsule polyosides. Serotypes and subtypes within serogroups can be defined from the external membrane proteins.

TESTING FOR SOLUBLE ANTIGENS

This can be performed on serum, CSF supernatant or urine using the sensitised latex particle agglutination method or by counter-immunoelectrophoresis. The latex method is fast and indicated for use in cases of meningitis which have been aborted by antibiotic therapy. Its sensitivity and specificity however are poor.

GENE AMPLIFICATION METHODS

PCR testing may be performed on CSF, blood and rhinopharyngeal samples.

TREATMENT

ANTIBIOTIC THERAPY

The β-lactams still have an important place amongst the antibiotics available despite an increase in strains exhibiting reduced sensitivity to penicillin. Current first line treatment of purulent *N.meningitidis* meningitis is with a 3rd generation cephalosporin (ceftriaxone or cefotaxime).

PROPHYLAXIS

This is well defined and is used for the patient and patient's "contacts". The contact is given antibiotic prophylaxis with rifampicin or spiramycin. If rifampicin is contraindicated and/or is vaccinated (if the Meningococcus belongs to groups A, C, Y or W135). The patient is isolated in an individual room and should also be given chemoprophylaxis after curative treatment to destroy organisms carried in the rhinopharynx.

In France, vaccination recommendations against invasive non-B serogroup Meningococcus infections (BEH April 09)

"Vaccination is recommended for the following at risk groups:

– Contacts of an invasive Meningococcus serogroup A, C, Y, or W135 infection when a vaccine exists. The vaccination must be administered no more than 10 days after the index case has been hospitalised;

– Children suffering from terminal complement fraction or properdin deficiency or who have anatomical or functional asplenism".

The oligoside C meningococcal vaccine can be used to vaccinate children from 2 months old using the following regimens:

– In children under 1 year old, 2 doses at an interval of at least 2 months with a booster during the second year of life, at least 6 months after the second dose

– From 1 year old, in adolescents and adults: a single injection.

Beyond the age of 2 years old, a polyoside A, C vaccine (one injection) can also be used to vaccinate contacts of a Meningococcus C index case or people living in defined areas where the incidence of Meningococcus C is particularly high.

On the other hand, the tetravalent A, C, Y, W135 polyoside vaccine is recommended for children over two years old suffering from terminal complement fraction or properdin deficiency or who have anatomical or functional asplenism, following a 3 yearly dose regimen.

Health recommendations for travellers (BEH 2 June 2009)

"Vaccination against invasive meningococcal infections is recommended:

– For people going to an endemic area (particularly the sub-Saharan African meningitis belt) during the dry season which encourages meningococcal transmission (usually winter and spring) or to any other epidemic area when under close prolonged contact conditions with the local population.

– From 6 months old with the polysaccharide A + C meningococcal vaccine in meningococcal serogroup A epidemics.

– From 2 months old with the conjugated oligoside C meningococcal vaccine for meningococcal serogroup C epidemics.

– From 2 years old with the polyoside A, C, Y, W135 meningococcal vaccine in meningococcal serogroup W135 or Y epidemics.

– People staying in these areas working in the health sector or with refugees.

– People on pilgrimages to Mecca (Hadj and Umra with the polyoside A, C, Y, W135 meningococcal vaccine)".

FOR FURTHER INFORMATION

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NEOPTERIN

DEFINITION - PATHOPHYSIOLOGY

Neopterin is a pteridin, derived metabolically from guanosine triphosphate (GTP). It is produced mostly by monocytes and macrophages during the synthesis of tetrahydrobiopterin, the enzyme cofactor for synthesis of the serotonin and noradrenaline neurotransmitters. It is an early specific marker of macrophage activation and cell proliferation, production of which is stimulated by gamma interferon. Because of this, serum and/or urine neopterin concentrations are raised in situations in which cell immunity is stimulated, such as, during infections, graft rejection, graft versus host disease, autoimmune diseases and malignancies.

INDICATIONS FOR MEASUREMENT

– In human immunodeficiency virus (HIV) infection, neopterin is a marker of disease progression and of the effectiveness of antiretroviral agents. It is also believed to be of prognostic use in Kaposi sarcoma.

– Neopterin is proposed as a marker to monitor non-Hodgkin's lymphoma, leukaemia and cancers (gynaecological, lung, renal and gastro-oesophageal).

– Neopterin is an early marker of rejection in transplantation (liver, kidney or bone marrow).

– It is proposed to monitor the effectiveness of immuno-modulatory therapy (interferons, TNF-α, and interleukins).

– Neopterin is occasionally measured to monitor progression of chronic inflammatory diseases (sarcoidosis, inflammatory bowel disease and rheumatoid arthritis).

– In CSF, neopterin is a marker of microglial cell activation, requested in HIV encephalopathy and in cerebral toxoplasmosis, in association with the measurement of β2-microglobulin.

INFORMATION

SAMPLE

Blood sample: Dry tube (serum).

Urine sample: First morning urine collection, kept away from light, before being measured promptly.

CSF: 0.5 ml into a sterile tube, at +4°C away from light.

QUESTIONS FOR THE PATIENT

In a cancer setting, type of tumour and current treatment, such as chemotherapy, radiotherapy and surgery (types and date of treatment).

SAMPLE STORAGE AND TRANSPORT

Samples should be processed within an hour after being taken or frozen at -20°C for subsequent analysis.

Transport frozen at – 20°C.

ASSAY METHODS

Radioimmunoassay.

NORMAL EXPECTED VALUES

For reference:

– Serum neopterin < 25 nmol/l

– Urine neopterin 80 to 200 nmol/mmol of creatinine (in adults)

– CSF > 5 µmol/l (chronic encephalitis) or > 20 µmol (acute form).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Urine neopterin excretion is increased in children. Normal expected age-related values are shown below:

0 – 3 months:	500 to 2800 nmol/mmol of creatinine
3 months – 1 year:	400 to 2500 nmol/mmol of creatinine
1 to 2 years:	400 to 2200 nmol/mmol of creatinine
2 to 6 years:	300 to 2100 nmol/mmol of creatinine
6 to 12 years :	200 to 1000 nmol/mmol of creatinine
12 to 18 years :	100 to 400 nmol/mmol of creatinine

PATHOLOGICAL VARIATIONS

- **Onco-haematology:** In oncology in general, serum and urinary neopterin concentrations rise early (cf. table). The rise however, is not specific and neopterin measurement is not widely used. Serum and urinary neopterin concentrations rise in parallel with disease progression and are proportional to tumour mass in recurrence or metastases, particularly in non-Hodgkin's lymphoma, chronic lymphoid leukaemia, chronic myeloid leukaemia and ovarian cancer.

Site of tumour	Diagnostic Sensitivity
Lung	50 – 60 %
Kidney	30 – 50 %
Gynaecological	40 – 70 %

- **Human immunodeficiency virus (HIV) infection:** Neopterin is a marker of disease progression; serum and urinary concentrations rise very early preceding seroconversion. They are then a good marker to monitor effectiveness of treatment.
- **Transplantation:** During patient monitoring, a rise in serum or urinary neopterin concentrations may indicate viral infection or acute rejection (values rise on average 48 hours before clinical rejection). It is therefore a good, although relatively non-specific, warning-sign.
- **Sarcoidosis and inflammatory bowel disease (Crohn's Disease or ulcerative colitis):** Serum and urinary neopterin concentrations rise during flares of the disease.
- **Other causes of raised serum and/or urinary neopterin concentration:** Various viral infections (varicella, CMV, EBV and viral hepatitis, etc.) and bacterial infections (particularly mycobacterial), cholestasis of pregnancy, etc.

FOR FURTHER INFORMATION

- Wirleitner B., Schroecksnadel K., Winckler C., Fuchs D., *Neopterin in HIV-1 infection*, Mol Immunol 2005; 42 (2): 183-14.
- Puy H., Westeel P.F., Fournier A., *Néoptérine urinaire*. In: Kamoun P., Fréjaville J.P., Guide des examens de laboratoire, 3rd Ed. Médecine Sciences Flammarion, Paris 1993.
- Wang Z., Dong M., Chu H., He J., *Increased serum levels of neopterin and soluble interleukin-2 receptor in intrahepatic cholestasis of pregnancy*, Acta Obstet Gynecol Scand 2004; 83: 1067-70.

NETILMICIN

DEFINITION

Netilmicin is an antibiotic belonging to the aminoglycoside or aminoside family, characterised by bactericidal activity on numerous Gram positive and negative bacteria. It is used in monotherapy or in association, particularly with β-lactams, for infections with sensitive organisms (particularly Gram negative bacilli). Its major disadvantage is its renal and auditory toxicity (class effect).

It is marketed in France in the form of an injectable 25 or 100 mg/ml solution.

The preferred route of administration is intramuscular, by one of two regimens:

– As two or three daily doses.

– As a single daily dose, in patients under 65 years old with a normal renal function and without neutropaenia when treatment is for < 10 days, and only for infections due to Gram negative organisms excluding Pseudomonas and Serratia. At least identical efficacy and occasionally better tolerability have been demonstrated with this treatment regimen.

It is also occasionally administered intravenously as a discontinuous 30 to 60 minutes infusion.

The usual doses are:

– In adults: 4 to 6 mg/kg/day (up to 7.5 mg/kg/day).

– In infants and children: 6 to 7.5 mg/kg every 8 hours.

– In the newborn < 10 days: 6 to 7.5 mg/kg as 2, IV, injections.

Aminoglycosides are concentration-dependent antibiotics characterised by a post antibiotic effect enabling the interval between two doses to be spread out without the concern of bacterial regrowth.

PHARMACOKINETICS

Bioavailability (parenteral route)	100%
Plasma peak	30 to 60 minutes after an intramuscular infusion
Time to steady state	24 hours (2nd day of treatment)
Protein binding	Practically non-existent
Metabolism	Nil
Elimination	In the unchanged form by the kidney
Half-life of elimination	- 2 hours 30 minutes in people with normal renal function - 4 hours in the newborn - Long in patients with renal failure - Variable depending on body weight in intensive care or major burns patients.

INDICATIONS FOR MEASUREMENT

Plasma measurements are usually performed during treatment, as netilmicin has a low therapeutic index and its pharmacokinetics are variable.

Because of the risk of toxicity (ototoxicity and nephrotoxicity) and the severity of the infections in which it is generally used, measurements are used to confirm that the dose is effective but not in the toxic range.

Measurements are essential when treatment needs to be for longer than 7 days in the following situations:

– People over 65 years old.

– Renal failure.

– Severe infection due to Gram negative bacilli.

– The newborn and young children.

– The obese.

INFORMATION

SAMPLE

Serum or plasma.

Measurements must be performed when treatment is started and once steady state has been reached, i.e. 24 hours after starting treatment or 24 hours before changing dose.

– Before the next injection for trough concentration measurements;

– 30 minutes after the end of IV infusion (contralateral arm to the infusion) or 1 hour after IM injection for peak measurement.

QUESTIONS FOR THE PATIENT

– Any request for drug measurement must include the reasons for the request (testing for efficacy or toxicity), the sampling time, date treatment was started and/or any change in dosage, dosage information, amount administered, frequency and route of administration, together with the age, height and weight of the person whenever possible.

– Treatment with other nephro- or oto-toxic drugs (risk of increased toxicity)?

– Pre-treatment with another aminoglycoside, treatment with a loop diuretic, such as bumetanide, furosemide or piretanide?

– Increased nephrotoxicity particularly with cyclosporin, tacrolimus, amphotericin B, polymyxin and cisplatin.

SAMPLE STORAGE AND TRANSPORT

Store and transport the serum at + 4°C.

ASSAY METHODS

Mostly immunological methods: FPIA, EMIT, immunoturbidimetry, etc.

NORMAL EXPECTED VALUES

Trough concentration < 2 mg/ml. A lower trough concentration indicates that the administration frequency is compatible with the patient's ability to remove the drug.

Peak concentration: 5 to 12 mg/ml. Lower concentrations are associated with treatment failures.

Dosages are adjusted according to serum antibiotic concentrations on an individual case basis, determined by the patient's state, the severity of the infection and administration details.

In general, the time between doses should be increased if the trough concentration is too high: low peak concentrations (after re-testing) suggest that the dosage should be increased.

FOR FURTHER INFORMATION

- Dictionnaire Vidal®.

NEUROPEPTIDE Y

DEFINITION

Neuropeptide Y (NPY) is a 36 amino acid peptide found mostly in the central nervous system in the hypothalamus and paraventricular nuclei. High concentrations have also been found in the cerebral cortex. Peripherally, neuropeptide Y is found in the sympathetic nervous system.

INTRODUCTION

Neuropeptide Y is involved in many central nervous system functions; in the control of anxiety, mood and libido regulation. In conjunction with orexins A and B, it contributes to regulating dietary behaviour (increasing appetite and adipogenesis). Leptin, which can adjust appetite to fat reserves, is believed to act by inhibiting hypothalamic NPY secretion. The hyperphagia associated with insulin deficiency in type 1 diabetics, is believed to be associated with increased NPY. It is also believed to have an anticonvulsant effect and to play a role in learning and memory processes.

Peripherally, neuropeptide Y is contained in the nerve endings of the sympathetic system surrounding blood vessels and has a vasoconstricting effect. Neuropeptide Y is also produced by adrenal chromaffin cells, where it appears to modulate catecholamine secretion. In endothelial cells, neuropeptide Y stimulates prostacycline secretion and it regulates *T helper* lymphocyte differentiation and *natural killer* lymphocyte activation in the immune system.

INDICATIONS FOR MEASUREMENT

Measurement has been proposed to assess activation of the sympathetic system in hypertensive patients, patients with coronary artery disease and in idiopathic postural hypotension.

INFORMATION

■ SAMPLE

The sample should be taken into a dry tube containing no additives or anticoagulant, or into a tube containing lithium or sodium heparinate.

The sample should be taken from the patient fasting in the morning, as concentrations follow a 24 hour circadian rhythm controlled by the hypothalamic suprachiasmic nuclei. The sample must be centrifuged, separated and frozen as soon as possible.

■ QUESTIONS FOR THE PATIENT

Suspected disease?

Current treatment?

■ SAMPLE STORAGE AND TRANSPORT

Samples can be stored for several weeks after freezing at -80°C. Transport the sample frozen on dry ice.

ASSAY METHODS

Immunoradiometric assay. The assay is not standardised against an international standard.

NORMAL EXPECTED VALUES

In healthy people with normal blood pressure: 223.6 ± 14.7 ng/ml.

PATHOLOGICAL VALUES

Serum neuropeptide Y concentrations are increased in phaeochromocytoma, chronic renal insufficiency, dialysis patients, asthma attacks and in flares of rhinitis.

FOR FURTHER INFORMATION

■ Silva A.P., Cavadas C., Grouzmann E., *Neuropeptide Y and its receptors as potential therapeutic drug targets*, Clin Chim Acta 2002; 326: 3-25.

NICKEL

DEFINITION

Nickel (Ni) is a shiny hard white metal, present in small amounts in the earth's crust, particularly as sulphides, oxides and silicates. It is widely used in the manufacture of solid, durable metal alloys (aluminium, copper and iron) and is found in numerous everyday objects such as stainless steel pans, coins, rechargeable batteries and nickel cadmium batteries.

INTRODUCTION

Most absorption occurs through the respiratory tract and the deposition, retention and pulmonary absorption depend on the physico-chemical properties and particle granule size. Average absorption is 20 to 35%, the remainder being expectorated, ingested or retained in the respiratory tract.

Nickel is also absorbed to a lesser extent by the gastro-intestinal tract and skin absorption increases with sweating, solvents, detergents, and oils which promote dissolution of nickel from some alloys. The most fully and rapidly absorbed nickel compound is nickel carbonyl.

It is mostly transported in the circulation in a protein bound form (plasma half-life of 20 to 35 hours) and is found above all, in the kidneys and lungs.

After pulmonary or percutaneous absorption, it is excreted mostly in urine. When ingested, approximately 90% is removed rapidly in faeces.

The soluble compounds have a half-life of elimination of 20 to 40 hours, compared to several weeks to years, for the insoluble compounds.

INDUSTRIAL POISONING

NICKEL DERMATITIS OR ECZEMA

Nickel plating workers develop nickel eczema (or "nickel itch"), an allergic process which can be confirmed by an epicutaneous test.

RESPIRATORY ALLERGY

Occasional cases of asthma associated with nickel inhalation have been reported.

RISK OF CANCER

An increased risk of nasal fossa (ethmoid sinus) and lung cancer has been found in factory workers exposed mostly to relatively insoluble compounds such as nickel bisulphide and oxide. Improved hygiene conditions in production factories, however, have almost abolished this risk.

A study of nasal biopsies in people exposed to nickel for more than 10 years has also shown a greater prevalence of keratinisation and atypical epithelium, compared to controls. Nickel accumulates in the nasal mucosa of exposed workers, where its concentration is proportional to duration of exposure. Given its long biological half-life (3 to 5 years), concentrations also remain high long after exposure has stopped.

OTHER RESPIRATORY TRACT DISORDERS

Features of the following conditions have been described in workers exposed to nickel dust:

– Chronic bronchitis.

– Hypertrophic rhinitis.

– Sinusitis associated with anosmia.

– Nasal polyposis.

– Perforation of the nasal septum.

INDICATION FOR MEASUREMENT

Blood and urinary nickel are markers of exposure to the metal, although there are few studies available, making results difficult to interpret. Blood and urinary concentrations correlate relatively well with atmospheric concentrations for the soluble compounds.

Blood and urinary nickel concentrations indicate recent exposure to soluble nickel compounds and recent or old exposure to insoluble compounds; they increase throughout the working week. Absence of raised values does not necessarily indicate absence of risk associated with exposure to some insoluble compounds (cancer of the lung or nasal cavities).

INFORMATION

TYPE OF SAMPLE

Serum (3 ml): Beware of the possible risk of contamination from the needle.

Urine sample (10 ml), non-acidified, collected into a new polyethylene or polystyrene bottle.

SAMPLE DETAILS

Serum and urine preferably collected at the end of the shift at the end of the week.

USEFUL INFORMATION

In order to interpret laboratory monitoring results, it is important to identify whether exposure is to soluble compounds (nickel chlorides, sulphates and nitrates) resulting in a rapid rise in urinary nickel, or to poorly soluble compounds (nickel sulphides, carbonates and oxides), which are eliminated slowly over a long period of time in the urine.

SAMPLE STORAGE AND TRANSPORT

Serum and urine samples may be stored and transported to the laboratory at between + 2 and + 8°C.

ASSAY METHODS

Electrothermal atomisation (graphite furnace) and atomic absorption spectrophotometry with Zeeman correction.

REFERENCE VALUES

Reference values in the general population

Serum nickel < 0.8 µg/l

Urinary nickel < 2.00 µg/g of creatinine in non-smokers; < 4.00 µg/g of creatinine in smokers.

Biological exposure index is not established.

FOR FURTHER INFORMATION

- Lauwerys R., *Nickel,* Toxicologie industrielle et intoxications professionnelles, 3e édition, Masson: p192-197.
- Guide BIOTOX 2001, INRS.

NSE

DEFINITION

Neurospecific enolase (NSE) is one of the enzymes involved in anaerobic glycolysis (the reaction converting glucose to lactate). Several isoenzymes have been described, resulting from the 2 by 2 combination of 3 sub-units,α, β and γ. In the nervous system, glial cells express the αα form and neurones more specifically express the γ dimer subunit. NSE is the γγ dimer which is only found in nervous or neuro-endocrine tissue and it is measured using a specific monoclonal antibody for the γ sub-unit.

INDICATIONS FOR MEASUREMENT

NSE is the serum marker of choice for treatment monitoring and early detection of recurrence of small cell anaplastic lung cancers. It is also used as a prognostic indicator and monitoring test for neuroblastomas (the most common of the solid childhood tumours).

INFORMATION

SAMPLE

Preferably serum (dry tube) as values in plasma and serum are different. Separate the serum promptly.

Discard haemolysed samples (red blood cells are rich in NSE). Also discard opalescent lipaemic samples. Fasting samples are not essential, although hyperlipidaemia may interfere with measurements.

No circadian rhythm has been described.

QUESTIONS FOR THE PATIENT

Interference may occur with other neuro-endocrine tissues. State the disease and current treatment(s): chemotherapy, radiotherapy and surgery (types and date of treatment).

SAMPLE STORAGE AND TRANSPORT

4 hours at + 4°C and at -20°C beyond this time. If the analysis is to be performed later or the sample is to be transported, freeze within 4 hours of sampling.

ASSAY METHODS

"Sandwich" method with 2 monoclonal Antibodies.

NORMAL EXPECTED VALUES

For reference, usual serum values are < 12.5 ng/ml (95th percentile). Values vary depending on the population (including between smokers and non smokers) and by method used. It is strongly recommended that each laboratory redefines its normal values, even when these are proposed by the manufacturer.

PATHOLOGICAL VARIATIONS

Increases in NSE in small cell anaplastic lung cancer

At diagnosis: Serum NSE concentrations are raised in 60 to 80% of small cell anaplastic lung cancers (SCALC). Mean serum concentrations are higher in SCALC than in other lung cancers.

As applies to the other tumour markers, NSE is not a screening test, although values over > 30 ng/ml are highly suggestive of SCALC.

NSE concentrations correlate with clinical stage at diagnosis. They are raised in approximately 50% of localised thoracic tumours and in 100% of diffuse cancers.

Monitoring treatment: In the initial days of SCALC chemotherapy, serum NSE concentrations can be used to assess the quality of response to treatment. NSE increases in serum 24 to 72 hours after chemotherapy is started. A rapid fall to pre-treatment values during the first week of treatment (or at the end of the first treatment cycle) indicates that the tumour is sensitive to chemotherapy. Conversely, concentrations increase progressively or remain outside of reference intervals in non-responders.

NSE returns to normal in 80 to 96% of patients in remission. A rise in the marker may precede clinical and/or radiological relapse by 1 to 4 months. In this situation it increases exponentially with a doubling time of 10 to 94 days.

Raised NSE in neuroblastomas

Serum NSE concentrations are raised in approximately 60% of cases of neuroblastoma (> 30 ng/ml).

– The major use of measurements is for prognostic purposes. Serum NSE concentrations at diagnosis correlate closely with stage of disease. They are low in stage 1 disease and high in stage IV disease and are associated with the mass of primary tumour and extension of the disease. A serum NSE concentration of > 100 ng/ml at the time of diagnosis is a very poor prognostic indicator in advanced stages of disease.

– A return of NSE concentrations to normal in the follow-up of treatment of these children generally indicates remission, whereas a recrudescence suggests relapse or metastases. Serum NSE concentrations appear to correlate closely with residual mass of primary tumour following effective treatment and are a better marker of response to chemotherapy than the urinary markers HVA or VMA.

Increases in other diseases

Apart from haemolysis, serum NSE concentrations can rise in the following situations (rarely > 30 ng/ml):

– Haemodialysis patients.

– Benign bronchopulmonary diseases.

– Other cancers, such as lung tumours other than small cell anaplastic lung cancers, APUDomas (APUD cell or *Amine Precursor Uptake and Decarboxylation* cell tumours of the neuro-endocrine system) such as insulinomas, medullary thyroid carcinomas, intestinal carcinoid tumours and phaeochromocytomas, in which a rise in serum NSE concentration indicates a malignant tumour.

FOR FURTHER INFORMATION

Chatelain et *al.*, IBS 1989; 16: 37-44.

5'- NUCLEOTIDASE

DEFINITION

5'-nucleotidase (5'Nu) is an enzyme present in many tissues, mostly in the liver and biliary tract but also in the bowel, placenta, kidneys, testes and nervous tissue, and in both erythrocytes and lymphocytes. It is mostly used as a marker of hepatobiliary cholestasis, which is less sensitive, but more specific, than other hepatocyte membrane enzymes, particularly alkaline phosphatase (ALP) or gamma-GT (GGT).

Synonym: 5'Nu.

INTRODUCTION

5'Nu is located principally on the cell plasma membrane. Different molecular forms have been described. A membrane or ecto 5'Nu form attached to the membrane by a glycosyl phosphatidyl inositol group, a soluble membrane form and two cytosolic forms. Physiologically, the role of ecto 5'Nu is to hydrolyse extracellular nucleotides into nucleosides (adenosine), which can cross the cell membrane and enrich the cytosol purine pool. 5'Nu is found in the liver in the hepatocyte plasma membrane and in the sinusoid wall, in the vascular endothelium and in Küppfer cells. It is released into the systemic circulation in hepatobiliary disorders, following enzymatic cleavage of the membrane form and the detergent action of bile acids.

INDICATIONS FOR MEASUREMENT

Second line test in the presence of raised ALP to confirm (or exclude) that the ALP rise is hepatobiliary in origin, particularly in pregnancy, in children and when bone disease is present.

Other indications: Testing for hepatic metastases and monitoring hepatobiliary disease after bone marrow transplant.

INFORMATION

■ SAMPLE

Serum (dry tube) or heparinised plasma. A fasting sample is preferred.

■ QUESTIONS FOR THE PATIENT

Are you taking any medical treatment? Hepatotoxic drugs are liable to cause an increase in serum 5'Nu activity.

■ SAMPLE STORAGE AND TRANSPORT

Serum can be stored for 1 week at + 4°C, for 2 weeks at –20°C and for several months at – 80°C.

ASSAY METHODS

Colourimetric chemical reactions.

NORMAL EXPECTED VALUES

These vary depending on the method. For reference < 5 U/l at 30°C and < 9 U/l at 37°C (Enzyline optimise 5'Nu® reagent, Biomérieux).

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Serum 5'Nu increases slightly in the second and third week of pregnancy and in people over 70 years old.

■ PATHOLOGICAL VARIATIONS

5'-nucleotidase is the most specific enzyme for hepatic cholestasis. Unlike ALP, its activity in serum does not rise in bone disease, with growth or in pregnancy and it is not affected by enzyme inducers or alcohol, as is GGT. It may however rise when potentially hepatotoxic drugs are taken, with some cancers (hepatocellular carcinoma or hepatic metastases and also other cancers including breast, cerebral metastases of a primary breast cancer and prostate cancers) and in various diseases (rheumatoid arthritis, sarcoidosis, lymphomas, etc.). The rise is generally interpreted in conjunction with the ALP result.

– Increased 5'-nucleotidase activity associated with a rise in ALP indicates hepatic cholestasis; cholestasis due to gallstones, cholestatic hepatitis, primary biliary cirrhosis, and liver or pancreatic cancer. It is also a very specific marker for hepatic metastases, although is less sensitive than gamma GGT.

– A rise in 5'-nucleotidase activity associated with normal or only slightly increased serum ALP activity is seen in liver disease secondary to bone marrow transplantation. In these situations serum 5'Nu rises early and to high levels (approximately 20 times the upper limit of normal or even higher).

– Normal 5'-nucleotidase activity associated with raised ALP is seen in childhood growth, during the 2nd and 3rd trimesters of pregnancy and in the elderly. Alternatively it may suggest bone or bowel disease.

FOR FURTHER INFORMATION

■ Clerc-Renaud P., *5'nucléotidase*, Encycl Med Biol, Elsevier Paris, 2003.

■ Friedman R.B., Young D.S., *Effects of disease on clinical laboratory tests*, 3rd Ed. AACC Press, Washington, 1997.

O'SULLIVAN TEST

DEFINITION

The O'Sullivan test is a simplified oral glucose tolerance test (OGTT) performed in pregnant women, used for early screening for gestational diabetes. It is performed between 24 and 28 weeks into the pregnancy or in the first antenatal consultation, if the woman has known risk factors (maternal age > 35 years old, obesity, excessive weight gain during the current pregnancy, family history of diabetes, personal history of gestational diabetes or *in-utero* foetal death). Gestational diabetes (GD) is defined as "an abnormal glucose tolerance developing and/or diagnosed during pregnancy" and affects 3 to 6% of pregnancies in Europe. The risks from GD affect both mother and child. These risks include hypertension of pregnancy and pre-eclampsia in the mother, increased perinatal mortality, foetal macrosomia, prematurity, traumatic neonatal injuries and metabolic abnormalities in the child.

PROTOCOLS

In France, the *Collège National des Gynécologues et Obstétriciens Français* recommend that the protocol is performed in 2 stages: a screening test (O'Sullivan test) followed by a confirmatory test known as a "diagnostic test" if the O'Sullivan test is positive.

The WHO recommends that the test be performed in a single stage with 75 g of glucose (instead of 50) and that samples are taken at T0 and T+120 min.

- **The O'Sullivan screening test** involves measuring blood glucose before and one hour after an oral dose of 50 grams of glucose. The test can be performed fasting or non-fasting at any time in the day, regardless of meal times.

Protocol:

– Blood glucose at T0

– Oral ingestion of 50 g of glucose dissolved in 100 ml of water over a time not exceeding 5 minutes.

– Blood glucose at T+60 min.

- **The diagnostic test** is performed if the screening test is positive and involves a 3 hours OGTT with an oral dose of 100 g of glucose (or 2 hours with a dose of 75 g of glucose).

Protocol:

– Blood glucose at T0.

– 100 g of glucose dissolved in 250 ml of water over a period of between 5 and 15 minutes.

– Blood glucoses at T+60 min, T+120 min, and T+180 min.

INTERPRETATION

SCREENING TEST

The blood glucose concentration 60 minutes after taking 50 g of glucose must not exceed 1.30 g/l (7.2 mmol/l). Some authors, however, recommend a threshold of 1.40 g/l (7.8 mmol/l). The result must be confirmed with a diagnostic test above this threshold.

DIAGNOSTIC TEST

– The blood glucose thresholds established by the Collège National des Obstétriciens Français (Carpenter and Coustant) from a test using a 100 g glucose load are shown below:

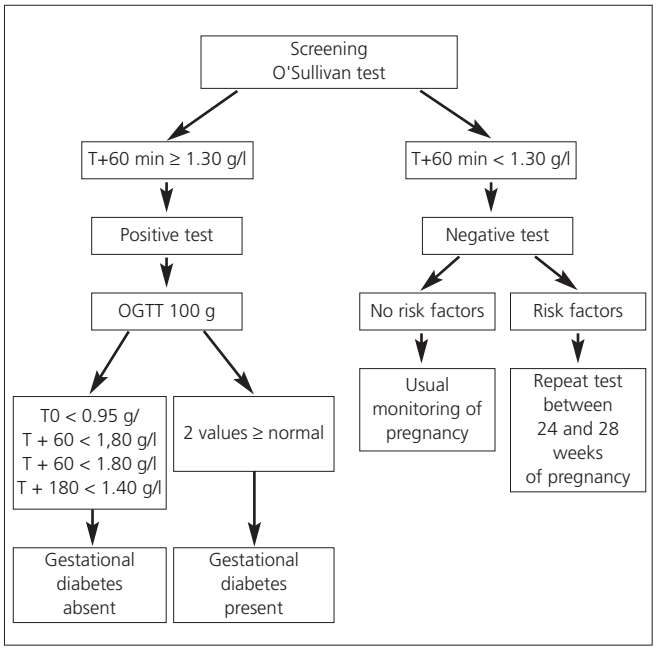
T0	< 0.95 g/l	< 5.3 mmol/l
T+60 min	< 1.80 g/l	< 10 mmol/l
T+120 min	< 1.55 g/l	< 8.6 mmol/l
T+180 min	< 1.40 g/l	< 7.8 mmol/l

The diagnosis of GD is made if 2 out of 4 values are abnormal, i.e. if 2 blood glucose concentrations are at or above the threshold values shown in the table above.

– The blood glucose thresholds adopted by the WHO (after a 75 g glucose load) are:

T0	< 1.26 g/l	< 7 mmol/l
T+120 min	< 1.40 g/l	< 7.8 mmol /l

The diagnosis of GD is made if 1 out of 2 values is abnormal, i.e. if one of the 2 values is equal to or greater than the limits shown above.



FOR FURTHER INFORMATION

- *Rapport de synthèse sur le dépistage et le diagnostic du diabète gestationnel*, HAS. 2005.
- Fénichel P., Hiéronimus S., Harter M., Gillet J.Y., *Diabète et grossesse*, Encycl Med Biol. Elsevier Paris; 1998.

OPIATES

DEFINITION

The opiates are a group of compounds extracted from the opium poppy. They have been used for a very long time for their analgesic and psychoactive properties. Morphine and codeine are natural and heroin is an illegal semi-synthetic substance responsible for many "overdose" deaths. Opium is now hardly used at all. Morphine is widely used in medicine for its analgesic properties and codeine for its antitussive properties. They may however be misused for drug abuse purposes. Abuse of heroin has fallen since 1996 through the introduction of buprenorphine or methadone substitution programmes. We are, however, seeing a large rise in legal opiate and opiod users: substitution products *(cf. above)*, morphine pain killers and over-the-counter opiates marketed as antitussives or misused as drug substitutes by drug addicts.

The opiates and opioids *(from Pépin G, Opiacés et opioïdes, 1998)*

Natural opiates	Opium, morphine, codeine
Semi-synthetic opiates	Heroin (or diacetyl-morphine), codethyline, pholcodine
Semi-synthetic opioids	Buprenorphine Naloxone
Synthetic opioids	Methadone, dextropropoxyphene, dextromoramide, pethidine, phenoperidine, fentanyl, alfentanil, sufentanil, pentazocine

Effects of the main opiates and substitution products
(from Pépin G, Revue française des laboratoires, 2000).

Substance	Main effects	Overdose effects	Withdrawal syndrome
Morphine (or opium)	Analgesia, depression, euphoria, semi-sleep, inducing, well-being	Respiratory depression, coma which may be fatal	Panic, seizures, vomiting, nausea, rigors, cold sweats
Heroin	Analgesia, depression, major euphoria, very great toxicity	Respiratory depression, coma which may be fatal	Panic, seizures, vomiting, nausea, rigors, cold sweats
Codeine	Weak analgesic, antitussive, variable dose - related effects	Variable, may be fatal	Variable, severe
Methadone	Analgesia, depression, euphoria, slower effect than morphine	Respiratory depression, seizures, coma which may be fatal	Seizures, vomiting, nausea
Buprenorphine	Analgesia, depression	Respiratory depression, coma which may be fatal	Less severe withdrawal syndrome than with morphine

INTRODUCTION

In view of their misuse for drug abuse purposes, detection and identification of opiates in urine (illegal substances, substitution products and over-the-counter products) are required to quantify and determine the type of substance in question, in occupational medicine (in France), in patients on substitution treatment and in automobile drivers (the French law stipulates systematic screening for narcotics in drivers involved in a fatal accident). All positive urinary test results must be confirmed in blood, using a specific analytical technique. Only blood measurements reflect cerebral exposure and the effect on behaviour.

INDICATIONS FOR MEASUREMENT

Testing and confirmation of opiate poisoning, either in a medical situation (agitation or coma), or in a medico-legal context (occupational medicine, road accident, crime, doping, etc.).

INFORMATION

■ SAMPLE

A 40 ml urine sample should be collected at the laboratory: Outside of the medico-legal context, a sample collected into a single MSU bottle is acceptable. Ensure no tampering occurs (substitution, addition of water or adulterating agents), by measuring urinary pH and density and confirming the sample temperature as soon as possible after it has been collected (temperature > 30°C). Close bottles firmly, identify them precisely and record the date, time and place of collection.

Blood sample: A few ml of serum or plasma. Do not use tubes with a phase separator.

■ QUESTIONS FOR THE PATIENT

Context of request: Medico-legal or monitoring a substitution treatment?

If possible, circumstances of poisoning, known current treatment?

■ SAMPLE STORAGE AND TRANSPORT

Urine can be stored at +4°C and then at -20°C for several months.

Serum must be separated and frozen at - 20°C within 4 hours of sampling, if the analysis is to be performed later.

ASSAY METHODS

Screening: Immunochemical (screening for the morphine ring) FPIA, EMIT (qualitative or semi-quantitative tests). All opiates are recognised except for methadone and buprenorphine, for which specific kits are available.

Specific confirmatory methods: gas chromatography linked to mass spectrometry, HPLC linked to mass spectrometry.

NORMAL EXPECTED VALUES

Negative test when the substances have not been taken.

INTERPRETATION

(See table)

	Metabolites	Detection window in urine after taking the substance	Interpretation – treatment
Heroin	- Heroin - 6-MonoAcetylMorphine (6MAM) - Morphine - Glucuronide-conjugated urine metabolites	- entirely metabolised - 7 h - 12 to 48 h - 36 to 72 h	Toxic, varies greatly between individuals. The presence of 6MAM indicates heroin exposure. Treatment of respiratory depression and/or on naloxone.
Morphine	Different glucuronide and sulpho-conjugated metabolites including the highly active morphine 6-glucuronide and codeine	- 12 to 48 h (morphine) - 36 to 72 h (morphine LP)	Theoretically, a positive result indicates that morphine has been taken (beware of interferences, *cf* below). Treatment of respiratory depression and/or on naloxone
Codeine	Morphine (10%) Norcodeine Sulpho- and glucuro-conjugates	24 to 54 h	In between 10 and 20 h after taking codeine, the codeine/morphine urine ratio is > 1 and the presence of norcodeine indicates that codeine has been taken as the primary drug. Between 20 and 40 h, the ratio becomes < 1, norcodeine is no longer present and it is no longer possible to distinguish the drug taken initially by the test. Treatment for massive ingestion: activated charcoal
Buprenorphine	Norbuprenorphine Glucuronoconjugates, 20% elimination in urine	- 20 to 25 h	A blood test is required to determine poisoning. Treatment of respiratory depression + naloxone
Methadone	Main metabolite: EDDP (2 ethylidene-1,5 dimethyl-3,3 diphenylpyrrolidine) inactive	- 72 h	Screening method: few interferences and satisfactory positivity threshold. Treatment of respiratory depression + naloxone

Opiate metabolism results in urinary excretion of free or conjugated morphine. Screening kit antibodies recognise the morphine ring. Urine containing compounds used therapeutically (codeine, codethylline or pholcodine) or illegal compounds (6-acetyl morphine, heroin metabolites) are recognised without distinction when their urine concentration exceeds the limit of detection of the method.

Conversely, morphine mimetics (buprenorphine, methadone, dextropropoxyphene) which have a chemical structure including a modified morphine ring are not recognised, even if abused.

Results must be interpreted in the context of pharmacokinetic knowledge and complex metabolites of the substances concerned (morphine for example is metabolised into glucurono-conjugated compounds which can be detected in urine up to 48 hours after morphine has been taken but also when codeine, pholcodine or heroin have been taken, as it is also a metabolite of these substances).

If the method is positive, it is essential that an appropriate confirmatory method be used to distinguish whether heroin, morphine or codeine has been taken and to provide an accurate measurement or even to establish whether specific potentiating agents (benzodiazepines, etc.) are present.

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OROSOMUCOID

DEFINITION

Orosomucoid is a very low molecular weight, extensively glycosylated, highly acid protein. It is mostly synthesised by the liver, although is also produced by polynuclear cells and monocytes, explaining the rise in serum concentration in patients suffering from severe infections. It is catabolised in the liver and 30% is removed by the kidney. It has a plasma half-life of approximately 3 days.

Synonym: Alpha1-acid glycoprotein.

INTRODUCTION

The physiological roles of orosomucoid are still poorly understood. It is probably involved in defence against infection, inhibiting lymphocyte proliferation, reducing polynuclear cell phagocytosis and platelet aggregation and stimulating fibroblast production. Because it is highly acidic, it binds strongly to basic molecules, notably some steroids (particularly progesterone) and some drugs, whose effect may be reduced in an acute inflammatory reaction (propranolol, erythromycin, penicillin, some steroids, quinidine, chlorpromazine and diazepam, etc.).

INDICATIONS FOR MEASUREMENT

Orosomucoid is an inflammatory protein. Its synthesis in hepatocytes is stimulated by the pro-inflammatory cytokines: IL-1, TNF-alpha and IL-6. Serum concentrations rise from the 3rd to 4th hour after the inflammatory stimulus, reaching a peak in 3 to 5 days.

It can therefore be used to detect and monitor an inflammatory reaction and is often requested in this situation, in conjunction with C-reactive protein and haptoglobin as the "inflammatory profile". Combined with CRP, it is also used for the diagnosis and monitoring of bacterial infections, particularly in neonatology. Finally, it also forms part of the "nutritional profile" alongside albumin and pre-albumin and is used with CRP, albumin and pre-albumin to calculate the PINI (Prognostic Inflammatory and Nutritional Index), mostly in geriatric practice.

PINI = CRP (mg/l) x orosomucoid (mg/l) / albumin (g/l).

INFORMATION

SAMPLE

Heparinised or EDTA plasma or serum, preferably withdrawn when the patient is fasting.

Discard hyperlipidaemic samples.

QUESTIONS FOR THE PATIENT

Are you taking any of the following medicines? Basic medicines may reduce serum orosomucoid concentration (by complexing): non-steroidal anti-inflammatory drugs, penicillin, erythromycin, furosemide, beta blockers, etc.

SAMPLE STORAGE AND TRANSPORT

Plasma or serum can be stored for 1 week at + 4°C and for several months at – 20°C.

Transport: + 4°C.

ASSAY METHODS

Immunoturbidimetry and immunonephelometry.

NORMAL EXPECTED VALUES

Adults: 0.50 to 1.20 g/l.

Reference material: CRM 470, titre 0.65 g/l orosomucoid.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

- **Age related:** Serum concentrations are extremely low in the newborn, in the region of 0.18 to 0.40 g/l in the first day after birth. They increase gradually to values of approximately 0.50 to 0.90 g/l 1 week after birth, reaching adult values at around six months to 1 year old.
- **Orosomucoid synthesis** is increased by androgens and glucocorticoids and is reduced by oestrogens. Because of this, "normal" values are slightly lower in 20 to 45 year old women and fall by approximately 20% in pregnancy.

PATHOLOGICAL VARIATIONS

Major reasons for changes in serum orosomucoid concentrations

Falls	Rises
• Hepatocellular insufficiency (late fall in orosomucoid)	Acute, sub-acute or chronic inflammation
• Severe malnutrition (in the absence of inflammation)	• Renal insufficiency (retention in plasma of the fraction normally filtered by the glomerulus)
• Urine loss (nephrotic syndrome +++), gastrointestinal loss (exsudative enteropathies) or skin loss (burns)	• Myocardial infarction: peak at day 5 returning to normal on day 21 (orosomucoid has no practical use in this situation)
• Basic drugs (cf above) + small fall with oestrogen therapy	
• Cancers (advanced stage)	

- **In an inflammatory reaction**

CRP, which is the first inflammatory protein to rise, is more a marker of the acute phase, whereas serum orosomucoid concentrations rise by a factor of 3 to 4 with a peak 3 to 5 days after the inflammatory stimulus and is more a marker of chronic infection. In practice, a combined interpretation of these two proteins can be used to monitor progress of inflammation. Only CRP is raised initially, both are raised in the established disease state and with effective treatment, CRP is the first to fall followed by orosomucoid, indicating recovery.

Inflammatory state	CRP	Orosomucoid	Haptoglobin
Early, acute	+++	+	N or +
Acute, established disease phase	+++	+++	+++
Resolution	N or +	+	++
Chronic	N or +	+ or ++	++

D'après J. Rousseaux, www.med.univ.lille2.fr

■ **Combined interpretation of haptoglobin and orosomucoid measurements in an inflammatory protein profile**

The main use of this combined interpretation is to diagnose intravascular haemolysis during which haptoglobin may be normal (as a result of an increase due to inflammation and a fall due to haemolysis).

In inflammation, the rise in orosomucoid usually correlates with the rise in haptoglobin, according to the following equation:

Haptoglobin ≈ orosomucoid x 1.3 (expressed as a percentage of normal values).

– Parallel fall in orosomucoid / haptoglobin
  - Malnutrition
  - Hepatocellular insufficiency
  - Gastro-intestinal or skin loss

– Dissociation between orosomucoid / haptoglobin (haptoglobin < 1.3 x orosomucoid)
  - Intravascular haemolysis
  - Renal insufficiency

– Dissociation between orosomucoid / haptoglobin (haptoglobin > 1.3 x orosomucoid)
  - Glomerular loss in urine
  - Oestrogen exposure
  - Complexing with basic drugs (erythromycin, penicillin, beta blockers, etc.)
  - Some neoplasms

■ **Monitoring chronic inflammatory diseases and/or cancers**

Studies have particularly examined Crohn's disease and rheumatoid arthritis, in which a return of orosomucoid concentrations to normal reflects effective treatment.

Stable serum orosomucoid concentrations reflect remission of some cancers (breast and bowel cancers, Hodgkin's disease, etc.), whereas a rise is seen in recurrence or infectious complications.

■ **Diagnosis and monitoring of neonatal infections**

A rise in orosomucoid combined with a rise in CRP in the first days of life is very useful in the early diagnosis of bacterial infection, as it is highly specific (haptoglobin cannot be used as serum concentrations are very low in the newborn). A rise is seen in more than 80% of severe neonatal sepsis (false negatives mostly been due to severe Streptococcus B infections) and there are very few other reasons for a rise in these proteins in neonates. They are also useful in monitoring treatment: CRP falls very quickly if antibiotic therapy is effective and a return of orosomucoid concentrations to normal, an indicator of recovery, enables the decision to be made to stop treatment (before the ESR has returned to normal).

■ **Assessment of nutritional status through the PINI** (according to Ingelbleek Y et al. Int J Vit Nutr Res 1985 ; 55: 91-101)

A PINI > 1 is a marker of protein and energy malnutrition.

– 1 < PINI < 10: low risk.

– 11 < PINI < 20: moderate risk.

– 21 < PINI < 30: high risk.

– PINI > 30: life-threatening risk.

FOR FURTHER INFORMATION

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OSTEOCALCIN

DEFINITION

Osteocalcin is a predominant protein in the Inter-fibrillar bone tissue, where it is one of the non-collagen proteins. It is a single chain, 49 amino acid peptide with a molecular mass of 5800 Da. After being produced by osteoblasts, 90% is incorporated into the organic bone matrix and 10% passes in the intact form into the systemic circulation; it has a short ½ life of 5 minutes. The major feature of this protein is that it contains numerous glutamic acid (Glu) groups on its surface. The Glu groups are carboxylated (a vitamin K dependent stage) into γ carboxyglutamic (Gla) groups, which bind ionised calcium. Osteocalcin therefore promotes the formation of hydroxyapatite crystals, which are essential components of bone mineral material and provides its rigidity and solidity.

Synonym: GLA protein, BGP (Bone GLA protein).

PATHOPHYSIOLOGY

A rise in serum osteocalcin directly reflects the extent of osteogenesis. It is used as a marker of bone remodelling and is more specific for bone formation.

INDICATIONS FOR MEASUREMENT

Documenting bone remodelling in diseases characterised by high level bone remodelling (primary or secondary hyperparathyroidism, hyperthyroidism etc.).

Monitoring bone treatment: Hormone replacement therapy for the menopause, SERM (Selective Estrogen Receptor Modulators), such as raloxifen and bisphosphonates.

NB: In the investigation of osteoporosis, a raised osteocalcin indicates high level bone remodelling and is evidence in favour of rapid bone loss or increased risk of fracture. It may therefore be an aid to deciding on treatment in women who have low but not very low bone mineral density and no fracture. The studies which have been conducted, however, have produced contradictory results and this index cannot be used alone in clinical practice.

INFORMATION

SAMPLE

1 ml of serum. EDTA plasma must not be used.

The sample should be taken fasting in the morning, preferably between 0730 and 0930 hrs. Osteocalcin follows a circadian rhythm with a peak at the end of the night and a low in the afternoon.

Avoid lipaemic and haemolysed specimens.

QUESTIONS FOR THE PATIENT

Current diseases, treatment and dosage? Patients should be asked particularly whether they have recently had corticosteroid therapy (such as infiltrations). In this situation wait for at least a month before measuring osteocalcin.

SAMPLE STORAGE AND TRANSPORT

Centrifuge and freeze the serum within an hour of sampling (the molecule degrades *in-vitro*).

ASSAY METHODS

Radioimmunoassay or electrochemoluminescence assay methods (sandwich method with two monoclonal antibodies, one against an epitope on the N-terminal end and one against the N-MID fragment).

NORMAL EXPECTED VALUES

Reference RIA values in children are shown as an indication in the table below.

Age band	Girls osteocalcin (µg/l)	Boys osteocalcin (µg/l)
3 to 6 years	45-86	50-90
6 to 12 years (Stage P1)	28-63	31-87
Stage P2	54-118	36-71
Stages P3-P4	43-86	62-105
Stage P5	11-69	53-115

In adult men:

19 to 30 years: 23.0 to 41.0 µg/l

31 to 40 years: 17.0 to 29.0 µg/l

41 to 50 years: 13.0 to 27.0 µg/l

51 to 60 years: 14.0 to 24.0 µg/l

61 to 70 years: 13.0 to 25.0 µg/l

71 to 85 years: 12.0 to 26.0 µg/l

In adult women:

21 to 30 years: 19.0 to 38.0 µg/l

Over 31 years: 9.5 to 23.0 µg/l

(Pre-menopausal reference values should be used regardless of the age of the patient).

INTERPRETATION

PHYSIOLOGICAL VARIATIONS

- **Circadian variation** (*cf.* Sample information)
- **Age and sex:** Serum osteocalcin concentrations vary with age. Changes in children parallel the growth curve. Highest concentrations are seen during the first year of life and subsequently fall slowly, remaining at relatively constant levels until the onset of puberty. Osteocalcin rises in puberty, earlier in girls than in boys and correlates with the pubertal growth surge. Osteocalcin concentrations then fall in adults and remain stable. Levels rise again in the post-menopausal period in women because of increased bone remodelling.
- **Physical activity:** Osteocalcin rises with physical exercise.

PATHOLOGICAL VARIATIONS

- **Rises**

Chronic renal insufficiency, because of defective renal excretion.

Hyperthyroidism.

Primary and secondary hyperparathyroidism.

Bone metastases and osteosarcoma.

Paget's Disease: Osteocalcin is raised but is not a good reflection of high level remodelling because of a higher proportion of native osteocalcin incorporated into bone.

Formation phase of bone callus.

- **Falls**

Hypoparathyroidism.

Cushing's Disease

Bone treatments: A fall in osteocalcin concentrations of more than 20% compared to the pre-treatment baseline after six months' treatment with oestrogens, SERM or bisphosphonates indicates a biological effect and predicts a significant gain in bone mineral density after treatment for 2 years.

FOR FURTHER INFORMATION

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OXALIC ACID

DEFINITION

Oxalic acid is a dicarboxylic acid (HOOC-COOH) synthesised in the body in two distinct metabolic pathways; one based on glycoxylic acid (serine and glycine) and the other on ascorbic acid. In addition, 15% of the oxalate found in the urine comes from the diet (exogenous origin: chocolate, tea, coffee, sorrel, spinach, etc.). It is normally excreted in the urine. Its calcium salts are highly insoluble.

Synonyms: Oxalaemia, Oxaluria.

INTRODUCTION

Hyperoxalaemia and hyperoxaluria may be primary but are more often due to excess intake of oxalate precursors, excess absorption in the gut, a metabolic abnormality or impaired urinary excretion. Hyperoxaluria is a risk factor for urinary lithiasis because calcium oxalate is so insoluble.

The three types of primary hyperoxaluria:

Type I: Deficiency in a peroxisome enzyme, alanine glyoxylate aminotransferase, which soon gives rise to nephrocalcinosis and chronic kidney failure.

Type II: Deficiency in D-glycerate dehydrogenase, which causes a less severe syndrome.

Type III: Hyperabsorption in the gut without intestinal involvement.

Secondary hyperoxaluria is more common:

– Chronic kidney failure (haemodialysis).

– Gut involvement: increased intestinal absorption

– Vitamin B1 and B6 deficiency: increased endogenous production.

– Ethylene glycol poisoning: the metabolism of ethylene glycol gives rise to oxalate.

INDICATIONS FOR MEASUREMENT

– Aetiological diagnosis of urinary lithiasis.

– Monitoring of kidney failure patients on dialysis.

– Oxalic acid, a urinary metabolite of ethylene glycol, is a reliable marker for deliberate or occupational poisoning (occupational medicine).

INFORMATION

SAMPLE

At least 1 ml of heparinised plasma or serum: Fasting sample is required. Ensure the sample is not lipaemic or haemolysed.

Within one hour of blood drawing, separate the red cells from the plasma.

Acidify the 24-hour urine collection with hydrochloric acid (HCl) but do not add any preservative. Acidification is performed at the laboratory by adding 12 N HCl to adjust the pH to between 2 and 3 (5-20 ml acid depending on the volume of the sample and its initial pH).

QUESTIONS FOR THE PATIENT

Are you taking any of the following products?

Vitamin C or sodium ascorbate; vitamin C metabolism gives rise to oxalate (in variable quantities in different individuals).

If this is the case, the subject should stop taking the vitamin C and the urine collection should be delayed for 48 hours.

SAMPLE STORAGE AND TRANSPORT

Serum and plasma: A few hours at +4°C. If the sample is to be transported, freeze it (-20°C) within one hour of blood drawing.

Urine: The urine already collected should be kept in a refrigerator during a 24-hour collection. Acidified urine can be kept for 8 days at +4°C. If the sample is being sent to a specialist laboratory, it should be well mixed before aliquotting (a volume of 50 ml is required for testing). Record the total volume of urine in the 24-hour collection.

ASSAY METHODS

Oxalate is isolated by precipitation or selective extraction from urine or, from serum by deproteinisation followed by precipitation. It is then assayed by either chromatography, gas phase chromatography (GPC) or high performance liquid chromatography (HPLC), or an enzyme-based method (oxalate oxidase or oxalate decarboxylase) with a spectrophotometric read-out.

NORMAL EXPECTED VALUES

For reference:

Oxalic acid in the serum: less than 3.0 mg/l, i.e. 33 µmol/l.

Oxalic acid in the urine:

Men: 7.0-44.0 mg/24h, i.e. 78-489 µmol/24h

Women: 4.0-31.0 mg/24h, i.e. 44-344 µmol/24h

Children (both boys and girls): 13.0-38.0 mg/24h, i.e. 144-422 µmol/24h.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

– Age: Children excrete less oxalic acid than adults. The rate of excretion rises with age to reach the adult level by about 14.

– Other factors: The rate of excretion will depend on diuresis and dietary calcium intake. The rate of excretion tends to be higher in the daytime than at night.

■ PATHOLOGICAL VARIATIONS

The main causes of hyperoxaluria are summarised in the following Table:

Excessive oxalate production
- Type I and II primary hyperoxaluria
- Pyridoxine deficiency
- Vitamin B1 deficiency
Excessive intake of metabolic precursors of oxalate
- Ethylene glycol poisoning
- Massive doses of xylitol
- Excessive ascorbic acid intake
- Anesthesia by methoxyflurane
- Bladder irrigation with glycine during urological surgery (prostatectomy)
- Aspergillosis
Excessive intake or gut absorption of oxalate
- Enteric hyperoxaluria
- Excessive dietary intake
- Type III primary hyperoxaluria
Moderate idiopathic hyperoxaluria

Taken from P. Jungers et al. Lithiases calciques secondaires. Chap. V. In "Lithiase urinaire". Médecine-Sciences - Flammarion - Ed. 1989.

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OXCARBAZEPINE

DEFINITION

Oxcarbazepine is a compound with anti-epileptic activity, marketed in the form of 150, 300 and 600 mg tablets and indicated for use in the treatment of partial epileptic seizures, with or without secondary generalisation, in monotherapy or in association with other anti-epileptic treatment in adults and children (6 years old and above). Treatment is administered as two daily doses; the initial dose may be increased depending on the clinical response.

Oxcarbazepine and its active metabolite (DMH, *cf. below*), act primarily by blocking the voltage-dependent sodium channels, stabilising hyper-excitable neuronal membranes, inhibiting repetitive neuronal discharges and reducing the propagation of synaptic impulses.

Oxcarbazepine is generally preferred to carbamazepine, because of its improved tolerability and lower propensity to hyponatraemia.

PHARMACOKINETICS

Absorption	Complete, extensively converted into an active metabolite DMH (monohydroxy derivative = 10-OH carbazepine)
Plasma peak (Tmax)	4.5 hours
Time to steady state	2 to 3 days
Plasma protein binding	40%, mostly to albumin
Metabolism	Hepatic, rapid, mostly into active DMH
Plasma half-life of elimination	23 to 37 h
Elimination	More than 95% in urine (approximately 80% as DMH glucuronides or unchanged DMH).

INDICATIONS FOR MEASUREMENT

Routine plasma oxcarbazepine measurement is not recommended for therapeutic monitoring. Some authors, however, believe that it is justified when therapeutic or toxic effects may be difficult to interpret and when treatment failure may have serious consequences. In practice, plasma measurements are used:

– To inform the prescriber about patient adherence to treatment.

– To identify and interpret possible pharmacokinetic interactions with concomitant treatments.

INFORMATION

■ SAMPLE

Serum or plasma collected into EDTA or heparin. Avoid tubes with separator gel.

Take the sample immediately before the next dose (trough concentration), usually before the morning dose.

■ QUESTIONS FOR THE PATIENT

– Any request for drug measurement must include the reasons for the request (testing for efficacy or toxicity), the sampling time, treatment start date and/or any change in dosage, dosage information (amount administered, frequency and route of administration) and the age, height and weight of the person whenever possible.

– Are you taking any other medical treatment?

Oxcarbazepine and DMH inhibit the hepatic enzyme CYP2C19, which causes therapeutic interactions with compounds that are extensively metabolised by this cytochrome, particularly phenobarbital and phenytoin. Potent cytochrome P450 inducers (carbamazepine, phenobarbital or phenytoin), and viloxazine also alter plasma DMH concentrations *(cf. table)*.

In addition, oxcarbazepine and DMH are enzyme inducers of cytochromes CYP3A4 and CYP3A5, causing a fall in the plasma concentrations of some drugs, such as dihydropyridine calcium channel blockers, oral contraceptives and carbamazepine.

Therapeutic interactions: impact on plasma concentrations

Concomitant treatment	Impact of the compound on plasma oxcarbazepine concentrations	Impact of oxcarbazepine on plasma concentrations of the concomitant treatment
Carbamazepine	40% reduction	0 to 22% reduction (30% increase in carbamazepine epoxide)
Phenobarbital	30% reduction	Approximately 15% increase
Phenytoin	29 to 35% reduction	0 to 40% increase
Valproic acid	0 to 18% reduction	No impact
Viloxazine	10% increase	

■ SAMPLE STORAGE AND TRANSPORT

Separated plasma/serum can be stored for 24 hours at room temperature, for 5 days at + 4°C or for 15 days at – 20°C; if the analysis is to be deferred, the sample should be transported frozen promptly after being collected.

ASSAY METHODS

High performance liquid chromatography.

NORMAL EXPECTED VALUES

There is currently no defined therapeutic range. For reference: 10-OH-carbazepine (DMH): 13 to 30 mg/l.

Signs of toxicity may develop over 30 mg/l. The symptoms described in overdose are nausea, vomiting, hyperkinesia, hyponatraemia, ataxia and nystagmus. Treatment is symptomatic in the absence of any specific antidote.

FOR FURTHER INFORMATION

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■ Péhourcq F., *Suivi thérapeutique des antiépileptiques: généralités*. In: Suivi thérapeutique pharmacologique pour l'adaptation de posologie des médicaments, Collection Option/Bio, Ed Elsevier, Paris. 2004: 377-82.

PAPILLOMAVIRUS

DEFINITION

The Papillomaviruses (HPV: *human papillomavirus*) belong to the papillomaviridae family and are agents responsible for mucocutaneous lesions in human beings and in many species of animal. More than 100 types of human HPV have been identified or partially characterised. Whilst most HPV causes benign skin diseases many studies have shown some types of HPV (high-risk HPV) to play a major role in the development of cervical cancer. The oncogenic potential of high risk HPV is closely linked to their ability to incorporate their genome into the cell genome. Deletion of viral genes E2 and/or E1 following integration of the viral genome, results in over-expression of the viral proteins E6 and E7. By interfering with the cell proteins involved in cell cycle control, these viral proteins are partly responsible for cell transformation and malignant change.

The high-risk HPV viruses are found in more than 99% of invasive cervical cancer and also in a large number of vulval, anal and penile cancers.

Cervical cancer is the second leading cause of cancer, in terms of incidence in women, throughout the world.

HPV viral infection is transmitted mostly sexually, although transmission may also occur from inoculation from skin verrucas, contaminated water (swimming pools or baths) and materno-foetal transmission. Although HPV is recognised to be the aetiological agent responsible for cervical cancer, cofactors appear to be involved (early age at first sexual intercourse, multiple partners, immunosuppression, past history of sexually transmitted infection, socio-economic conditions, lack of smear screening for dysplasia and smoking).

Synonym: HPV: Human Papilloma Virus – family: papillomaviridae, genus: papillomavirus.

SYMPTOMS

The HPV have specific tropism for cutaneous and mucosal epithelia. Some types of HPV are preferentially associated with ***skin lesions*** (papilloma and warts). HPV types 1, 2, 3 and 4 are commonly found in plantar veruccas, common veruccas and planar verrucas. Verruciform epidermodysplasia is a rare skin disease found in people predisposed to the disorder and linked to a type 5 HPV (immunogenetic susceptibility). Other HPV are associated with ***lesions affecting*** *the anogenital and oropharyngeal* ***mucosa*** (genital or laryngeal condylomata). At least 23 types of HPV are found specifically in the male or female genital tract. Two groups of these can be distinguished by their oncogenic potential, a high risk or intermediary risk group (types 16, 18, 31, 33, 39, 45, 52, 58 and 69) and a low risk group (types 6, 11, 42, 43, 44, 53).

HPV infection is benign in the great majority of cases. According to epidemiological studies, the prevalence of HPV infection ranges from 5 to 50% with a peak found around the age of 25 years old, followed by a gradual fall after the age of 30 years old. Viral carriage is transient (median time 6 to 25 months) and in 70 to 80% of cases development of immunity results in the virus being removed and resolution of any lesions which are present.

Persistent HPV infection lasting more than 25 months is abnormally long and should be monitored. As the virus is not removed the harmful effects of incorporation of the genome may result in development and then worsening of intra-epithelial lesions and ultimately in a risk of cancer *(figure 1)*. Depending on the studies, HPV DNA is found in 20 to 65% of ASC-US smears, in 45% to 80% of low grade epithelial lesion smears (L-SIL) and in 65-90% of high grade lesions (H-SIL). HPV prevalence in histologically classified CIN-1 low-grade lesions is 30% to 95% compared to 33 to 98% in CIN-2 and 45 to 98% in CIN-3 (high- grade). The minimum carrier time for cervical cancer to develop varies between studies from 8 to 20 years.

SEARCH INDICATIONS

In cervical screening: suspected HPV infection from an abnormal smear cytology result – triage of ASC-US *(atypical squamous cells of undetermined significance)* cytological abnormalities.

Monitoring treated CIN-2 and 3 pre-malignant lesions.

Clinical suspicion of HPV infection (veruccas or condylomata).

INFORMATION

SAMPLE

HPV test testing for high-risk HPV: The sample is taken from women using a cytology brushing by swabbing the endo-exo-cervical junction, and in men and children by scratching lesions. Freshly collected biopsy specimens (condylomata and verrucas) are also used.

HPV genotyping: muco-cutaneous and genital samples and biopsies (condylomata and verrucas).

SAMPLE STORAGE AND TRANSPORT

Cytology brushings are deposited in a transport medium and sent to the laboratory at room temperature.

Biopsy specimens are placed in a transport medium and then frozen immediately at -20°C. Biopsies under 2 mm in diameter should not be used. Avoid histology fixing media.

From a thin layer smear, HPV testing and typing can be performed on the liquid medium used for the smear. Only some liquid media are validated for HPV testing using molecular methods.

PAPILLOMAVIRUS DETECTION AND TYPING METHODS

There is no simple culture system or serological test at present to confirm the diagnosis of HPV disease. Although development of a specific HPV immune response (cellular and humoral immunity) plays an important role in removing the viruses, the mechanisms involved are still not fully understood. Serological diagnostic methods are available (detection of HPV, IgG and IgA), but these are not widely used because the immune response is variable. Direct diagnosis of HPV infections therefore relies on detecting the viral genome using molecular biology techniques.

Methods based on single hybridisation

Since the start of the 1980s the first commercial tests to detect the HPV viral genome were based on the Southern-Blot method and required oligonucleotide probes, radio-labelled with 32 phosphorous (^{32}P) to be used. Variants were developed to simplify the operating procedures for clinical use (Dot-Blot and FISH: *fluorescent in-situ hybridisation*) or to enable direct testing on slide-fixed tissues (ISH: *in-situ hybridisation*). Because of poor sensitivity (Dot-Blot), specificity (ISH) or inability to automate (Southern) these initial diagnostic methods could not be used routinely.

Method based on hybridisation in a homogeneous medium with signal amplification

HPV are routinely identified and typed usually using a commercial kit (Hybrid Capture I test), based on hybridising RNA probes in a homogeneous liquid medium and then amplifying the signal developed by chemoluminescence. This is a semi-automated test and has been extensively evaluated. It can detect 5 types of HPV belonging to the low risk group (6, 11, 42, 43, 44) and 13 types belonging to the high-risk group (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). Detection sensitivity is between 1 and 5 pg/ml of DNA or approximately 120,000 to 600,000 genome equivalents per ml of sample. The method does not provide precise viral typing but can be used on female cervical samples and on anal samples from both sexes.

Methods based on gene amplification

Various HPV methods using gene amplification (PCR: *polymerase chain reaction*) have been published. These methods use consensus or generic primers (MY09/MY11, GP5/GP6, GP5 +/GP6 +, SPF, PGMY primers) specific for preserved genomic regions across the HPV. Although the "consensus" PCR method can amplify a large number of HPV genotypes, an additional stage is required for genotyping. Depending on the primer couple used, the detection sensitivity varies for some genotypes. HPV detection and typing tests using PCR are commercially available in the form of micro-titre plates or strips (reverse hybridisation on a strip or *line probe assay*). Finally, HPV can be typed following PCR using sequencing or pyrosequencing methods or by analysing the amplification product by hybridising to DNA probes or *Microarrays*, or by real-time PCR.

TREATMENT

HPV infections can be treated by cryotherapy, vaporisation, laser, electrocoagulation, surgical resection (cone biopsy) and local application of trichloracetic acid, podophyllin, podophyllotoxin or immunostimulants, such as interferon and imiquimod.

MANAGEMENT OF CERVICAL CANCER SCREENING

Cervical cancer screening in France is based on a conventional cervico-vaginal smear or liquid medium taken at three yearly intervals (after 2 normal smears one year apart). Testing to detect HPV by primary screening appears to be "premature" (French National Health Authority, 2010). The only current indication reimbursed in France for the HBV test is triage of ASCUS smears. If the HPV test is positive, the woman can be referred for colposcopy and a lesion will be detected in approximately 50% of cases *(figure 2)*.

Interpretation of a positive HPV test:

A positive HPV test indicates that a high-risk (HR) HPV-DNA amongst these sub-types tested is present. It does not indicate whether lesions are present.

A positive HPV test is only truly significant after the age of 30-35 years old. The test is positive in at least 25 to 30% of cases in younger women (high prevalence of HPV infection) and is positive in approximately 10% of women over 35 years old, i.e. in those who have not removed the virus after several years but have developed persistent infection. A positive HPV test must be always interpreted against the cytology and/or clinical history. The main role of this test is its very high negative predictive value (> 95%).

Several medical organisations in other countries including the United States and Germany have published cancer-screening guidelines, which include the HPV test. The specific guidelines governing the management of a woman found to be a carrier of HPV were published in the October 2007 edition of the *American Journal of Obstetrics & Gynaecology.*

	Normal smear	Inconclusive smear	Abnormal smear
Non-carrier of HPV	Repeat test (HPV test and smear) every three years.	Repeat cervical smear after 12 months.	Colposcopy
Carrier of HPV	Repeat the HPV test and smear after 12 months. If any of the tests are abnormal: colposcopy	Colposcopy	Colposcopy

In addition, according to the *American College of Obstetricians and Gynaecologists* (ACOG 2005), the HPV screening test can also be used to assess the effectiveness of treatment in a woman treated for pre-malignant cervical cells (CIN-2 and 3).

ANTI-HPV VACCINATION

Vaccines available

Commercial name (Company)	Gardasil® (Merck)	Cervarix® (GSK)
Type of vaccine	Tetravalent Active on HPV 16, 18, 11 and 6	Bivalent Active on HPV 16 and 18
Adjuvant	Hydroxyaluminium	Hydroxyaluminium + MPL
Date of injections	0, 2, 6 months	0, 1 and 6 months

Vaccine recommendations

Anti-HPV vaccination is recommended in France in all 14-year-old girls, in order to protect them before they are exposed to risk of infection with the viruses. The vaccine should also be offered to girls and young women between 15 and 23 years old who have not had sexual intercourse, or no later than a year after their first sexual intercourse.

Why target 14-year-old girls for vaccination?

– After the age of 14 years old, the proportion of girls who have had sexual intercourse increases rapidly with age (the expected benefits of the vaccine are reduced).

– The 15-23 age group is the age at which autoimmune diseases may be seen (commonest fortuitous pathological associations).

– The protective antibody titres achieved are higher with younger age.

There is no danger in vaccinating a woman who has already come into contact with the virus. The only potential risk is that knowing she has been vaccinated she may consider she no longer needs screening.

Mechanism of action of vaccination

This is based on the production of neutralising antibody (Ab) on the surface of the cervix. Once the virus comes into contact with the genital mucosa and reaches the cervix, the neutralising Ab prevents the virus entering cells.

We know that in a naive population (which has not been in contact with HPV), vaccination causes seroconversion with the production of neutralising Ab at titres very much higher than are obtained after a natural infection. Immunological findings from clinical studies suggest a long-term response (more than 6 years).

Practical limits of immunogenicity

The minimum protective Ab titre is not known and there may not be a correlation between Ab titres and the clinical effects of the vaccine (this titre is not recognised as an "efficacy end point" by the WHO).

Effectiveness of vaccination

With an experience of approximately 10 years the effectiveness of the anti-HPV vaccines is almost 100% against pre-invasive cervical lesions and also vaginal, vulval lesions and condylomata acuminata (for Gardasil®) due to the viral types targeted by the vaccine. Cross-protection against other types of HPV not contained in the vaccines is liable to provide additional benefit.

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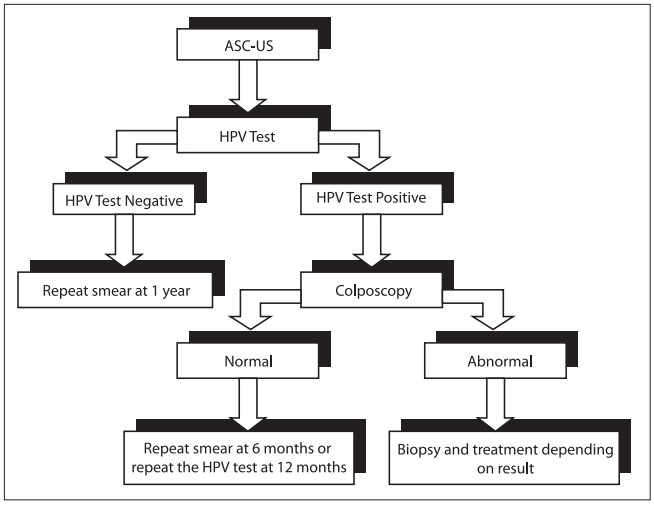

Fig. 2. Identification of HPV and triage of ASC-US atypical cells – French recommendations (Anaes 2002)

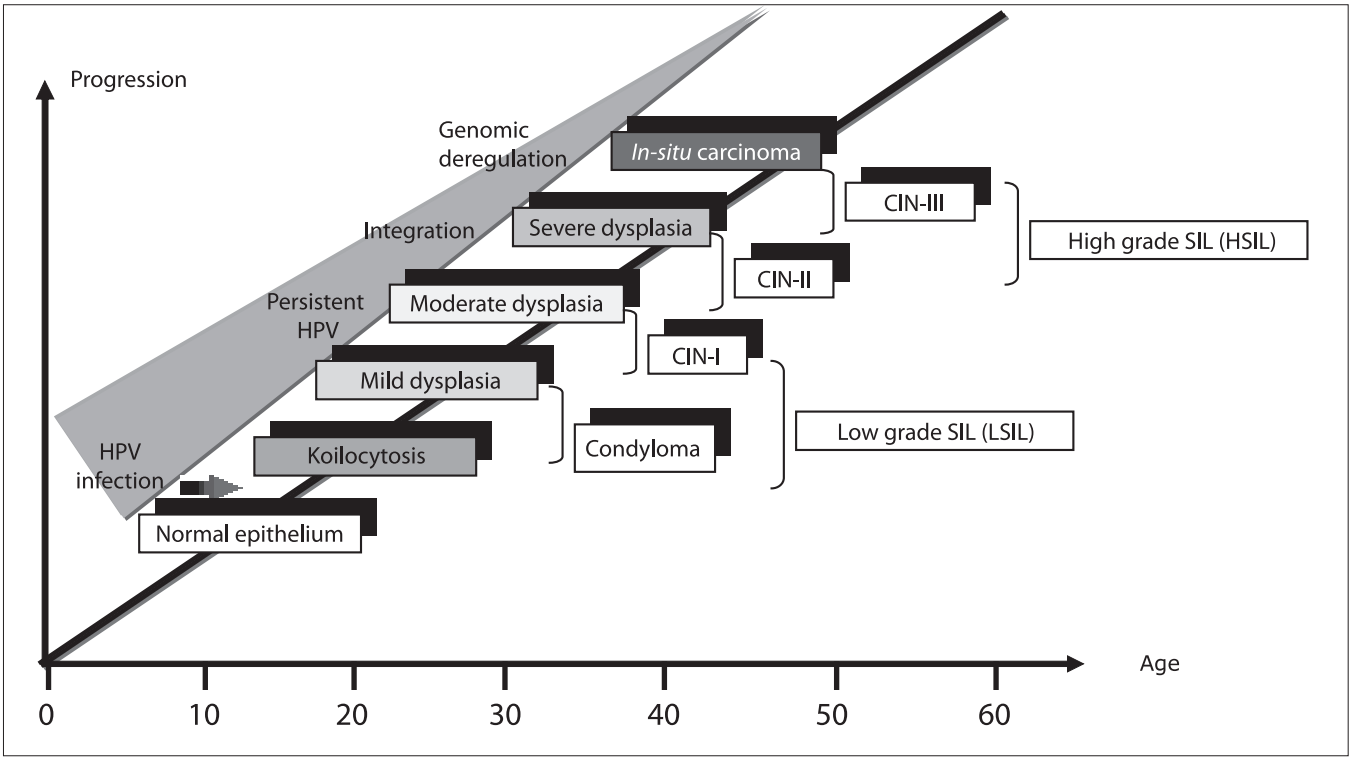

Fig. 1. Natural history of HPV - Classification of cervical lesions *by the Richart, WHO and Bethesda criteria.*

PARACETAMOL

DEFINITION

Paracetamol or para-acetylaminophenol is used for its antipyretic and analgesic properties.

Synonym: acetaminophen.

PHARMACOKINETICS

Paracetamol is rapidly and almost entirely absorbed, mostly in the stomach. The plasma peak is reached 30 to 60 minutes after an oral dose, 30% of the substance is bound to plasma proteins. Steady state is reached after 5 to 20 hours with continuous oral treatment. It has a half-life in the region of 2 hours. Paracetamol is metabolised mostly in the liver by conjugation with glucuronic acid (60%), sulphuric acid (35%) and cysteine (3%). The metabolites are then excreted by the kidney. Only 3% of the dose administered is removed in the unmetabolised form.

INDICATIONS FOR MEASUREMENT

Paracetamol is a very widely used drug and can cause acute or chronic poisoning responsible for liver disease. Measurement is indicated:

– In any patient with pain who has liver problems, in order to confirm undeclared or forgotten paracetamol use. Chronic poisoning develops after long-term ingestion of excessive amounts of paracetamol. A cumulative dose of 5 g over 24 to 36 hours or even less in people with liver disease, or if predisposing factors are present (alcoholism, malnourishment or concomitant treatment with enzyme inducers, such as rifampicin or anti-epileptic drugs).

– In suspected acute poisoning. An overdose of 10 g or more in adults (100 mg/kg in children) may cause hepatitic changes liable to produce complete irreversible necrosis. This results in hepatocellular failure, metabolic acidosis and encephalopathy, which may progress to coma and death. 12 to 48 hours after ingestion, a simultaneous rise in hepatic transaminases, LDH, bilirubin and a fall in PT develop. The treatment of overdose includes rapid removal of the substance taken by gastric lavage and administration of the antidote oral or intravenous N-acetyl-cysteine as early as possible. Paracetamol measurement in blood is usually performed as an emergency.

INFORMATION

■ SAMPLE

Dry tube (serum).

For therapeutic monitoring, the sample peak time is 1 hour post dose.

For acute poisoning, the sample should be taken in the initial hours following ingestion, preferably at 4 hours.

■ QUESTIONS FOR THE PATIENT

Any request for drug measurement must include the reasons for the request (testing for efficacy or toxicity), the sampling time, treatment start date and/or any change in dosage, dosage information (amount administered, frequency and route of administration) and the age, height and weight of the person whenever possible.

■ SAMPLE STORAGE AND TRANSPORT

24 hours at room temperature; up to 7 days between + 2 and + 8°C; – 20°C beyond this time. Transport at + 4°C.

ASSAY METHODS

Colourimetric chemical methods.

Enzymatic methods.

Immunological methods (EMIT or FPIA).

NORMAL EXPECTED VALUES

The therapeutic range one hour post dose is between 10 and 20 mg/l.

In poisoning, severity depends on the dose taken and on how soon specific treatment is started. Hepatotoxicity is classically seen at paracetamol values of between 150 and 250 mg/l, 4 hours after ingestion or between 30 and 50 mg/l, 15 hours after ingestion. The potentially fatal serum concentration is between 160 and 400 mg/l.

FOR FURTHER INFORMATION

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■ Dictionnaire Vidal®.

PARAINFLUENZA VIRUS

DEFINITION

The human parainfluenza viruses (PIV) are responsible for numerous respiratory infections in infants and young children, particularly laryngotracheitis and bronchiolitis. More rarely, they cause pneumonia in adults, particularly in the immunosuppressed. The PIV belong to the *Paramyxoviridae* family, *Paramyxovirinae* sub-family and *Paramyxovirus* genus, in which 4 serotypes (from 1 to 4) and two sub-types: 4A and 4B can be distinguished. They are RNA viruses measuring 100 to 350 nm, containing an internal helicoidal nucleocapsid surrounded by an external envelope.

INTRODUCTION

EPIDEMIOLOGY

This varies depending on the serotype: PIV 1 and 2 infections occur in the autumn as small epidemics, which recur every 2 or 3 years; PIV 3 infections are seen endemically, particularly in winter and spring. The 3 serotype causes half of childhood PIV infections, whereas PIV 4 infections are rare. Primary PIV infection generally occurs before the age of 5 years old and re-infection is common. Human-to-human transmission occurs directly, from respiratory secretions.

SYMPTOMS

The incubation period is from approximately 3 to 5 days. It is then followed by rhinitis, which progresses to laryngitis (with serotypes 1 and 2) or bronchiolitis (with serotype 3). Serotype 4 is responsible for mild rhinopharyngitis. The PIV are also responsible for acute bronchitis and pneumonia in the elderly and immunosuppressed.

SEARCH INDICATIONS

Investigation of the cause of respiratory infection in a young child, particularly if this occurs in a community (day-care centre) or hospital.

Investigation of the cause of respiratory infection in an immunosuppressed person.

As part of an epidemiological study.

Differential diagnosis of RSV bronchiolitis.

INFORMATION

SAMPLE

Nasal or tracheobronchial secretions, obtained by aspirating with a probe or swabbing for direct testing for the virus.

Serum (1 ml) for serological diagnosis.

QUESTIONS FOR THE PATIENT

Age of patient?

Clinical symptoms?

Immunosuppression?

SAMPLE STORAGE AND TRANSPORT

Samples for direct viral antigen testing should be sent, providing they are stored at room temperature for no more than 1 to 2 hours.

Smear slides are dried, fixed with acetone and transported at room temperature.

Samples for culture are collected into a transport medium and stored at + 4°C.

Serum samples are stored at + 4°C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

- **Direct testing for viral antigens:** The laboratory diagnosis of parainfluenza virus infections relies mostly on testing for viral antigens by immunofluorescence in respiratory samples, using anti-PIV1, PIV2 and PIV3 monoclonal antibodies which produce different fluorescent appearances depending on the virus (large cloudy inclusion bodies are seen with PIV1, fine granulations with a few large inclusion bodies with PIV2 and fine regular granulations with PIV3).
- **Viral culture:** This has limited use, as it is difficult to perform and the cytopathogenic effect is slow to develop.
- **Molecular biology:** RT-PCR methods still have limited use.

INDIRECT DIAGNOSIS

The complement fixation reaction (CFR) is limited, because it lacks sensitivity, particularly in young children. It is therefore used mostly in adults, looking for seroconversion in 2 samples taken 15 days apart.

INTERPRETATION

Direct immunofluorescence testing is quick to perform and demonstrates the presence of active viral infection. For this reason it is considered to be the method of choice to diagnose PIV infections.

Serology is useful in older children or adults in whom a nasal or bronchial sample cannot be obtained .

TREATMENT

At present there is no vaccine and the treatment of bronchiolitis and laryngitis is symptomatic with removal of nasopharyngeal obstruction in bronchiolitis. Administration of antipyretics and humidification of ambient air is recommended for laryngitis. Corticosteroids can be used in respiratory difficulty.

FOR FURTHER INFORMATION

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- Société Française de microbiologie, *Virus parainfluenza*, Référentiel en virologie 2000 ; 26: 107-108.

PAROXYSMAL NOCTURNAL HAEMOGLOBINURIA

DEFINITION

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare disease due to an acquired clonal mutation of the PIG-A (phosphotidylinositol glycane A) gene involved in the synthesis of a sugar, glycosylphosphatidylinositol (GPI), used to anchor membrane proteins. This mutation occurs in a haemopoetic stem cell and the clonal abnormality involves all cell lines. It therefore leads to the production of blood cells which are deficient in surface proteins which protect the cells against attack from complement.

The disease can present initially as an acquired haemolytic anaemia or secondarily, from the development of a PNH clone in bone marrow aplasia.

Classically, PNH is expressed as haemolytic anaemia developing in a young adult (average age 33 years old at the time of diagnosis although 15% are under 16 years old), accompanied by dark urine in the morning and occasionally by jaundice. The major potentially life-threatening complication is thrombosis which can also be a presenting feature.

Synonym: Marchiafava-Michelli syndrome.

INTRODUCTION

Since the first descriptions of the disease at the start of the last century, red blood cells have been shown to be abnormally sensitive to the lytic action of complement. Several authors in 1980 demonstrated that two proteins which had the role of inhibiting the action of complement failed to be expressed on the surface of red blood cells of patients suffering from PNH. These proteins are the *Decay accelerating factor* (DAF or CD55) and *Membrane inhibitor of reactive* lysis (MIRL or CD59).

PNH is therefore explained by defective expression of several membrane proteins occurring as the result of failure of synthesis of a glycosylphosphatidylinositol (GPI) anchor caused by a mutation in the *PIG A* gene, a gene formed from 6 exons and found on the short arm of chromosome X. The genetic mutation involves a clonal population of pluri-potent haemopoetic stem cells. The deficiency of membrane proteins which require GPI for anchoring therefore affects all haemopoetic stem cells (red blood cells, polynuclear cells, monocytes, platelets and lymphocytes).

In 75% of cases the *PIG-A* mutation responsible for the deficiencies causes complete abolition of gene activity. An active fraction remains in 25%. The mutations identified to date (174 described in 2000) are located over the entire gene, complicating their testing.

SYMPTOMS

The main feature of the disease is intravascular haemolysis and the resultant haemoglobinuria. Episodes of haemolysis occur particularly at night when the blood pH falls. CD55 and CD59 deficiency greatly increase red blood cell sensitivity to lysis from complement which is activated at acid pH.

The main complications are thromboembolic (high incidence of 25% at 5 years), particularly at unusual sites (portal vein thromboses, supra-hepatic vein thromboses, cerebral thromboses, etc.). Other complications include recurrent infections and attacks of abdominal pain (mesenteric microthromboses?).

It progresses to severe bone marrow failure, and even very occasionally to acute leukaemia. Forms of the disease which are stable over the long-term or even regress are however seen.

INDICATIONS FOR MEASUREMENT

Suspected PNH in the following contexts:

– Coombs negative intravascular haemolytic anaemia,

– Haemoglobinuria,

– Venous thrombosis at unusual site.

INFORMATION

SAMPLE

Whole blood taken into EDTA. Heparinised samples must not be used for molecular testing.

QUESTIONS FOR THE PATIENT

Recent transfusion? It is recommended that at least 1 month be left after a blood transfusion to assess the affected erythrocyte population.

SAMPLE STORAGE AND TRANSPORT

The samples must be transported to the laboratory at + 4°C as soon as possible (transport < 24 h); they cannot be frozen.

ASSAY METHODS

For reference, the "historic" tests used to diagnose PNH rely on identifying *in-vitro* hyperhaemolysis in the presence of acid solutions: haemolysis test in acidified serum (Ham-Dacie test) and the haemolysis test at low ionic force (Sucrose test). In the Ham-Dacie test, the patient's red blood cells were incubated with normal plasma at acid pH. Under these conditions, activated complement destroys the deficient red blood cells. The tests were significant if haemolysis exceeded 10%, although the tests lacked sensitivity.

The diagnosis of PNH is now made mostly by flow cytometry, when a PNH clone is examined for, primarily on erythrocyte and granulocyte cell lines. Studying the leukocyte population is more precise as the half-life of leukocytes is not reduced in PNH, unlike that of the red blood cells. In addition, the number of leukocytes present is independent of any past history of transfusions. The antibodies used are against CD55 and CD59 on red blood cells and granulocytes, CD16 on granulocytes and CD14 on monocytes. Some authors recommend that at least two GPI system proteins are examined to avoid interpretation errors if a specific deficiency of one of the proteins is present.

The results are expressed as % negative cells (relative size of the clone) and/or mean intensity of fluorescence (arbitrary logarithmic units). Sensitivity of flow cytometry to diagnose GPI molecule deficiency enables small PNH clones to be detected. In practice the deficiency is significant when the percentage of affected cells exceeds 5%.

Finally, molecular analysis of the *PIG -A* gene can also be performed. In view of the large number of mutations described, the use of DNA probes may facilitate the analysis by allowing the set of known *PIG-A* mutations to be screened.

NORMAL EXPECTED VALUES

Healthy people do not have PNH clones in any cell line.

PATHOLOGICAL VARIATIONS

In PNH, flow cytometry reveals reduced expression of CD16 on granulocytes, CD14 on monocytes, and CD55 and CD59 on red blood cells and granulocytes (all GPI-dependent membrane antigens).

Non-specific diagnosis: Patients suffering from PNH have high plasma LDH concentrations. Leukopaenia and neutropaenia are associated with the anaemia in approximately 50% of cases and thrombocytopaenia (moderate) in 80% of cases.

NB: Once the diagnosis has been made, it is essential that a reassessment is performed every 6 months for two years to confirm that the disease is stable.

TREATMENT

This involves mostly blood transfusion, with bone marrow allotransplantation in severe disease. Eculizumab, a recombinant monoclonal antibody which inhibits activation of the terminal complement components has been shown to be effective in reducing intra-vascular haemolysis, stabilising haemoglobin concentrations and avoiding red cell transfusions in patients suffering from PNH.

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PARVOVIRUS B19

DEFINITION

Parvovirus B19 has successively been called SPLV *(Serum Parvovirus-Like Virus)*, HPV *(Human Parvovirus)* and then B19, the initials which identify the blood bag in which it was first found.

It belongs to the *Parvoviridae* family of which it is the only pathogenic member to human beings, *Parvovirinae* sub-family and *Erythrovirus* genus in view of its tropism for erythrocyte precursors.

In principle it is strictly human, is highly resistant and is transmitted via the respiratory tract and in blood.

It is clearly associated with two types of clinical features. An eruptive form called "epidermal megalerythema" or 5th disease in children and joint features in adults. Its tropism for red blood cell haemopoietic cell progenitors makes it responsible for episodes deglobulisation in patients with chronic constitutional anaemia, chronic anaemia in the immunosuppressed and foeto-placental anasarca in pregnancy.

Synonyms: Erythrovirus B19, "epidemic erythema infectiosum" or 5th disease agent.

INTRODUCTION

PATHOGENIC POTENTIAL

It is an unenveloped virus approximately 20 nm in diameter (the small size gives it its name), containing a single strand DNA molecule of positive or negative polarity enclosed in an icosahedric capsule. Its genome codes for a small number of transcripts resulting in:

– The structural proteins PV1 and PV2 which can self-assemble in-vitro and become 5 and 95% respectively incorporated into the capsids; both proteins are immunogenic and generate specific neutralising antibodies. Irreversible protection is conferred following the first infection in immunocompetent people.

– The non-structural protein NS1, a pleiotropic regulatory protein which is believed to be partially responsible for the cytotoxicity of Parvovirus, particularly as a result of apoptosis of erythroid progenitor cells.

Parvovirus infects target cells via its receptor, the blood group P antigen, which is present on many haemopoietic cells (megakaryocytes, platelets and granulocytes) and also on pulmonary, hepatic, renal, muscle and endothelial cells. Its lytic viral cycle, however, only occurs in erythroid, bone marrow and progenitor cells (permissive cells), whereas semi-permissive cells are only graded by secondary toxicity from protein NS1 (e.g. by induction of thrombocytopaenia in primary infection). People with the "p" phenotype (without the "P" antigen), are resistant to infection.

Parvovirus cannot be grown on classical cells used for routine diagnostic purposes.

Although the variability between Parvovirus isolates is extremely small, a variant known as kV9 diverging by more than 10% from other B19 isolates has been described.

EPIDEMIOLOGY

The parvovirus is highly resistant and ubiquitous. Infections occur in epidemics at the end of winter and beginning of spring and are transmitted through the respiratory tract from which the virus may be excreted for up to 7 days after symptoms have developed. 50% of non-immune contacts are infected during an epidemic. Seroprevalence increases with age, from 10% between 1 and 5 years old, to 40% between 20 and 30 years old, rising to more than 60% after 50 years old. Primary infection is more common in children between 5 and 10 years old, although is not particularly rare in young adults, particularly in women of child-bearing age, when it is then associated with a 25 to 30% risk of vertical transmission. As a high viraemia occurs (up to 10^{14} particles/ml of serum), infection may occur from blood.

SYMPTOMS

Infection in healthy people

Primary infection in children (epidemic erythema infectiosum)

This is still called 5th disease and results in slap-like maculo-papular rash beginning on the face and then extending over 2 to 4 days to the trunk and extremities, which develops 18 days after infection. The exanthema is preceded by mild infectious disease (slight fever, general malaise and possibly rhinopharyngitis). The rash disappears over 5 to 9 days. Infection however is clinically silent in 50% of cases.

Primary infection in adults (joint features)

Joint symptoms are present in 70 to 80% of adult infections compared to only 10% of childhood infections, and are particularly common in women. The most affected joints are the hands, wrists, ankles and knees and the pain disappears over 2 to 3 weeks, although the clinical course is often long in adults and may persist for several months or in some cases even for several years with recurrent flares. These features may be entirely isolated or accompanied by erythematous or vesicular rashes or preceded by various, occasionally gastro-intestinal, problems.

Acute erythroblastopenia in people with constitutional haemolytic anaemia

Parvovirus infection in sickle cell anaemia, hereditary spherocytosis, thalassaemia or pyruvate kinase deficiency, results in a transient erythroblastopaenia crisis, characterised by sudden onset of profound anaemia which may require transfusion of concentrated red blood cells. Reticulo-cytopaenia without anaemia is seen in healthy people because of the long lifespan of red blood cells (120 days), although haemolytic anaemia or iron deficiency may also be worsened by primary infection.

Chronic anaemia in the immunosuppressed

This may occur following Parvovirus infection in patients with congenital or acquired immunodeficiency (particularly HIV +), in bone marrow transplant patients and in leukaemia patients treated with chemotherapy. As the humeral response is unable to control the infection, the chronic viraemia may cause profound sustained erythroblastopaenia or even bone marrow aplasia because of the toxicity of the virus on other cell lines.

This complication can be treated by injection of polyvalent human immunoglobulins, 0.4 g/Kg for 5 days, possibly as several courses (2 to 3).

Materno-foetal infection

Primary maternal infection is responsible for approximately 10% of cases of miscarriage, foetal death or foeto-placental anasarca. The foetus is particularly sensitive to the virus as its haemopoiesis is extremely active and the lifespan of foetal erythrocytes is very short (45 to 70 days). Anasarca typically occurs during the second trimester and the absence of cases after 27 weeks of pregnancy may be due to less intense multiplication of stem cells. Early infection is usually believed to cause spontaneous miscarriage. The pathophysiological mechanisms proposed to explain the anasarca are partially understood. Foetal anaemia, often severe, may cause congestive heart failure resulting in generalised anaemia. Direct viral damage to the myocardium may contribute to the heart failure. The foetal disease is generally found on a routine ultrasound and the signs of anasarca develop between 3 and 12 weeks after maternal infection. The anasarca should be monitored for persistence and worsening, in which case cordocentesis is indicated with a view to exchange transfusion as the anasarca may resolve spontaneously in 10% of case. Parvovirus is not teratogenic.

Other clinical features

Parvovirus has occasionally been found to be responsible for myocarditis, vascular purpura, immune thrombocytopaenia purpura, the Guillain- Barré syndrome, etc. Its involvement in fulminant childhood hepatitis is still uncertain.

It appears to be very occasionally responsible for chronic diseases (polyarthritis and anaemia) outside of a context of immunosuppression. This is believed to be due to persistence/latency of the B19 virus and its reactivation.

SEARCH INDICATIONS

– To confirm involvement of Parvovirus in a clinical picture compatible with primary infection in an immunocompetent patient.

– To establish the responsibility of the virus in acute or chronic anaemia in specific situations (constitutional anaemia and immunosuppression).

– To diagnose foetal infection in the presence of maternal primary infection or foetal ultrasound abnormalities.

INFORMATION

SAMPLES

Serological diagnosis

Serum: 5 ml of serum into a dry tube.

Foetal blood sample: Ensure no contamination occurs from maternal blood or amniotic fluid; maternal serum should be analysed in parallel.

Amniotic fluid or foetal ascitic fluid.

Direct PCR diagnosis

EDTA blood in the viraemic phase.

Bone marrow.

Amniotic fluid or foetal ascitic fluid.

SAMPLE STORAGE AND TRANSPORT

All sample types can be stored and transported (within a reasonable period) at + 4°C.

DIAGNOSTIC METHODS

INDIRECT DIAGNOSIS

Serology plays a key role in the virological diagnosis. Immunofluorescence methods most widely used are ELISA, with a purified antigen or usually, recombinant proteins produced by the Baculovirus (the structural proteins VP1 and/or VP2, targets for the neutralising antibodies). Samples are tested for the presence of IgM which develops a few days after the exanthema (12 to 14 days after infection) and which lasts for an average of 2 to 4 months or even longer. IgG develops late, approximately one week after IgM and persists throughout life in immunocompetent people.

Identification of IgM in acute erythroblastopaenia confirms the diagnosis, although IgM may not be detectable in the initial days of deglobulisation. The sample is then repeated 8 to 10 days later to retest serology or test for the genome by molecular biology techniques.

IgM may not be found in chronic anaemia in the immunosuppressed because of long-standing seroconversion and the patient's clinical state. In this case, it is reasonable to test for viral genome by PCR in bone marrow or blood.

DIRECT DIAGNOSIS

The only direct diagnostic method which can be used routinely is detection of the parvovirus genome by PCR as it can only be cultured in primary bone marrow cells, foetal liver or cord blood. These are "in-house" PCR methods which are usually qualitative, although quantitative amplification methods are being developed. *In-situ* hybridisation is also occasionally used to identify the virus in different tissues.

THE SPECIFIC CASE OF ANTENATAL DIAGNOSIS

This must be performed under the supervision of an accredited laboratory specialist.

PCR needs to be combined with serological tests. The finding of IgM in maternal blood does not indicate viral transmission to the foetus. Similarly, absence of maternal IgM does not unequivocally exclude foetal infection as the IgM may have sufficient time to disappear because of the long time interval (3 to 12 weeks) between maternal symptoms and foetal ultrasound signs. On the other hand, seronegativity in the mother excludes the diagnosis.

Investigations are therefore based on:

– Combined testing of antibodies in maternal and foetal blood (in which IgM is only found in 50% of cases of foetal infection);

– Testing for the Parvovirus genome by PCR on amniotic fluid or foetal ascitic fluid combined with testing in foetal blood. Measurement of foetal haemoglobin is used to determine whether in-utero transfusion is required (the currently accepted threshold is 8 g/dl). PCR has a specificity of 100% and a sensitivity of almost 90% under these conditions.

PREVENTION AND TREATMENT

There is no vaccine or specific antiviral treatment at present.

Primary parvovirus infection in patients suffering from chronic haemolysis may require urgent concentrated red blood cell transfusion. In some cases, injection of polyvalent gamma globulins may help to control the sudden fall in red blood cell count.

Chronic infections in the immunosuppressed must be treated by injection of polyvalent gamma globulins.

As foetal complications are rare after primary material infection, routine serological screening during pregnancy does not appear to be justified. When a contact history is found in a pregnant woman the woman's immune status must be confirmed promptly. If specific IgG is absent, injections of standard gamma globulins may be effective, although no specific studies have been conducted in pregnancy. If the mother is seronegative, the serology must be rechecked 15 days later (whether or not the woman has been given gamma globulins). If IgM develops, close interval ultrasound monitoring is required (every 8 to 15 days) until at least the 28th week of pregnancy, invasive samples only being taken if anasarca develops. No specific surveillance is required if anti-Parvovirus IgM remains negative.

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PASTEURELLOSIS

DEFINITION

The pasteurelloses are anthropozoonoses caused by bacteria belonging to the Pasteurella genus and Pasteurellacae family. They are widespread throughout the world. Animal pasteurellosis usually results in respiratory disease. Human pasteurellosis is due to inoculation of the bacterium *"Pasteurella multocida"* into human beings.

INTRODUCTION

EPIDEMIOLOGY

Pasteurella multocida is an opportunistic commensal pathogenic bacterium of the oral cavity and saliva of most domestic animals (cats and dogs) and also of some wild animals. Generally, human infection occurs through an animal bite or scratch or through a plant sting. Infection can also develop in pre-existing skin lesions, as a result of inoculation of the bacterium contained in the oropharyngeal flora from an animal lick, or indirectly through objects soiled by animal waste.

SYMPTOMS

After a short incubation period (average 3 to 6 hours and always less than 24 hours), severe pain develops at the inoculation site usually on the hands and arms. The wound becomes very inflamed, oedematous and discharges pus and serous fluid. Loco-regional reactions may develop particularly without treatment and involve inflamed lymphadenopathy, arthritis of neighbouring joints and lymphangitis. The skin lesions recover although late complications occur producing clinical features of a chronic algo-dystrophy syndrome of the affected limb. Inoculation pasteurelloses occurring in a healthy people usually have a benign clinical course, although the same does not apply for frail patients (with cirrhosis, cancer, diabetes, respiratory insufficiency, etc.) who may progress to a systemic infection with bronchopneumonia developing on top of respiratory disease, septicaemia, neuro-meningeal disease, endocarditis and gastro-intestinal problems.

SEARCH INDICATIONS

Diagnosis of human pasteurellosis in the presence of suggestive symptoms and/or a history of animal bite or scratch.

Diagnosis of human pasteurellosis in an inoculation disease in an exposed person (veterinary practitioner or abattoir staff).

Diagnosis of human pasteurellosis in systemic infection in a frail patient.

Differential diagnosis with other animal inoculation diseases, such as cat scratch disease, etc

INFORMATION

SAMPLE

Serous fluid from the wound collected onto a swab or aspirated with a syringe.

Various samples depending on the symptoms for systemic disease: bronchial brushing, CSF, pleural fluid, etc.

Blood cultures.

Serum samples.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

Associated disease (diabetes, immunosuppression, etc.) ?

A history of an animal bite or scratch?

Person exposed through their occupation?

Current antibiotic therapy?

SAMPLE STORAGE AND TRANSPORT

Serous samples from wounds or other pathological products must be cultured as quickly as possible.

Serum samples may be stored for a few days at + 4°C.

Blood cultures should be transported as promptly as possible at room temperature. Bottles should be placed in an incubator at 37°C as soon as possible.

DIAGNOSTIC METHODS

BACTERIOLOGICAL DIAGNOSIS

- **Direct examination:** testing for Gram-negative coccobaccili with, often pronounced, bipolar staining and appearances of small canoes.
- **Culture:** isolation of the organism from wound serous fluid or various other samples. The media used are fresh blood agar or supplemented chocolate agar. Translucent greyish coloured, occasionally mucous-like colonies grow over 24 to 48 hours at 37°C.
- **Identification:** based on characteristic features (catalase + oxidase variable) and on the specific biochemical features tested by inoculated into an API 20® gallery.

SEROLOGICAL DIAGNOSIS

The serological method used is passive haemagglutination, which involves detecting anti-type A or D anti-capsule antibodies (A and D are the human strains of the 5 types which exist: A, B, D, E and F). This is difficult to interpret as antibodies are found in people in contact with household animals and those who are exposed through their occupation. Serology is also almost always negative in acute or sub-acute localised pasteurelloses, which are the commonest diseases. It is mostly of epidemiological interest although is also used when culture has not been possible or in systemic disease. The Reilly pasteurellin intra-dermal reaction is no longer performed.

TREATMENT

Following an animal bite or scratch in human beings: scrupulous wound cleansing with prompt application of an antiseptic combined with antibiotic therapy. The antibiotics used are the beta-lactams (amoxicillin), tetracyclines and the fluoroquinolones. Acquired resistance to these antibiotics in France is rare for human strains although this does not apply to animals, particular bovine strains, which often produce beta-lactamase. The association of amoxicillin + clavulanic acid is highly recommended in these situations.

Systemic disease is treated with an intravenous 3rd generation cephalosporin and amino glycoside.

FOR FURTHER INFORMATION

■ Avril J.L., Donnio P.Y., *Pasteurelloses*, Encycl Med Chir. Elsevier Paris; 8-035-C-10 1996: 4 p.

PCA 3 (*PROSTATE CANCER GENE 3*)

DEFINITION

A "pseudo-gene" called DD3, then PCA3 *(Prostate cancer gene 3)* was discovered in 1999. PCA3 expression is limited to the prostate and is extremely low in healthy prostate cells, although PCA3 is over-expressed by malignant prostate cells (≅ 60 to 100 times).

INTRODUCTION

Prostate cancer is the commonest cancer in men over fifty years old and is the second leading cause of deaths from cancer. Early diagnosis (or screening) via a rectal examination and measurement of serum PSA *(Prostate Specific Antigen)* concentration is offered in France to men between 50 and 75 years old. This age is reduced to 45 years old in men with risk factors (family history).

If the rectal examination and/or serum PSA concentration is abnormal a transrectal prostate biopsy is required under ultrasound guidance to investigate for the presence or absence of prostate cancer.

If the biopsy is positive a diagnosis of cancer is made. Treatment depends on age, life-expectancy and how aggressive the cancer is (surgery, radiotherapy, hormone therapy or monitoring).

If the biopsy is negative, monitoring is offered via PSA measurements and repeated biopsies depending on the patient's age and/or changes in the PSA (kinetics of PSA increase or "PSA velocity").

PSA antigen is a specific marker for the prostate but not for prostate cancer. Benign prostatic hyperplasia (or prostatic adenoma) and infections or inflammation may also cause a rise in PSA. PSA values are also normal, i.e. < 4.0 ng/ml in approximately 15% of cases of prostate cancer. The PSA test is therefore poorly sensitive and is not specific (producing many false negatives, false positives and unnecessary biopsies). Each positive PSA leads to one or more biopsies, which have several disadvantages, they are expensive to the health care system, result in discomfort to the patient and are a cause of concern and worry and a risk of infection and/or of bleeding.

INDICATIONS FOR MEASUREMENT

2nd line test:

– Aid to the diagnosis of prostate cancer.

– Prognostic indicator in patients with abnormal serum PSA and a normal biopsy result.

– Aid to the clinician in deciding whether or not to repeat biopsies in a patient.

INFORMATION

SAMPLE

A rectal examination should be performed with sufficient pressure on the prostate in order to release a maximum number of prostate cells into the urine and 20 to 30 ml of the next urine pass should be collected; 5 ml transferred into 2 tubes of transport media at room temperature and then sent to the laboratory.

SAMPLE STORAGE AND TRANSPORT

Sample stability: 7 days at between + 8°C and + 30°C or 14 days at between + 2°C and + 8°C.

ASSAY METHODS

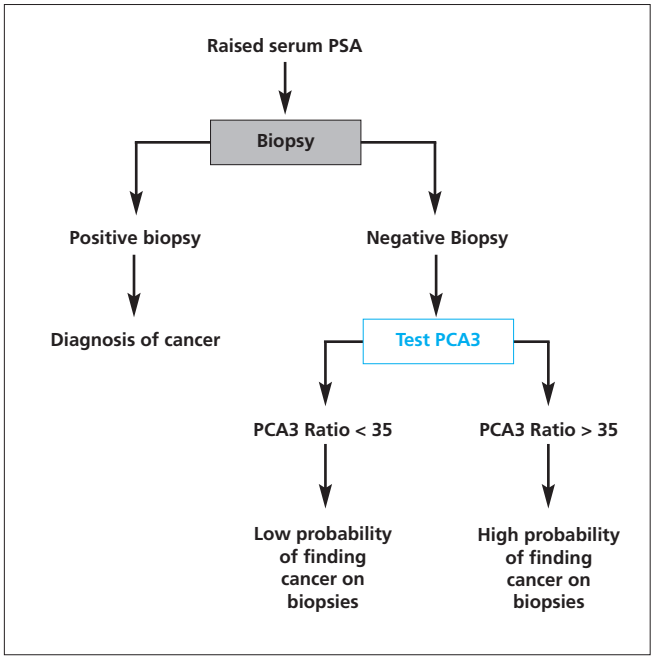
PROGENSA™ PCA3 test. Molecular biology method on prostate cells.

INTERPRETATION

This is based on analysis of the PCA3/PSA ratio, which is independent of prostate volume.

– PCA3/PSA < 35 → ratio: Low probability of cancer being diagnosed on subsequent biopsies.

– PCA3/PSA > 35 → ratio: High probability of cancer on subsequent biopsies.



FOR FURTHER INFORMATION

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PHENCYCLIDINE

DEFINITION AND SYNONYMS

Phencyclidine or PCP is an anaesthetic developed in the 1950s, which has no longer been used in human treatment since 1965 because of its side effects, including agitation and confusion. It has since been synthesised illegally and sold under the name "angel dust" or "ozone" and is occasionally associated with cannabis under the name *"killer joints"* or *"crystal supergrass"* or with other drugs such as LSD, amphetamines or cocaine. It is a white crystalline powder, soluble in water and alcohol and has a bitter taste. It can be easily mixed with dyes and is found in different forms of tablets, capsules and powder. It can be smoked (in which case it can be applied to mint or marijuana leaves), sniffed, ingested or administered intravenously.

INTRODUCTION

Phencyclidine is both hallucinogenic and a psychostimulant. Its peak effect is reached a few minutes after a dose and lasts 2 to 48 hours. It is highly lipid soluble and is stored in fats from which it is released slowly, explaining firstly why the withdrawal syndrome is poorly understood and secondly why metabolites are found long after a dose. Phencyclidine is metabolised by the liver with a half-life of 18 hours, although this can be shorter if urine pH is acid. It induces moderate to strong psychological dependency, unlike the other hallucinogens.

Phencyclidine has a narrow safety margin and its effects vary depending on the dose taken:

– At low dose, stimulant effects are seen including agitation, excitation, coordination disorders, dysarthria, analgesia, nystagmus, polypnoea, hypertension, flushing, sweating, hyperacousia, distorted body image and disordered thinking.

– At high dose the sedative effects occur with hypersalivation, visual disturbance, vomiting, fever, myoclonia, rhabdomyolysis, hypotension, bradypnoea, ataxia, seizures and coma.

– Death from multifactorial causes may occur with respiratory congestion and depression, hypertension causing hypertensive encephalopathy, cerebral vasospasm and cerebral haemorrhage.

Following single use, subjects develop a psychosis similar to schizophrenia, amnesia, diarrhoea, abdominal pain and tooth grinding. With chronic use, insomnia, anorexia and depression occur, associated with an increased risk of suicide, psychosis, growth retardation and learning disorders in adolescents, together with memory problems, confusion and language disorders which may last for several months after the last dose.

INDICATIONS FOR MEASUREMENT

Testing, possibly combined with quantitative determination of phencyclidine and its metabolites, mostly in urine and possibly in blood, is designed to identify phencyclidine having been taken within the days or weeks before the sample is taken. As the molecule is lipophilic, a storage compartment exists within the body's fatty tissues from which phencyclidine can be released, sometimes for up to several months after it has stopped being taken, particularly following chronic use in a person with a large amount of fatty tissue.

INFORMATION

■ SAMPLE

In blood the measurement is performed on serum (dry tube). Do not use a tube with phase separator. The sample can be taken at any time of the day. The tube should be centrifuged and the serum separated and stored at + 4°C.

The urine sample is a random urine collected at any time of the day, preferably obtained at the laboratory in order to avoid the risk of substitution. The patient's identity and sample temperature which should be between 32 and 38°C, with a pH between 3 and 11, a nitrate concentration less than 500 µg/ml, a density between 1.0010 and 1.0200 and a creatinine level between 20 and 200 mg/l, should be checked according to the American recommendations published in April 2004.

■ QUESTIONS FOR THE PATIENT

It is important to establish from the patient when he/she last took phencyclidine and whether this was single or chronic use. As phencyclidine can be associated with other drugs, patients should be asked if they are using other drugs. Phenothiazines potentiate the anticholinergic effects of phencyclidine and patients should be asked if they have taken these (phenothiazines may produce a false positive result for LSD, use of which may be associated with phencyclidine).

■ SAMPLE STORAGE AND TRANSPORT

Samples are stable for several days when refrigerated and for several months when frozen.

ASSAY METHODS

The main methods available are immunoenzymatic screening tests and confirmatory methods using chromatography linked to mass spectrometry.

NORMAL EXPECTED VALUES

Phencyclidine is normally absent in blood and urine samples.

The *National Institute on Drug Abuse* (NIDA) has set a positivity threshold in urine of 25 ng/ml.

PATHOPHYSIOLOGICAL VARIATIONS

Phencyclidine is found in urine between a few days and a few weeks after it was last taken (15 to 30 days on average). This time varies depending on whether the drug has been taken on a single occasion or chronically, the amounts taken, the person's fat mass and urine pH, as acid pH promotes its removal.

FOR FURTHER INFORMATION

■ NIDA info facts, *PCP (Phencyclidine)*. www.drugabuse.gov

PHENOBARBITAL

DEFINITION

Phenobarbital (5-ethyl, 5-phenyl barbituric acid) is the class leader of the barbiturates, used in human therapy for their anti-epileptic, sedative and hypnotic properties.

INDICATIONS FOR MEASUREMENT

Measurement of serum phenobarbital concentrations is used to confirm that the dose is effective and not within the toxic range, particularly when concomitantly administered with other medicinal products which influence the metabolism or compete with phenobarbital for protein binding.

Measurement is indicated:

– When treatment is started (after reaching steady state in order to adjust dosage).

– If treatment is ineffective, to monitor adherence to treatment or consider a change in treatment.

– If signs of overdose appear, such as nausea, vomiting, headache, clouded consciousness, confusion, coma, accompanied by a characteristic autonomic syndrome (irregular bradycardia, tracheo-bronchial congestion and hypotension). These symptoms develop within an hour after a massive dose.

– When associated with other medicinal products or in some clinical situations which may alter the metabolism of phenobarbital:

- Phenobarbital is a potent enzyme inducer which alters its own pharmacokinetics and those of many associated medicinal products, such as anti vitamin K, cyclosporin, tacrolimus, corticosteroids, digitoxin, dihydropyridine, disopyramide, doxycycline, oestrogen-progestogens and non-contraceptive progestogens, folates, thyroid hormones, hydroquinidine, quinidine, itraconazole, theophylline and aminophylline, zidovudine and other anti-epileptic agents, etc.

- Association with valproic acid or valpromide carries a risk of increased plasma phenobarbital concentrations.

INFORMATION

SAMPLE

Serum or EDTA or heparinised plasma.

For therapeutic monitoring, the sample must be taken before the next dose of the drug (trough level), always at the same time. The sample should wait until pharmacokinetic steady state has been reached, after 5 half-lives or 15 - 30 days after starting treatment. In suspected overdose the samples are taken whenever clinical signs develop.

QUESTIONS FOR THE PATIENT

Any request for drug measurement must include the reasons for the request (testing for efficacy or toxicity), the sampling time, treatment start date and/or any change in dosage, dosage information (amount administered, frequency and route of administration) and the age, height and weight of the person, whenever possible.

Current treatment, such as valproic acid, sodium valproate, valpromide, carbamazepine, phenytoin, anti vitamin K drugs and oestrogen-progestogens, etc.

SAMPLE STORAGE AND TRANSPORT

8 hours at room temperature; 7 days between 2 and 8°C; and for several weeks at –20°C.

Transport at +4°C.

ASSAY METHODS

Most methods used are immunoassays. HPLC is not widely used.

NORMAL EXPECTED VALUES

The therapeutic range for anticonvulsant therapy is between 15 and 40 µg/ml (65 and 175 µmol/l). The main side effect which develops at concentrations in excess of 40 µg/ml (175 µmol/l) is drowsiness. Concentrations over 50 µg/ml (215 µmol/l) are considered to be toxic.

FOR FURTHER INFORMATION

■ Berny C., *Dosage des médicaments*, tome II, Cahier BIOFORMA N° 18, Paris, janvier 2000, chapitre phénobarbital: 58-68.

■ Dictionnaire Vidal®.

PHENOL

DEFINITION AND SYNONYMS

Phenol or monohydroxybenzene (molecular mass 94.11) is a solid at room temperature (20°C) in the form of colourless, hydroscopic, sharp and sickly- tasting, soft needles or crystals. It is 8.7% m/m soluble in water at 25°C and in all proportions at 66°C. It is also soluble in numerous organic solvents (particularly ethanol and diethyl oxide). Phenol is used in the plastics and synthetic fibre industry, in petrol refining, in the pharmaceutical industry and in the manufacture of detergents, pesticides, dyes and oil additives.

INTRODUCTION

Phenol enters the body rapidly through all routes, particularly the skin and respiratory tract, which are most frequently involved in poisoning. After absorption, phenol is partly sulpho- (approximately 20%) and predominantly glucurono-conjugated (80%) and then rapidly removed in urine, usually over 24 hours. Its half-life of elimination is approximately 3.5 hours. Traces of pyrocatechol and hydroquinone are also found in urine. It does not accumulate in the body. Its metabolism may vary, depending on the dose absorbed and on diet.

Acute poisoning is rare and mostly occurs through the skin, as inhalation exposure is rarer because of the low volatility of phenol. The severity of poisoning depends on the contact time, size of area exposed, concentration of the solution and individual susceptibility. Symptoms develop over fifteen to twenty minutes and include headache, muscle weakness, dizziness, visual and auditory disturbances, tachypnoea, slow pulse and loss of consciousness. Renal (oliguria and anuria) or hepatic (necrosis) damage may also be seen. Death may occur between 30 minutes and a few hours after contact. Inhalation poisoning also produces signs of respiratory irritation (cough and dyspnoea) and in addition to the caustic effect on the gastro-intestinal tract, poisoning from ingestion is characterised by neurological (coma and seizures), cardiovascular (bradycardia, excitability disorders and collapse) and hepatitic changes, methaemoglobinaemia with haemolysis and renal tubular necrosis. Late onset pulmonary oedema occurs commonly. Because of the analgesic action of the substance, pain from burning of the area of skin exposed may be treated after some time, worsening symptoms.

Chronic toxicity involves gastro-intestinal problems (vomiting, swallowing difficulties, drooling, diarrhoea and anorexia), nervous disorders (headaches, fainting, dizziness and mental disorders) and skin problems (erythema and eczema). These problems are known by the term "phenol marasmus". In severe cases, hepatic and renal disease may occur and fatal cases have been reported.

INDICATIONS FOR MEASUREMENT

Blood or particularly urine phenol measurement is indicated for the laboratory monitoring of phenol exposure.

INFORMATION

SAMPLE

Samples for blood measurements must be collected into lithium heparin tubes.

Blood or urine samples (into a plastic bottle), should be taken at the end of shift on any day of the week. Use of substances containing phenol, such as detergents or antiseptics other than those used occupationally for which exposure is to be assessed, should be avoided for 24 hours before the sample.

QUESTIONS FOR THE PATIENT

Is the patient exposed to phenol in their occupation?

Is the patient exposed to benzene?

Is the patient exposed to household products (disinfectants and antiseptics)?

Is the patient taking medicinal products, such as phenylsalicylates, non-steroidal anti-inflammatory drugs, analgesics, antibiotics or allopurinol?

Does the patient suffer from renal or hepatic insufficiency or gastro-intestinal disorders?

When was the sample taken with respect to phenol exposure?

Does the patient smoke?

SAMPLE STORAGE AND TRANSPORT

Urine or plasma samples are stable for several days refrigerated and for several weeks when frozen.

ASSAY METHODS

Assay methods use colourimetry, gas phase chromatography or high performance liquid chromatography. Gas phase chromatography methods are preferred.

NORMAL EXPECTED VALUES

The reference value in the general population is below 30 mg of phenol/g of creatinine for urinary phenol. The French, Swiss and Quebec guideline value is 250 mg/g of creatinine at the end of shift for urinary phenol (300 mg/g of creatinine in Finland). No reference values are available in the general population nor are there guideline values for blood concentrations in exposed people.

PATHOPHYSIOLOGICAL VARIATIONS

Phenol concentrations rise in proportion to exposure. Phenol measurement, however, is non-specific and interferences with benzene, household products (disinfectants and antiseptics) and drugs (phenylsalicylates, NSAID, analgesics, antibiotics and allopurinol) should be taken into account. Renal or hepatic disease can also alter its removal. Individual variations associated with smoking, sex, circadian rhythm and aromatic amino acid metabolism may have a large impact on the phenol concentrations found.

FOR FURTHER INFORMATION

- Fiche Biotox, *Phénol*. www.inrs.fr

PHENYTOIN

DEFINITION

Phenytoin is a derivative of hydantoin, 5,5-diphenyl-2,4-imidazolidinedione, mostly used for its anticonvulsant properties in the treatment of epilepsy.

INDICATIONS FOR MEASUREMENT

The steady state relationship between phenytoin dosage/serum concentrations is highly variable and even unpredictable. Measurement in blood can be used to confirm that the dosage is effective and not reaching the toxic range.

Measurement is indicated:

– When treatment is started, after reaching steady state, to adjust dosage.

– If treatment is ineffective, to monitor adherence to treatment or suggest a change of treatment.

– If signs of overdose develop, such as nausea, dizziness, confusion and gingival erythema.

– In acute poisoning: The signs of this are calmness, hypotonic, hyporeflexic coma with respiratory depression and miosis.

– When associated with other medicinal products or in some clinical situations which may alter phenytoin metabolism.

INFORMATION

■ SAMPLE

Serum or heparinised plasma. The sample must be taken before the dose of the drug (trough concentration), always at the same time. For therapeutic monitoring the sample should wait until pharmacological steady state is obtained after 5 half-lives or, for phenytoin, 7 to 8 days after starting treatment or changing dose. If overdose is suspected the sample is taken when clinical signs develop.

■ ESSENTIAL INFORMATION

Any request for drug measurement must include the reasons for the request (testing for efficacy or toxicity), the sampling time, treatment start date and/or any change in dosage, dosage information (amount administered, frequency and route of administration) and the age, height and weight of the person whenever possible.

■ QUESTION FOR THE PATIENT

Are you taking any medicines? Phenytoin is an enzyme inducer which alters its own pharmacokinetics and those of many concomitant drugs.

Reduced serum/plasma phenytoin concentrations in association:

– With carbamazepine: reciprocal reduction in plasma concentrations with no apparent change in anti-epilepsy effectiveness.

– With folates: due to an increase in its metabolism, for which folate is a cofactor.

– With sucralfate (reduced gastro-intestinal absorption of phenytoin).

Increased serum/plasma phenytoin concentrations (with development of signs of overdose) when associated with chloramphenicol, cimetidine, disulfuram, phenylbutazone, sulfamethoxazole, sulfafurazol, sulfamethizol, amiodarone, fluconazole, miconazole, fluoxetine, fluvoxamine, isoniazid, nifedipine, rifampicin, ticlopidine and viloxazine.

Variations (unpredictable) in serum/plasma phenytoin concentrations and when associated with valproic acid, valpromide, phenobarbital, primidone or diazepam.

■ SAMPLE STORAGE AND TRANSPORT

Serum or plasma: 24 hours at room temperature; 7 days between + 2 and + 8°C; - 20°C beyond this time.

ASSAY METHODS

Chromatographic methods (gas phase chromatography, HPLC) are not commonly used.

The most commonly used methods are homogeneous or heterogeneous phase competitive immunological methods: FPIA and EMIT are the most widely used.

NORMAL EXPECTED VALUES

The trough therapeutic range at steady state is between 10 and 20 µg/ml. Concentrations over 25 µg/ml are considered to be toxic.

FOR FURTHER INFORMATION

■ Berny C., *Dosage des médicaments*, tome II, Cahier BIOFORMA N° 18. Paris, janvier 2000; chapitre phénytoïne: 69-80.

■ Dictionnaire Vidal®.

PHILADELPHIA CHROMOSOME (PH+)

DEFINITION

Chronic myeloid leukaemia (CML) is a malignant blood disease characterised by clonal myeloid cell proliferation affecting the stem cells and their descendants (without maturation blockage) in the bone marrow and peripheral blood.

It is characterised by the presence of a chromosomal abnormality, Philadelphia chromosome or Ph+.

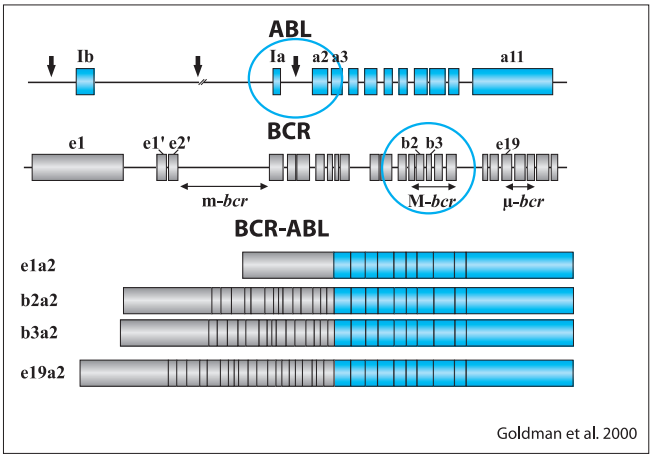
Since the last decade, specific treatment for CML has been available with a new treatment class called the tyrosine kinase inhibitor (TKI), the group leader being GLIVEC® (Imatinib mesylate).

PATHOPHYSIOLOGY

The Philadelphia chromosome is the result of a reciprocal translocation between chromosomes 9 and 22 in the 9q34 position in the Abelson gene (ABL) and position 22q11 in the BCR gene *(Break point Cluster Region)* respectively. It was described for the first time in 1960 by Nowell and Hungerford.

On a molecular basis, the translocation induces the formation of a fusion gene, *BCR-ABL*, coding for a chimeric protein, BCR-ABL, with high tyrosine kinase activity (constitutional activity). This tyrosine kinase activity is involved in different cell signalling pathways and is responsible for the disease. The site of the breakage point on the genes varies, resulting in different mRNA.

In the great majority of cases (95%), the breakage in chromosome 22 occurs in the M-BCR (M for major) region. Transcripts from this breakage are translated into a 210 kD protein(b2a2 and b3a2 transcripts). Rarer rearrangements also exist, with breakage points in the m-BCR region (m for minor, e1a2 transcript) forming a 109 kD protein or in the µ-BCR region (e19a2 transcript) forming a 230 kD protein. Other rare transcripts are also described.



The BCR-ABL chimeric protein is a membrane protein with a cytoplasmic domain. The SH1 domain of the ABL part contains the kinase site which binds ATP. The BCR part of the protein contains the dimerisation domain. Dimerisation of BCR-ABL causes constitutional activation of the BCR-ABL kinase site.

Many substrates are phosphorylated by BCR-ABL: GRB-2, SHC, STAT5, DOK and CRK. Phosphorylation of these substrates activates different cell signalling pathways and the MAP-RAS kinase pathway, the STAT pathway (increasing production of myeloid growth factors: IL3, GM-CSF and G-CSF), PIK3, MYC, SRC *family kinase* and NF-KB. On a cellular level, the consequences of activation of these different pathways are an increase in cell proliferation, a reduction in adherence to the bone marrow stroma and reduced apoptosis.

INFORMATION

SAMPLE

For conventional cytogenetics and FISH, the samples must be obtained sterile: bone marrow, blood, and effusion fluids. Blood and bone marrow samples must be taken into a heparinised tube (or heparinised syringe for bone marrow).

For molecular biology: whole blood taken into an EDTA tube or PAX gene tube.

The sample must be accompanied by a clinical information form stating the proposed diagnosis or known diagnosis of the disease during follow-up, the stage of the disease, current treatment and other haematological laboratory findings.

SAMPLE STORAGE AND TRANSPORT

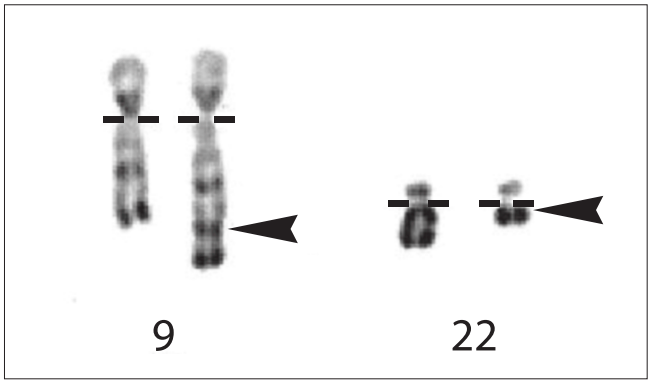
The sample must be transported as quickly as possible to the laboratory that will be performing the karyotype analysis.

For molecular biology: the EDTA tubes must be sent to the laboratory within 3 hours of sampling; PAX gene tubes must be frozen within 4 hours of sampling and transported frozen.

DIAGNOSTIC METHODS

BONE MARROW KARYOTYPE

In conventional cytogenetics the Ph chromosome can be identified by finding t(9;22)(q34;q11) in the karyotype. The bone marrow karyotype remains the reference method for the management of patients suffering from CML. Its use is essential in the diagnosis and in follow-up. It also enables additional chromosomal abnormalities to the diagnosis to be identified together with those which develop during the course of the disease (trisomy 8, isochromosome i(17q), trisomy 19, duplication of Philadelphia chromosome or t(3;21)(26;q22)).



It is important to note however, that the Ph1 chromosome is not visible in the karyotype in 10% of patients suffering from CML (a hidden "Phi" due to insertion between chromosomes 9 and 22 or the existence of a complex rearrangement). In this case, another method should be used.

FLUORESCENCE *IN-SITU* HYBRIDISATION OR FISH

This is a molecular cytogenic method used to identify the *BCR-ABL* fusion gene. It involves hybridising nucleic acid probes labelled with a fluorochrome to interphase or metaphase preparations.

The fluorescent probes used are produced in order to identify all of the possible *BCR-ABL* rearrangements: M, m and µ.

This method can also identify the presence of a deletion in band 9q34 of the 9 translocated t(9;22) product, which is reported in approximately 15% of patients suffering from CML.

If karyotype culture fails the FISH method can be used to easily identify the *BCR-ABL* fusion gene.

MOLECULAR BIOLOGY: PCR METHODS

Molecular biology analysis of *BCR-ABL* transcripts is now essential for the management of CML. It is performed after amplification using the PCR method. Because of the many breakage points on the BCR and ABL genes, it is not the DNA which is amplified directly but the corresponding mRNA, using RT-PCR techniques. Technically, the RMA is extracted from leukocytes and the complementary DNA (cDNA) is then synthesised using a reverse transcriptase (RT). This is amplified in turn by PCR.

Many methods are available depending on the indications:

Multiplex RT-PCR: qualitative method

This method is used to test for all *BCR-ABL* transcripts.

Several primer couples are used simultaneously to detect the different possible rearrangements: e1a2, b2a2, b3a2, e19a2, etc.

This is a qualitative method used to identify a type of *BCR-ABL* transcript for a given patient. It is useful to confirm the diagnosis of CML in a Ph- karyotype patient and to assess residual disease (quantification of the transcript on treatment requires it to have been previously identified).

RQ-PCR: quantitative method

This is a real time RT-PCR, i.e. quantiative RT-PCR. It is used to measure the number of *BCR-ABL* transcripts present and to assess their kinetics of change over time. For this reason, it is the reference method for the management and follow up of residual disease in patients suffering from CML.

The *BCR-ABL* transcript can be quantified in bone marrow or blood. It is measured as a ratio to expression of a reference gene (generally the *ABL* gene).

The method can be used to precisely assess tumour mass at diagnosis and during follow-up. It can also be used to assess the effectiveness of treatment, particularly for patients in complete haematological and cytogenetic remission, as it can quantify residual molecular disease.

A qualitative RT-PCR at the time of diagnosis is essential to determine the transcript type and to use the corresponding primers.

INDICATIONS FOR INVESTIGATION

	Bone marrow karyotype	FISH	RT-PCR	RQ-PCR
Diagnosis and/or before starting treatment	Mandatory	Not mandatory at diagnosis (except if Ph- with suggestive clinical picture or culture failure)	Recommended	Recommended
Evaluation of response to treatment/ residual disease	Recommended every 6 months in the first year and then annually	If Ph- at diagnosis or culture fails	No	Recommended every 3 months for 1 to 2 years then every 6 months.

MONITORING DISEASE ON TREATMENT: INTERPRETATION OF RESULTS

Assessment of cytogenetic and molecular response is essential in the evaluation of response to treatment. The aim of treatment is to abolish Ph+ cells.

Monitoring cytogenetic and molecular response.

Response to treatment		% of Ph+ cells
Major	Complete	0%
	Partial	1 -35%
Minor		36 -65%
Minimal		66-95%
No response		96-100%

The MMolR or major molecular response is a primary treatment objective. It is defined as a reduction in the *Bcr-Abl* transcript of 3 log units or more, since diagnosis.

Complete molecular response is a *Bcr-Abl* level of less than the level of detection by RQ-PCR (undetectable residual disease).

The aim of treatment is to achieve MMolR at 18 months and then to obtain a complete molecular response.

Time	Failure	Suboptimal response	Warnings	Optimal response
Diagnosis	N/A	N/A	High risk, del 9q, additional chromosomal abnormalities	N/A
3 months after diagnosis	No HR	Less than HR	N/A	CHR
6 months after diagnosis	Less than CHR, no CgR (Ph+ >95%)	Less than PCgR (Ph+>35%)	N/A	At least PcGR (Ph+> 35%)
12 months after diagnosis	Less than PCgR (Ph+>35%)	Less than CCgR	Less than MMolR	CCgR
18 months after diagnosis	Less than CCgR	Less than MMolR	N/A	MMolR

A suboptimal response is defined as a decrease in the Bcr-Abl transcript level not achieving the MMolR at 18 months. It should be noted that failure to achieve MMolR at 12 months is a warning signal and requires increased patient monitoring.

Finally, resistance to treatment is reflected by an arrest in the fall in the *Bcr-Abl* transcript level and treatment escape by an increase in the Bcr-Abl level confirmed on two successive RQ-PCR tests.

N/A: not applicable

HR: haematological response (Pt <450, WBC <10, no myelaemia and basophil count < 5%)

CHR: complete haematological response

CgR: cytogenetic response CCgR: complete CgR
PCgR: partial CgR

MMolR: major molecular response.

ACTION TO TAKE FOR FAILURE TO ACHIEVE OR LOSS OF MMOLR

If a molecular response is not achieved or is inadequate, the first action is to ensure correct adherence.

The following may be considered if adherence is good:

- Plasma imatinib measurement: some patients even when treated correctly at a dose of 400 mg/day, do not achieve effective plasma concentrations. In addition, some drug interactions may interfere with effective imatinib concentrations.
- Investigation for point mutations in the BCR-ABL tyrosine kinase domain must be performed. This is a relatively common resistance mechanism. Disease progression is preceded by the emergence of certain mutations. Mutations located in the P loop are a poor prognostic indicator. The T315I mutation confers resistance to treatment regardless of the TKI uses.

FOR FURTHER INFORMATION

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PHOSPHOLIPIDS

DEFINITION ET INTRODUCTION

Phospholipids are either glycerides containing a basic structure of phosphatidic acid consisting of a glycerol molecule esterified with two fatty acids and a phosphoric acid, or sphingomyelins.

The main phospholipids in the body are phosphatidylcholine (70%) and the sphingomyelins (20%). Monitoring phospholipid composition in serum has been proposed for the assessment of special fatty acid diets.

Phosphatidylcholin (lecithin)	70%
Sphingomyelin	20%
Phosphatidyl serine and ethanolamine	3% to 6%
Lysophosphatidyl choline	4% to 9%

Serum phospholipids have two origins, hepatic and intestinal (dietary).

Within the blood and lymph circulation, phospholipids are present in the composition of lipoproteins (LP): 25% of HDL, 20% of LDL and 15% of VLDL. Lipoproteins consist of a lipid monolayer containing non-polar lipids. The apoproteins are proteins, which bind to the phospholipid monolayer. Lipoproteins are the main circulating form of serum phospholipids.

Phospholipids are the target for LCAT (lecithine-cholesterol-acyl transferase), which is involved in the metabolism of cholesterol. They are also present in bile and together with bile acids solubilise cholesterol.

INDICATIONS FOR MEASUREMENT

Testing for familial hyperlipidaemia (defect of LP metabolism).

Testing for rare familial hypolipidaemias (abetalipoproteinaemia, Tangier's Disease and LCAT deficiency).

INFORMATION

■ SAMPLE

The sample should be collected into a dry tube and the patient must have been fasting for 12 to 14 hours.

■ QUESTIONS FOR THE PATIENT

Medicines taken (corticosteroids or oral contraceptives)? Specific organ system disease (liver, kidney or endocrine disorder)? Family history of lipid disorders? Diet?

■ SAMPLE STORAGE AND TRANSPORT

Serum can be stored for up to one month in a refrigerator at between + 2°C and + 8°C. The sample can be stored for longer and is stable if frozen.

ASSAY METHODS

Serum phospholipid measurement includes all of the phospholipids (free and those bound in lipoproteins).

The enzymatic method after precipitation uses the action of phospholipase D on phospholipids (more than 90% circulating phospholipids). The choline released is oxidised by a choline oxidase into betaine, releasing hydrogen peroxide. In the presence of phenol and 4-aminoantipyrine the hydrogen peroxide produces a coloured quinine-immine compound following the action of a peroxidise and is read by spectrophotometer at 500 nm. The method only measures lecithins, sphingomyelins and lysolecithins (95 to 99%). It does not measure cephalins.

Other methods are available to assess total phospholipid concentrations. A method is available which directly measures phospholipid phosphorous (lipid extraction, conversion into inorganic phosphore and colorimetric measurement) to measure total phospholipid concentrations.

Finally, methods are available to determine the composition of the different phospholipids contained in biological fluids (HPLC).

NORMAL EXPECTED VALUES

0 to 1 month	0.7 to 1.3 g/l	0.9 to 1.7 mmol/l
1 month to 1 year	1.3 to 2.0 g/l	1.7 to 2.6 mmol/l
From 1 year to adult	1.8 to 2.5 g/l	2.3 to 3.2 mmol/l

PATHOPHYSIOLOGICAL VARIATIONS

Serum phospholipid concentrations vary as a function of circulating lipoprotein concentrations. Total serum phospholipid concentrations in the dyslipoproteinaemias do not provide any additional information over specific lipoprotein measurement.

Results must be interpreted taking account of conditions, which may increase phospholipid concentrations: some treatments (oral contraceptives and corticosteroids), alcoholism, stress (emotional stress, myocardial infarction, burns and acute infections).

The major causes of the hypo and hyper phospholipoproteinaemias are shown in the table below.

Disorder	Etiology	Effect on phospholipid concentrations
Lipid	familial hyperlipidaemia (defective lipoprotein metabolism)	Rise
Endocrine and metabolic	diabetes mellitus, Cushing's syndrome, acromegaly, hypothyroidism	Rise
Renal **Hepatic**	nephrotic syndrome, uraemia primary biliary cirrhosis, extra-hepatic biliary obstruction	Rise Rise
Immunological	Lupus	Rise
Drugs and stress	oral contraceptives, corticosteroids, alcohol, emotional and organic stress	Rise
Diet	malnutrition with choline deficiency	Fall
Lipid	familial hypolipidaemia (rare): abetalipoproteinaemia (infants), Tangier's disease (children), LCAT deficiency (young adults)	Fall

FOR FURTHER INFORMATION

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■ Suchocka Z., Gronostajska D., Suchocki P., Pachecka J., *New HPLC method for separation of blood plasma phospholipids*, J Pharm Biomed Anal. 2003; 32: 859-65.

PICORNAVIRUS

DEFINITION

The picornaviruses or human enteroviruses (EV) belong to the Picornaviridae family and Enterovirus genus. According to the new internal taxonomy committee classification (2000), they contain 5 species (poliovirus and human enteroviruses A, B, C and D) and currently group together more than 65 serotypes (table 1). Enteroviruses E22 and E23 are classified separately and belong to the Parechovirus (PV) genus. They are small, single chain linear RNA viruses, the genome of which varies greatly between species. The messenger RNA codes for a giant protein which divides into 4 structural capsid proteins (VP1 to VP4) and non-structural proteins. They also have 2 non-coding regions at the 5' and 3' ends. Their capsid is icosahedric and they have no envelope and are therefore very resistant in the external environment.

Genus	Species	Number of serotypes	Names and numbers of serotypes
Enterovirus	Poliovirus	3	poliovirus 1-3
	Human enterovirus A	12	coxsackievirus A2 to A8,10, 12,14,16, enterovirus 71
	Human enterovirus B	36	coxsackievirus A9, B1-6 Echovirus 1 to 7, 9, 11 to 21, 24 to 27, 29 to 33. Enterovirus 69
	Human enterovirus C	11	coxsackievirus A1 to 11,13, 15, 17 to 22,24
	Human enterovirus D	2	enterovirus 68,70

Table 1: current classification of Enteroviruses pathogenic to human beings.

INTRODUCTION

EPIDEMIOLOGY

The Enteroviruses are ubiquitous. They are resistant in the external environment and circulate widely in nature. Infections are very common and occur predominantly in summer and autumn. Human beings are affected at all ages, particularly during childhood. Because of the many serotypes, several EV infections may occur during a person's life. Some serotypes are transmitted endemically and other endemo-epidemically or even epidemically. Transmission is mostly faeco-oral (by ingesting contaminated water or food), although respiratory and muco-cutaneous transmission may also occur.

Transplacental transmission may occur with a risk of serious clinical consequences in the newborn.

SYMPTOMS

EV binds to many cell receptors explaining the large variety of target organs which can be affected including the central nervous system, respiratory system, skin and mucosa, gastro-intestinal system, myocardium and muscles.

The incubation period lasts for 7 to 15 days for orally transmitted infections. Multiplication may be limited to the portal of entry and neighbouring lymphoid collections. In massive multiplication the virus passes into the systemic circulation (viraemia) reaching the target organs. Most infections are asymptomatic and clinical forms are extremely varied: they are acute, often mild and paucisymptomatic (flu-like illness, rhinopharyngitis, etc.) but may be more worrying (meningitis, paralysis, pericarditis, or myocarditis). In some situations (immunosuppression) they can become chronic and more serious *(table 2)*.

Table 2: main diseases associated with the Enteroviruses and Parechoviruses

Acute diseases		Chronic diseases
Latent infections	Very common (all of the EV and PV)	
Generalised infections	Febrile illness (all EV and PV) Bornholm's disease (CVB) Neonatal infections (CVB, ECV, PV)	Chronic post-viral fatigue syndrome (CVB?)
CNS infections	Lymphocytic meningitis (all EV and PV) Meningo-encephalitis (CVA, CVB, ECV, EV 70-71, PV) (CVA, CVB, ECV, EV 70-71, PV) Paralysis (PoV, CVA, CVB, ECV, EV 70-71, PV)	Post-polio syndrome (PoV) Meningo-encephalitis in the immunosuppressed (PoV, CVA, ECV)
Respiratory infections	Colds, bronchiolitis, pneumonia (CVA, CVB, ECV, EV68, PV)	
Muco-cutaneous infections	Herpangina (CVA) Hand, foot and mouth disease (CVA, CVB, EV71) Exanthema, eruptive rash (all EV and PV) Haemorrhagic conjunctivitis (EV 70)	
Gastro-intestinal tract infections	Diarrhoea (CVB, ECV, PV) Neonatal hepatitis (CVB, ECV)	Insulin-dependent diabetes (CVB)
Muscle and cardiac infections	Pericarditis (CVB) Myocarditis (CVB, EcV) Myositis (CVB)	Myocarditis (CVB) Dilated cardiomyopathy (CVB)

PoV: poliovirus; CVA: coxsackie A; CVB: coxsackie B; ECH: echovirus; PV: parechovirus

SEARCH INDICATIONS

Diagnosis of acute neuromeningeal disease.

Diagnosis of acute myocarditis or pericarditis.

Diagnosis of muco-cutaneous rash.

Diagnosis of materno-foetal or neonatal infection.

INFORMATION

SAMPLE

The samples must be taken as early as possible from several sites depending on localisation.

– Faeces, nasopharyngeal swabs or aspirate, respiratory secretions, CSF, skin or mucosal lesions, conjunctival samples, more rarely biopsies or amniotic fluid. Viral isolation testing may be performed on these samples.

– A stool sample (in a pot kept at refrigerated temperature) must always be obtained for suspected enteroviral infection regardless of the site. The enteroviruses are excreted for long periods of time by the gastro-intestinal tract.

– CSF, whole blood, amniotic fluid or biopsies to test for the viral genome.

– Serum: 5 ml of blood into a dry tube for serology in mother and child for materno-foetal infection.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

Are your pregnant?

SAMPLE STORAGE AND TRANSPORT

Samples for viral genome testing should be frozen at a temperature of < 20°C. Serum should be stored at + 4°C.

Transport: CSF should be transported within 4 hours of sampling, failing which it must be frozen.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

- **Cell culture:** this is the reference method. The sample is inoculated onto human diploid fibroblast cells (MRC5), onto a continuous simian (BGM, Vero, MK2) or human (KB, Hela, Hep2, RD…) cell line. Some EV are easier to isolate and culture than others. The majority of Coxsackie A viruses do not grow on cell culture. In order to isolate these, the sample must be inoculated into newborn mice and the cause of some enteroviral infections is not therefore found. The cytopathic effect (CPE) develops over culture for 2 to 12 days. Cell lesions are characteristic to a trained observer. A rapid cell culture method is available for genus diagnosis (EV) using immunoenzymatic development with a monoclonal antibody against a capsid antigen common to most of the enteroviruses. The serotype responsible can be identified in some specialist laboratories by ECP sero-neutralisation using polyclonal antibodies. This can exclude the presence of a poliovirus.
- **Gene amplification:** viral RNA amplification is used for rapid diagnosis of acute or chronic EV infection. This is used particularly to diagnose EV meningitis in CSF. The RT-PCR method is very sensitive and provides a diagnosis of group.

  In order to identify the type of virus responsible the viral genome must be sequenced. This is rarely performed outside of reference centres. Real-time PCR quantification tests of viral load are being developed.

INDIRECT DIAGNOSIS

Delayed development of antibodies, possible prolonged persistence of IgM and the wide range of serotypes make a serological diagnosis of limited use.

– Seroneutralisation of viral replication on cell culture is the reference method. This requires strains of the test viruses to be maintained in laboratories. Use of this method is obviously limited to EV serotypes which can be cultured on cells.

Neutralising antibodies are protective and last for long periods of time. They are said to be specific for the type of virus responsible although serotypic reactions have been reported in successive infections with different EV.

The indications for these tests are:

- Identification of the presence of a neutralising antibody against the 3 types of poliovirus in order to assess post-vaccine protection.
- Confirmation of the involvement of an isolated virus in a patient and identification of seroconversion to this virus on 2 samples taken 15 days apart.
- Retrospective serological diagnosis of EV infection in epidemics due to a particular serotype.

– The complement fixation reaction (CFR) uses a mixture of different enterovirus antigens. In view of the cross-reactions detected by this test, it can be used to titre total "EV group" antibodies without prejudging the serotype responsible. It is not particularly sensitive and the antibodies detected only last for a few months after infection. Other than finding seroconversion, a significant antibody titre enables a presumptive diagnosis of recent or semi-recent EV infection to be made.

– The ELISA method provides overall the same information as the CFR, although it is more sensitive. It is automatable and can be used to detect IgM antibodies. The antigens generally consist of a mixture of purified viral particles. Use of this method, however, is limited due to a lack of commercially available reagents.

TREATMENT - PROPHYLAXIS

There is no specific effective treatment. Antiviral compounds are being studied. Only prophylaxis is available and involves testing, waste water and foods and epidemiological surveillance of EV circulation, particularly polioviruses.

Anti-poliovirus is mandatory (in France). This is very effective and has resulted in disappearance of the disease from industrialised countries. The medium term WHO plan is to completely eradicate poliomyelitis.

FOR FURTHER INFORMATION

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- http://www.microbe-edu.org/etudiant/picornaviridae2.html

PLASMINOGEN ACTIVATOR INHIBITOR

DEFINITION

Plasminogen activator inhibitor 1 (PAI-1) is a glycoprotein and the main inhibitor of t-PA (tissue plasminogen activator) and two-chain urokinase (*Two-chain urokinase Plasminogen activator* or tcu-PA). It therefore plays an essential controlling role in any excessive activation of fibrinolysis.

PAI-1 is synthesised mostly by vascular endothelial cells and by adipose tissue stromal cells, hepatocytes, smooth muscle cells and fibroblasts. It is found in plasma, bound to a carrier protein (vitronectin) and in platelet alpha granules.

PAI-1 circulates in three forms in plasma: an active form bound to vitronectin, a latent free form (the active form loses its activity spontaneously with a half-life of approximately 90 minutes, being converted into this form) and an inactive form bound to t-PA. In addition, the PAI-1 contained in platelets (90% of total PAI-1, mostly in a latent form) is released into plasma during platelet activation and therefore at the time of thrombus formation, for which it is understood to prevent premature lysis.

In practice, measurement of "PAI-1 antigen" is more a reflection of free PAI-1 or PAI-1 bound to plasminogen activators, whereas measurement of "activated PAI-1" only assesses the PAI-1 bound to vitronectin ("active").

Synonym: Plasminogen activator inhibitor 1.

Note: There is also a 2nd placental inhibitor called PAI-2, levels of which increase in the 3rd trimester of pregnancy and a 3rd inhibitor, PAI-3, which in reality is activated protein C inhibitor. This also inhibits urokinase, thrombin, the thrombin-thrombomodulin complex, factors Xa, XIa, kallikrein, trypsin and chymotrypsin.

INTRODUCTION

Fibrinolysis is a physiological effect of the fibrin thombus being solubilised by plasmin, which is an enzyme generated from bound plasminogen and adsorbed onto fibrin. The main activation pathways for plasminogen into plasmin involve t-PA and urokinase. By inhibiting t-PA and urokinase, PAI-1 reduces plasminogen activation and controls the degradation of the fibrin thrombus. It also plays a role in regulating cellular adhesion mechanisms and tissue remodelling.

An equilibrium is needed between fibrinolysis and coagulation for physiological haemostasis. Reduced fibrinolytic activity promotes thrombosis, whereas excess fibrinolysis results in bleeding.

INDICATIONS FOR MEASUREMENT

Measurement may be indicated for unexplained haemorrhage in children or young adults to test for hyperfibrinolysis. To do this, it must be measured in plasma and possibly in platelets and combined with the euglobulin lysis time and measurement of t-PA antigen. These measurements are second line tests reserved for a few specialist laboratories.

Increased plasma PAI-1 concentrations causing hypofibrinolysis have been reported in a large number of arterial and venous prothrombotic or thrombotic states. PAI-1, however, does not appear to be a risk factor for deep vein thrombosis and its measurement is not indicated in the aetiological assessment of these disorders. Conversely, PAI-1 measurement has been very widely used in epidemiological studies to assess cardiovascular risk, highlighting the involvement of the fibrinolytic system in development of acute complications of atherosclerosis. Plasma concentrations are high in different pathological situations such as myocardial infarction, obesity, hypertriglyceridaemia, insulin-resistance and inflammatory states and measurement may therefore be useful in assessing vascular risk. There are at present, however, no recommendations about this measurement on an individual basis.

PAI-1 measurement has also been proposed as a prognostic indicator in meningococcal septicaemia.

INFORMATION

SAMPLE

Citrated tubes containing anti-platelet agents (theophylline, adenine, dipyridamole: CTAD tubes) or containing an acidified citrate solution (such as Stabilyte®) are recommended. Failing this, the sample should be taken into a concentration of 3.2% (0.109 M) 1/10 citrate (0.5 ml per 4.5 ml of blood); 3.8% citrated tubes (0.129 M) are acceptable. (cf: *General sampling conditions in haemostasis*).

As PAI-1 is labile, sampling conditions must be observed scrupulously:

– The sample must be taken in the morning between 0800 and 1000 hours from a person who has been fasting or has had a light, fat-free breakfast, after resting for at least 20 minutes (circadian variations and increase in plasma t-PA concentrations after physical activity).

– Tobacco and drinks containing caffeine should be avoided for at least an hour before the sample is taken and alcohol consumption avoided for at least 18 to 24 hours before the sample is taken.

– The sample should be taken distant to any thrombotic, infectious or acute inflammatory event.

– It should be taken without tourniquet (or with a loose tourniquet) to limit venous stasis.

– It is recommended that the first few millilitres of blood be discarded and that the measurement is performed on the second tube taken.

QUESTIONS FOR THE PATIENT

Are you taking medical treatment? Gemfibrozil and clofibrate (withdrawn from the market in France) reduce hepatocyte PAI-1 synthesis by 40 to 50% over 24 hours.

Do you have a past history of venous or arterial thrombosis or symptoms of bleeding?.

SAMPLE STORAGE AND TRANSPORT

As 90% of circulating PAI-1 is contained in platelets, for specific measurement of plasma PAI-1 the sample should be totally stripped of platelets and any platelet activation fully prevented. To do this it is essential that the sample is processed as soon as possible after it is taken (within 30 minutes).

Centrifuge for 30 minutes at 2500 g at + 4°C, separate the plasma, retaining only the upper 2/3 (to avoid any contamination from the leukocyte and platelet layer), and freeze rapidly (the whole process to be completed within an hour of sampling).

The frozen plasma can be stored for 15 days to 1 month at – 30°C or for several months at – 80°C.

Transport the sample frozen.

ASSAY METHODS

PAI-1 antigen: ELISA immunoenzymatic assay.

PAI-1 activity: Chromogenic method (amidolytic), bio-immunoassay.

REFERENCE VALUES

PAI-1 antigen: As an indication, normal plasma concentrations in a healthy non-obese person are a few ng/ml (< 10, 30 or 100 ng/ml depending on method used).

PAI-1 activity: 0 to 50 U/ml or < 5 ng/ml (bio-immunoassay).

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

A circadian rhythm is seen, with higher plasma concentrations at the beginning of the morning.

Plasma PAI-1 concentrations increase in pregnancy.

■ PATHOLOGICAL VARIATIONS

- **Failure to observe pre-analytical conditions:** Platelet contamination or activation.
- **Increased plasma PAI-1 concentrations** are seen in the following situations:

  – Inflammation (particularly post-operative period),

  – Hypertriglyceridaemia,

  – Android obesity,

  – Type 2 diabetes and insulin resistance,

  – Myocardial infarction (MI). The ECAT study showed that an increase in plasma PAI-1 antigen activity and concentrations was associated with MI. By multivariate analysis, however, PAI-1 was no longer an independent risk factor of MI but was related to insulin resistance. Other authors have shown that increased PAI-1 activity predicts MI in patients with angina or a past history of recent MI. There is at present, however, no specific treatment for this abnormality.

  Raised plasma PAI-1 concentrations in meningococcal septicaemia are associated with increased mortality.

  Similarly, increased plasma PAI-1 concentrations are associated with poor prognosis in complicated pregnancy.

- **Reduced plasma PAI-1 concentrations**

  A few cases of inherited PAI-1 deficiency have been described in the literature, responsible for mild bleeding syndromes, particularly spontaneous bleeding following surgery. The euglobulin lysis time is reduced in these cases (indicating hyperfibrinolysis) and the t-PA antigen is normal.

FOR FURTHER INFORMATION

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PLASMINOGEN

DEFINITION

Plasminogen is the zymogen of plasmin, the key enzyme in the fibrinolytic system, which converts insoluble fibrin into soluble fibrin degradation products.

It is a β2 globulin, synthesised mostly in the liver but also by eosinophils, kidney and cornea. The plasminogen polypeptide chain is folded into 5 loops known as "kringles" carrying the "lysine" binding sites responsible for the high affinity the molecule has for fibrin, alpha-2 antiplasmin and synthetic fibrinolysis inhibitors such as tranexamic acid and ε-aminocaproic acid.

Plasminogen exists in two molecular forms: glu-plasminogen (native form) and lys-plasminogen (the more active form). Lys-plasminogen is derived from glu-plasminogen, the NH_2-terminal end of which is cleaved by tissue plasminogen activators. Both forms can be converted into plasmin.

The plasma half-life of plasminogen is approximately 50 hours. The gene coding for it is located on the long arm of chromosome 6 in human beings.

INTRODUCTION

Fibrinolysis is a physiological reaction involving the dissolution of the fibrin thrombus by plasmin, an enzyme generated from plasminogen bound and adsorbed to fibrin. The main routes of plasminogen activation into plasmin involve t-PA and pro-urokinase. The main purpose of the plasminogen-plasmin system is to lyse the formed clot and prevent excessive fibrin formation.

An equilibrium is required between fibrinolysis and coagulation for physiological haemostasis. Reduced fibrinolytic activity may predispose to thrombosis, whereas excessive fibrinolysis leads to bleeding.

Qualitative (dysplasminogenaemia) or functional defects of plasminogen (hypoplasminogenaemia) have been described as risk factors for venous thrombosis. These deficiencies are far rarer than AT, PC and PS deficiencies.

INDICATIONS FOR MEASUREMENT

Measurement of plasminogen is a second line test in the assessment of deep venous thrombosis and is reserved for a few specialist laboratories. Functional measurement can be used to test for plasminogen deficiency and immunological measurement is used to type whether the deficiency as qualitative (normal antigen, reduced activity) or quantitative (reduced antigen and activity).

The test is occasionally indicated in bleeding disorders accompanied by defibrination.

It may also be useful in monitoring some thrombolytic treatments.

INFORMATION

SAMPLE

The sample should be collected into 3.2% citrate (0.109 M), volume 1/10 (0.5 ml per 4.5 ml of blood). 3.8% citrate tubes (0.129 M) are acceptable. Blood can also be collected into CTAD (citrate, theophylline, adenine, dipyridamole) tubes which improve sample preservation (this should be preferred when the transport time for the tube is more than 2 hours). No other anticoagulant must be used.

The sample should preferably be taken in the morning between 0800 and 1000 hours from a fasting person who has been resting for at least 10 minutes.

It should be taken without a tourniquet (or with a loose tourniquet) to limit venous stasis.

It is recommended that the first few millilitres of blood be discarded and that the measurement is performed on the second tube taken.

The sample should preferably be taken distant to any thrombotic, infectious or acute inflammatory episode.

QUESTIONS FOR THE PATIENT

Do you have a personal or family history of venous or arterial thrombosis?

Have you had thrombolytic treatment? Streptokinase, urokinase, rt-PA, reteplase or tenecteplase. These treatments lyse the fibrin clot causing consumption of plasminogen.

SAMPLE STORAGE AND TRANSPORT

Samples can be stored for 2 to 4 hours at temperatures of between 15 and 20°C. Never place the sample at + 4°C (risk of factor VII activation).

The plasma can be stored for 1 month at –20°C or for several months at – 80°C; rapid thawing in a water bath at 37°C (5 to 10 minutes) is recommended before measurement.

Transport: centrifuged sample (dual centrifugation is recommended), separated and frozen within 2 hours of sampling.

ASSAY METHODS

Functional assay: chromogenic or chronometric method.

Immunoassay of the protein (electro-immunodiffusion) to differentiate quantitative from qualitative deficiency.

Molecular biology gene analysis (chromosome 6): testing for the mutation responsible.

REFERENCE VALUES

Plasminogen antigen: 130 to 200 mg/l.

Functional plasminogen: results are usually expressed as percentage of normal or as kIU/l, 1 kIU/l = 100%. "Normal" values: 80 to 120% (0.80 to 1.20 kIU/l).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

- **Age:** Values are very low in premature infants and half normal in term neonates (normal: 40 to 60%).
- **Pregnancy:** Rises during the third trimester (128 to 220%).

PATHOLOGICAL VARIATIONS

- **Increased plasminogen concentration** in inflammatory disorders and infections.
- **Reduced plasminogen concentration**

Acquired deficiencies:

Liver disease.

Thrombolytic treatment.

Constitutional deficiencies:

Quantitative (reduced plasminogen antigen and activity) or dysplasminogenaemia (normal antigen, reduced activity) have been described as risk factors predisposing to venous thrombosis (thrombophilia). This association is still however debated. The prevalence of plasminogen deficiency in a population of patients with a past history of thrombo-embolism is similar to that in the general population (< 1%). In addition, a large number of patients with hypo- or dysplasminogenaemia are asymptomatic. The role of plasminogen measurement in the aetiological investigation of thrombosis therefore remains to be established.

In addition, occasional cases of homozygous plasminogen deficiency have been reported in children suffering from ligneous conjunctivitis (fibrin deposition on the conjunctiva in children).

The diagnosis of constitutional deficiency is only made after rechecking the deficiency on a sample taken some time later and after excluding causes of acquired deficiency which are far more common. Constitutional deficiency is rare (is believed to be more common in the Japanese population); family screening is required in these cases.

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PNEUMOCOCCUS

DEFINITION

Streptococcus pneumoniae (Sp) still called the Pneumococcus belongs to the *Streptococcacae* family, the *Streptococcus* genus and oral Streptococci group. It is a commensal bacterium of the upper respiratory tract in human beings, infections with which are a current public health problem, as they are serious or may even be fatal, if invasive. There are currently 90 serotypes of Pneumococci recognised and classified according to the composition of their bacterial capsule.

Synonym: *Streptococcus pneumoniae*, Pneumococcus.

INTRODUCTION

EPIDEMIOLOGY

Streptococcus pneumoniae colonises the rhinopharynx of children from an early age (all children have come into contact with the organism by the age of 2 years old). This reservoir is primarily human, and human-human transmission occurs through aerial droplets.

SYMPTOMS

Pneumococcus causes various infections:

– ***Community-acquired bacterial pneumonia*** occurs particularly in the elderly or in predisposed patients (immunosuppression, respiratory viral infections, asplenism and diabetics, etc.). It may present as sudden onset frank, acute lobar pneumonia or occasionally as post-viral superinfection pneumonia.

– ***Acute otitis media in children under 2 years old:*** It is the second leading organism responsible after *Haemophilus influenzae*.

– ***Sinusitis, bronchitis, mastoiditis and conjunctivitis.***

– ***Bacterial meningitis in children over 3 months old and in adults over 50 years old.***

– ***Septicaemia and bacteraemia which*** often complicate primary Pneumococcal infections.

PATHOPHYSIOLOGY

The pathogenic potential of *Streptococcus pneumoniae* is mostly due to the presence of 2 virulence factors, the ***bacterial capsule*** and the ***pneumolysin***. The capsule provides resistance to phagocytosis by macrophages in the pulmonary alveolae, whereas the pneumolysin has direct cytotoxic activity on respiratory endothelial cells.

SEARCH INDICATIONS

The diagnosis of community-acquired adult pneumonia, particularly in predisposed people.

The diagnosis of bronchitis and bronchopneumonia in patients with chronic respiratory insufficiency or following viral respiratory diseases.

The diagnosis of acute otitis in children under 2 years old.

The diagnosis of bacterial meningitis in adults and infants.

The diagnosis of invasive infections such as septicaemia or meningitis.

Testing after vaccination (investigation for humoral immunodeficiency).

INFORMATION

SAMPLE

Bronchial samples: Expectoration in community-acquired pneumonia, broncho-alveolar lavage (BAL), protected distal sample (PDS), protected bronchial brushing (PBB) or transtracheal aspirate in severe pneumonia.

Paracentesis pus in otitis.

CSF in suspected meningitis.

Blood cultures

Urine

Serum

Pleural aspirate fluids.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

Age?

Underlying diseases, such as diabetes, sickle cell anaemia, asplenism or immunosuppression?

Current or past antibiotic treatment?

SAMPLE STORAGE AND TRANSPORT

Bacteriology samples should be transported promptly to the laboratory (within 2 hours) protected from cold, as *Streptococcus pneumoniae* is extremely fragile.

Serum (for serology) can be stored for a few days at + 4°C and should be transported at + 4°C.

DIAGNOSTIC METHODS

DIRECT EXAMINATION

The Pneumococcus is easy to recognise on Gram staining: Gram positive diplococci arranged in "candle flames" or "short chains".

CULTURE

This is the reference method to diagnose Pneumococcal infection. The diagnosis is suggested by the Gram stain appearance and appearance of the colonies (alpha-haemolytic, smooth, concave and shiny after incubation for 18 to 24 h at 37°C). The most widely used confirmatory test is optochin sensitivity, with a diameter of > 14 mm around a disc loaded with 5 µg suggesting Pneumococcus, although 0.5 to 5% of Pneumococci are resistant to optochin and some *Streptococcus viridans* are sensitive. If any doubt exists, a bile lysis test which is more specific is recommended (a dense suspension of the test bacterium lightens after adding a few drops of a 10% sodium deoxycholate solution and incubating for 30 min at 37°C). An agglutination method using latex particles sensitised with antibodies that recognise the different capsule serotypes (Slidex® pneumo-kit Mérieux) can also

help towards identification. This test, however, may produce false positive results, particularly with *S. oralis* and *S. mitis*. The reagent can be used directly on pathological specimens.

The Pneumococci grow well in blood cultures, although the positive blood culture rate in adults with acute community-acquired pneumonia (ACAP) is only 20% (10% in children). Blood cultures are positive in more than 50% of cases of meningitis. These results are probably explained by the possibly low numbers of bacteria in blood, the prevalent use of antibiotics and intermittent nature of the bacteraemia, hence the merit of complementary tests.

■ DIRECT TESTING FOR SOLUBLE BACTERIAL ANTIGENS

This is performed using an immunological method, such as sensitised latex particle agglutination or an immunochromatographic test. The Binax *Now S. pneumoniae*® immunochromatographic test (ICT) represents an important advance in identification of Pneumococcal infection. This is a test for soluble antigen and can be performed in 15 minutes on a sample of urine or CSF.

Testing is performed in urine in ACAP, with a specificity of > 90% and sensitivity of 77 to 89% in bacteraemic ACAP and 44 to 64% in non-bacteraemic ACAP. The test can be used in patients with exacerbations of COPD (occasional false positives occur) and in those who have been on antibiotics, even for several days (80% of people still have a positive test on day 3 after starting antibiotics). It is recommended in France as part of the management of serious ACAP in adults (intensive care) in parallel with testing for Legionella antigen in urine.

The benefit of the test in children is its high negative predictive value (NPV). A negative test almost certainly excludes Pneumococcal infection, although a positive test cannot be interpreted because of the high incidence of Pneumococcal infections in this age group. The test is not currently recommended in children.

The test has also been validated in CSF, if the clinical context suggests meningitis, when it has a sensitivity of between 95 and 100% and a specificity of > 99%. The 2008 consensus conference on meningitis in France recommended its use in CSF in suggestive situations (but not in urine because of the risk of false negatives).

■ MOLECULAR BIOLOGY

The method used at present is real-time PCR. Different target genes can be amplified and the best performing are lytA (the gene coding for autolysin) and spn9802 (which codes for a gene of unknown function). Amplification of other targets (*ply* or *psaA*) may produce false positive reactions in the presence of *S. viridans* or *S. pseudopneumoniae* (a recently described species similar to the Pneumococcus, with intermediary sensitivity or resistance to optochin under 5% CO2, but sensitive if the dish is incubated in ambient air).

In CSF, PCR has a sensitivity of 92 to 100% and a specificity of 100%. It can be used particularly if direct examination is positive and culture is negative (which may be explained by antibiotic treatment). The consensus conference on meningitis in France recommended its use, unless an ICT test has been performed.

The sensitivity of PCR is 71% in pleural fluid (*vs.* 28% for culture). PCR performed on plasma is positive in 29% to 100% of cases depending on the study in ACAP; it is positive in 68 to 100% of cases in respiratory samples, although it is difficult to distinguish between "colonising" Pneumococci and those responsible for infection.

■ SEROGROUPING/TYPING

This is particularly of epidemiological use.

■ SEROLOGY

Post-vaccination testing in a diagnostic context of immunodeficiency.

TREATMENT

■ Antibiotic therapy:

PENICILLINS

Penicillins bind covalently to penicillin binding protein (PBP), enzymes involved in the peptidoglycan synthesis in the bacterial wall. The resistance mechanism relies on changes in these PBP causing an increase in the MIC for all of the β-lactams, the magnitude of which varies depending on the compound.

In practice, RPSP (reduced penicillin sensitivity Pneumococci) are detected with a 5 µg oxacillin disc (RPSP if diameter < 26 mm) and are defined as having a penicillin MIC of ≥ 0.1 mg/l (for sensitive strains, MIC: 0.06 mg/l): within the RPSP; intermediary sensitivity strains (or low resistance strains) for which 0.1 mg/l < MIC ≤ 2 mg/l are distinguished from resistant strains (MIC > 2 mg/l) (European recommendations).

In terms of the other β-lactams, such as amoxicillin (AMX), ampicillin (AMP), cefotaxime (CTX), and ceftriaxone (CRO), a strain is said to be "sensitive" (S) if the MIC is ≤ 0.5 mg/l; of "intermediary sensitivity" (I) between 1 and 2 mg/l and "resistant" (R) if the MIC is > 2 mg/l. The CA-SFM in France states that "category I (or low level R) strains "should be considered to be resistant in meningitis but sensitive to high doses in respiratory infections".

The recommendations in France are that the MIC of at least one β-lactam with pharmacodynamic properties compatible with effective treatment be established, particularly for the antibiotic prescribed. The MIC must be determined in severe infection, clinical failure after treatment with a β-lactam and suspected RPSP in *microbiological screening*.

The most widely used methods to determine the MIC are those involving the E-test® strips. It is fundamental that strips used are correctly stored. To do this, check absence of moisture (by looking at the colour of desiccating agents), and strictly observe the storage temperature (if frozen, do not open the kit more than 30 minutes before use, failing which the strips absorb moisture). To read the strips, it must be possible to see small colonies clearly, i.e. reading in light with a magnifying glass. In practice, it is very rare to find MIC values of > 8 mg/l for AMX or > 4 mg/l for CTX. If these MIC readings are found, they must be confirmed; if necessary by sending to a reference centre.

It is not unusual to find AMX MIC > penicillin MIC for low values (11% of cases, 2007 data), and a strain which is sensitive to penicillin is not necessarily therefore sensitive to amoxicillin.

AMINOGLYCOSIDES

The *Streptococcus* genus exhibits low level resistance to all of the aminoglycosides, although synergistic bactericidal activity may be seen. What must be detected is acquisition of additional resistance conferring high level resistance (HLR) onto a strain. To do this, high load (500 µg) streptomycin, gentamicin and kanamycin (which tests for amikacin) should be used. The laboratory specialist may therefore classify strains not as S, I or R, but as "low level resistance" (LLR = association permitted) or HLR (association not permitted). Bactericidal associations can be used for severe Pneumococcal infections, such as endocarditis (although these are extremely rare).

MACROLIDES

These antibiotics are less active in an acid environment, hence the recommendations that antibiotic sensitivity profiles for Pneumococci should not be obtained after incubating in a CO_2-enriched atmosphere. This effect is seen particularly with telithromycin (an organism should not be defined as R to this antibiotic if tested under CO_2). A CO_2-enriched atmosphere should be reserved for strains which do not grow in ambient air.

The main resistance mechanism is a change in target due to ribosomal methylation. This is MLS_B resistance *(erm genes)*, which can usually be induced in the Pneumococcus.

MLS_B resistance affects macrolides with a 14 (erythromycin, oleandomycin, roxithromycin, clarithromycin and dirithromycin), 15 (azithromycin) and 16 (josamycin and spiramycin) atom lactone ring and the lincosamides (lincomycin and clindamycin), streptogramin B (a component of pristinamycin and quinupristine). This mechanism was detected by identifying antagonism between erythromycin (inducer) and clinda- or lincomycin (non-inducers), flattening of the inhibition area around the non-inducing antibiotic, forming a D zone. In the MLS_B phenotype, erythromycin, lincomycin, spiramycin and clindamycin are classified as R, whereas pristinamycin and telithromycin remain sensitive.

In the constitutional MLS_B phenotype (no induction between erythromycin and telithromycine), telithromycine is no longer sensitive, although these strains are rare.

Another possible resistance mechanism is efflux (lack of antagonism between erythro- and lincomycin where the antibiotic sensitivity profile should not be interpreted), causing erythromycin resistance although lincomycin, clindamycine, spiramycin, telithromycin and pristinamycin remain sensitive.

COTRIMOXAZOLE

This antibiotic must be tested on haemolysed horse blood agar, as on Mueller Hinton blood agar the diameters are reduced and may falsely suggest resistance.

FLUOROQUINOLONES

The fluoroquinolones act through selective inhibition of bacterial DNA synthesis by an effect on the gyrase responsible for DNA coiling (*gyrA* and *gyrB* genes), or IV topoisomerase causing DNA untangling at the end of replication, either on 2 C sub-units dependent on the *ParC* gene or on 2 E sub-units dependent on the *Par E* gene.

The mechanisms involved in resistance may be a change in target following a chromosomal mutation in a very highly preserved region of the gene (QRDR gene) or mutations, mostly the Pneumococcus, in the *gyrA* and *parC* genes, or by an active efflux mechanism. As soon as the resistance mechanism has been acquired, (generally a first parC mutation), the MIC of the anti-Pneumococcal fluroquinolones (levofloxacin and moxifloxacin) increase slightly compared to the wild strains, although this does not justify classification as I or R. These strains must be detected as they are liable to require high level resistance on treatment. To do this, it is recommended that norfloxacin be used (disc loaded with 5 µg), for which the MIC increases from 2-4 mg/l to > 16 mg/l as soon as a mutation is acquired (diameter < 10 mm).

VACCINATION

Two vaccines are available. The Pneumo 23® polysaccharide vaccine is effective against 85% of the most common strains but ineffective in children under 2 years old. It is particularly used to prevent respiratory Pneumococcal infections in people over 65 years old.

The 7-valent conjugated vaccine which has been available since 2003 (increased to 13 valencies at the end of 2010) is effective from the initial months after birth. It is indicated for use in children between 2 months and 2 years old, using a vaccine regimen of 2 doses at the ages of 2 and 4 months old followed by a booster at 12 months (an additional dose is recommended at 3 months for premature babies and infants with disease putting them at high risk of invasive Pneumococcal infection). It is also indicated in children from 2 years to 5 year old who have not previously been vaccinated and have a risk factor of developing invasive Pneumococcal infection, using a vaccine regimen of two doses at a two month interval followed by a dose of the Pneumo23® vaccine at least 2 months later.

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PNEUMOCYSTIS CARINII

DEFINITION

The agent responsible for human pneumocystosis was discovered at the start of the 20th century by M. Carini and Chagas. It was called the *Pneumocystis carinii* var. *hominis*, and its taxonomic position has long been debated. Some consider it to be a parasite based on morphological criteria and its sensitivity to pentamidine, and others to be a fungus because it takes on silver staining. Following recent molecular biology studies it has been classified amongst the atypical fungi and renamed *Pneumocystis jiroveci*.

P. jiroveci takes the form of trophozoites, 1 to 3 microns in diameter, or 5 to 6 micron cysts which are round or ovular and contain 8 sporozoites of 1 to 2 µm arranged in a rosette. Some cysts are empty and appear as small cups.

Pneumocystosis is a cosmopolitan opportunistic disorder which has long been confined to services treating immunosuppressed patients or premature infants. Since the emergence of AIDS before the triple therapy era, it became very common (more than a third of the first manifestations of AIDS were pneumocystosis). Its incidence has fallen since the use of prophylactic treatments and it now occurs later, still affecting approximately 10% of patients infected by the human immunodeficiency virus (HIV). It is generally diagnosed in hospital but its follow-up and maintenance treatment are now usually carried out in primary care.

Synonym: *Pneumocystis jiroveci*.

INTRODUCTION

EPIDEMIOLOGY - SYMPTOMS

Infection occurs through the respiratory tract by inhalation of cysts present in their natural state in the ground or by between-human transmission. The cysts enter the lungs, rupture and release the sporozoites which transform into trophozoites and then cysts, where new trophozoites reform. The trophozoites adhere to the wall of alveolar cells causing influx of inflammatory cells (macrophages and endothelial cells). The *Pneumocystis* cycle in the lungs lasts for a few hours explaining why all of the forms may be found in broncho-alveolar samples. Immunocompetent people remove the fungi spontaneously which are rapidly destroyed by macrophages. The full cycle only occurs in the immunosuppressed, in whom the infection may occur as a result of *de novo* infection or reactivation of latent disease.

The disease is characterised in the immunosuppressed by insidious-onset pneumonia with dry cough and respiratory difficulty, and initially no fever. Fever then develops and the cough and dyspnoea worsen, the lungs become white with a "ground glass" appearance on radiography, sparing the base and apex. Symptoms occur mostly in HIV infected people with less than 200 CD4 lymphocytes/mm^3, who are not receiving effective prophylaxis. In premature infants, pneumocystosis begins with polypnoea and cough, followed by acute pneumonia with tachycardia and cyanosis which may progress to severe respiratory distress.

SEARCH INDICATIONS

Development of respiratory problems:

– In people with severe immunosuppression: mostly HIV infected patients with less than 200 CD4 lymphocytes/mm^3 who are not receiving effective prophylaxis, cancer patients, transplant patients and patients receiving immunosuppressant therapy or long term corticosteroid therapy;

– In premature infants.

INFORMATION

SAMPLE

The sample of choice is broncho-alveolar lavage fluid (BAL), obtained during fibroscopy. This provides the best sensitivity for the fungus (75 to 99%).

Pneumocystis may be found from other respiratory samples, such as expectoration, induced expectoration and bronchial brushings. Induced expectoration is most frequently used, particularly if BAL cannot be performed (if the patient is too hypoxic or dyspnoeic). This is performed in a fasting patient after stimulating expectoration with a 3% hypertonic NaCl solution administered 15 to 20 minutes via an ultrasound nebuliser. Secretions are collected into a sterile container (2 to 5 ml are required).

Lung biopsies are used particularly for histology and deep organ biopsies are occasionally taken to diagnose extra-pulmonary or systemic forms of the disease.

QUESTIONS FOR THE PATIENT

Are you being treated with long-term immunosuppressants or oral corticosteroids?

Are you seropositive for HIV?

SAMPLE STORAGE AND TRANSPORT

Store and transport as promptly as possible at + 4°C, as it is an urgent test.

DIAGNOSTIC METHODS

Direct microscopy on BAL or induced expectoration centrifugation pellets:

– At least two of the following methods are used after staining: May Grünwald Giemsa (MGG), RAL®, toluidine blue, Gomori-Grocott, Gram-Weigert.

– Or by direct immunofluorescence after labelling with anti-*Pneumocystis* monoclonal antibodies.

– A PCR method has been described but is not routinely available.

NB: *Pneumocystis* does not culture and serology does not contribute to the diagnosis.

INTERPRETATION

The diagnosis is based on finding the fungus on direct examination. Two stains are recommended, one to reveal the trophozoites and cysts (MGG or RAL®) and the other to show the cyst wall (Grocott or toluidine blue).

– With MGG, clusters of trophozoites stained blue with red tips corresponding to their nuclei; the cysts are in the middle

of the clusters. RAL® staining shows the trophozoites as red-violet cloudy clusters with numerous red dots. Isolated or group cysts are surrounded by a clear halo.

–Toluidine blue selectively stains the cysts which are seen as saucers or small blue cups.

–Gomori-Grocott (silver staining) reveals the cyst wall without the content in black on a green background.

–Gram-Weigert stains the cysts and intra-cyst bodies purple on a pink background.

– Direct immunofluorescence is a useful alternative as it is very specific. The round or ovular cysts are fluorescent green.

Pneumocystosis is associated with an acute phase inflammatory reaction (increased ESR and increased plasma fibrinogen concentration) and blood gas analysis shows moderate hypoxia, hypercapnoea and respiratory alkalosis. The blood count is not particularly abnormal (a slight neutrophilia is typically seen). A rise in LDH above 450 IU/l is considered to be a poor prognostic indicator.

TREATMENT

Treatment is with co-trimoxazole or the association pyrimethamine-sulfadoxine. More recently, atovaquone (used as an antimalarial agent) has been shown to be effective. Prophylaxis is recommended in HIV+ patients with fewer than 200 CD4/mm^3 with cotrimoxazole, dapsone or nebulised pentamidine.

FOR FURTHER INFORMATION

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POLYOMAVIRUS

DEFINITION

The polyomaviruses (PMV) belong to the Papovaviridae family. The human PMV are the BK (or BKV) and JC (or JCV) viruses, named after the initials of the patients from whom they were isolated. They are small non-enveloped viruses with an icosahedric capsid, the viral genomes of which are circular, double-stranded DNA sequences which are very similar in genetic organisation (the JC and BK viral genomes exhibit 75% homology). 5 genotypes of the JC virus (from 1 to 5) and 4 of BK virus (from I to IV) are recognised.

INTRODUCTION

EPIDEMIOLOGY

The majority of primary BKV or JCV virus infections occur during childhood. They are ubiquitous viruses which do not grow in-vitro except in a very limited number of cells. Their methods of transmission and infection are still widely disputed. After entering the body (faeco-orally?) they are believed to circulate through the blood stream to the target organs. After a latent period (the length of which is still not known), reactivation is believed to result simply in urine viral excretion or in clinical features in the presence of immunosuppression.

SYMPTOMS

Primary infections are generally asymptomatic. There are 2 types of clinical features:

- **Progressive multifocal leukoencephalopathy (PMLE):**

  After long being considered a complication of malignant blood dyscrasias, this is now seen as an opportunistic infection in AIDS (although far less as a result of the antiviral treatments). It is caused by the JCV virus and causes CNS demyelinisation. Neurological damage is common and varied, affecting all regions of the brain *(table 1)*. The disease often progresses quickly and is fatal.

- **Urinary tract infections:**

  BKV or JVC are excreted asymptomatically in urine in renal or bone marrow transplant patients (25 to 44%) for several months. Urinary BKV infections in renal transplant patients may progress to ureteric stenosis and interstitial nephritis; haemorrhagic cystitis has also been reported in bone marrow transplant patients. Urine infections are described in pregnant women at the end of the second trimester, until childbirth, and carry no clinical risks either to the mother or foetus. They are also seen in HIV + patients and cause haemorrhagic cystitis and ureteric stenoses (BK virus). The oncogenic role of the human MPV is debated (particularly for BKV).

Clinical features	Percentage (%)
Muscle weakness	42
Speech disorders	40
Cognitive abnormalities	36
Headache	32
Altered taste	29
Sensory defect	19
Visual disturbance	19
Seizures	9
Diplopia	9
Mal co-ordination of the lower limbs	6

Table 1: main clinical features of PMLE in HIV-infected patients *(adapted from JR Berger et al.)*

INFORMATION

SAMPLE

EDTA whole blood (1 ml), urine (10 ml) or CSF (1 ml) for PCR detection of viral DNA.

QUESTIONS FOR THE PATIENT

Clinical context?

Immunosuppression?

SAMPLE STORAGE AND TRANSPORT

CSF must be stored and transported frozen.

Whole blood and urine must be stored and transported at + 4°C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

- **Cell culture:** Isolation of the virus is extremely difficult and therefore not widely used.
- **Molecular biology:** This is far more useful both with *in-situ* hybridisation or particularly, gene amplification techniques (PCR) adapted mostly to CSF samples from patients suffering from PMLE. The virus can also be tested for in whole blood and urine using the same method. This is a major aid to diagnosis in conjunction with clinical features and medical imaging results and is the current reference technique (sensitivity between 82 and 100% and specificity of up to 99 to 100%).

INDIRECT DIAGNOSIS

Serological diagnosis is of limited use, apart from comparative titration of anti JC virus antibodies in serum and CSF, which is used in the diagnosis of PMLE. Haemagglutinin inhibition, immuno-enzymatic and CFR methods are available to test for anti-BK antibodies.

TREATMENT

There is no specific antiviral treatment. Various treatments tried have been either of no use or toxic.

FOR FURTHER INFORMATION

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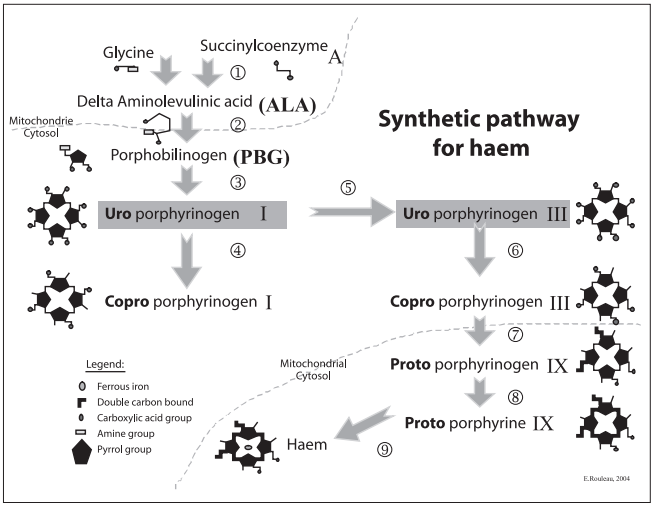
PORPHYRINS

DEFINITION

Porphyrins are intermediary products in haem synthesis. The precursors of haem synthesis are delta-aminolevulinic acid (ALA) and porphobilinogen (PBG); the three intermediary products are uroporphyrin (uro), coproporphyrin (copro) and protoporphyrin (proto), which lead to the synthesis of haem. This synthetic pathway occurs mostly in the bone marrow (80%) and in the liver and other tissues (20%).



From Fischbach F "Porphyrias" in "Common laboratory and diagnostic tests" Lippincott; 3rd Edition; New York 2002.

INTRODUCTION

The hereditary porphyrias are a group of seven rare diseases (they affect approximately 20,000 people in France), which have either autosomal dominant or recessive transmission and are due to a partial deficiency of the enzymes involved in the synthesis of haem, causing abnormal production of porphyrins and/or porphyrin precursors in the liver and/or bone marrow. Each porphyria occurs as a result of a deficiency of one of the enzymes involved in the synthesis of haem. Clinical expression varies greatly between people and between attacks.

CLASSIFICATION OF THE PORPHYRIAS

The porphyrias are usually classified according to the tissue in which predominant porphyrin accumulation occurs:

– The hepatic porphyrias (accumulation in the liver): include porphyria cutanea tarda (PCT) which only produces skin signs and the acute hepatic porphyrias, acute intermittent porphyria (AIP), hereditary coproporphyria (CH), porphyria *variegata* (PV) and DOSS porphyria or hereditary ALA dehydratase deficiency (dALA, very rare), which are characterised by acute neurovisceral attacks sometimes associated with skin signs.

– Erythropoietic porphyrias (accumulation in the bone marrow): these porphyrias affect children and are congenital erythropoietic porphyria (CEP or Gunther's disease) with severe skin features often associated with serious haemolytic crises and erythropoietic protoporphyria (EPP) which is characterised mostly by acute episodes of painful photosensitisation, associated with liver features in 1/4 of cases.

MOLECULAR GENETICS AND CLASSIFICATION OF THE PORPHYRIAS: HEREDITARY MUTATIONS OF GERM CELL DNA

– Autosomal dominant porphyrias (a mutated allele). The enzyme activity is approximately 50%. This applies to all of the hepatic porphyrias: AIH, HC and PV.

– Autosomal recessive porphyrias (two mutated alleles). Enzyme activity is well below 50% (but not zero). These are the erythropoietic porphyrias.

– One specific case is erythropoietic protoporphyria (one mutated allele and one weak allele).

SYMPTOMS

- **Skin lesions** caused by sunlight (photosensitivity), due to the accumulation of porphyrins in the skin. These are present in all of the porphyrias apart from AIP.
- **Acute neurovisceral attacks** are due to accumulation of precursors (ALA, PBG) and/or relative haem deficiency. These only occur in the acute hepatic porphyrias: AIP (100% of cases), HC (acute attacks + skin lesions), PV (particularly skin lesions and fewer acute attacks).

  80% of acute neurovisceral attacks affect women. They occur between the ages of 20 and 45 years old, and very rarely before puberty. They mostly produce abdominal pain. Triggering factors are young age, infections (particularly urinary), the premenstrual period, medical drugs, alcohol and stress. The attacks occur in less than 1 out of 10 carriers. As the symptoms are relatively non-specific the diagnosis is rarely considered.

INCIDENCE OF SYMPTOMS IN AN ACUTE ATTACK

Abdominal and low back pain	98%
Vomiting, constipation	72%
Muscle weakness	68%
Tachycardia	62%
Insomnia, anxiety, agitation	60%
Hypertensive crises	45%
Seizures	15%
Paralysis	10%
Red coloured urine (when left exposed to light, 30 to 60 min after being passed)	80%
Hyponatraemia	65%

PORPHYRIA CUTANEA TARDA (PCT)

PCT is due to a partial uroporphyrinogen decarboxylase (UROD) deficiency. In the familial form (PCF: 25% of cases), erythrocyte UROD is deficient and in the sporadic form (PCS: 75% of cases), erythrocyte UROD is normal.

Symptoms are fragile skin, bullae, skin lesions, coloured or achromic scars and hypertrichosis (particularly in the hands and then forearm, face, legs and feet). Triggering factors are a mutation of the UROD gene (PCF), alcohol, iron overload (haemochromatosis), hepatitis C and oestrogens-progestogens.

Porphyria	Enzyme defect	Type	Photo sensitivity	Neuro-psychiatric involvement	Hepatic involvement
DOSS porphyria	ALA dehydratase	Hepatic	-	+	-
Acute intermittent porphyria (AIP)	PBG deaminase	Hepatic	-	+	-
Porphyria cutanea tarda (PCT)	Uroporphyrinogen decarboxylase	Hepatic	+	-	+
Günther's disease or congenital erythropoietic porphyria (CEP)	Uroporphyrinogen synthetase	Erythropoïetic	++++	-	+
Hereditary coproporphyria (CH)	Coproporphyrinogen oxidase	Hepatic	+	+	-
Porphyrie variegata (PV)	Protoporphyrinogen oxidase	Hepatic	+ + +	+	-
Erythropoietic protoporphyria (EPP)	Ferrochelatase	Erythropoïetic	+ +	-	+

Porphyria	Enzyme defect	Transmission	Epidemiological features	Prevalence
DOSS porphyria	ALA dehydratase	Recessive	Less then ten patients known	Very rare
Acute intermittent porphyria	PBG desaminase	Dominant	Adolescence Female > Male	Uncommon
Porphyria cutanea tarda	Uroporphyrinogen Decarboxylase	Dominant	Adult Familial and acquired	Common
Günther's disease or congenital erythropoietic porphyria	Uroporphyrinogen Synthetase	Recessive	Childhood	Very rare
Hereditary coproporphyria	Coproporphyrinogen Oxidase	Dominant	Young adult Female > Male	Rare
Porphyrie variegata	Protoporphyrinogen Oxidase	Dominant	Young adult	Rare
Erythropoietic protoporphyria	Ferrochelatase	Dominant	Childhood	Common

From Fischbach F., Porphyrias, in: Common laboratory and diagnostic tests, Edition Lippincott; 3rd Edition; New York 2002.

INDICATIONS FOR MEASUREMENT

Suspected diagnosis of porphyria in a suggestive clinical context usually associated with dark red or "porto" urine.

For diagnosis of porphyria requested in the presence of neurovisceral and/or psychiatric signs: measure ALA and PBG in an urgent urine sample (morning urine if possible). The absence of raised ALA and particularly PBG excludes the diagnosis of an attack of acute porphyria. Conversely, a very high PBG (10, 50 or even 100 times normal) is highly suggestive.

For the diagnosis of porphyria requested in the context of skin signs (bullous photodermatosis), measure ALA, PBG and total porphyrins in urine; measure total porphyrins and perform stool chromatography and where applicable measure porphyrins in blood to distinguish a cutaneous porphyria from porphyria *variegata*.

INFORMATION

■ SAMPLE STORAGE AND TRANSPORT

– Urine: collect a urine sample (preferably morning urine) in a container covered so that it is protected from light and store in a refrigerator. It is important to avoid (wherever possible) medical drugs within 12 hours before the collection.

– Stool samples: collect a 24 hour stool sample and store in a refrigerator away from light. Ask the patient to avoid red meat in their diet for 3 days before the collection. A few grams of faeces can be sent to a specialist laboratory (indicate the weight of the 24 hour stool sample).

– Blood: whole heparinised blood stored at room temperature away from light.

■ QUESTIONS FOR THE PATIENT

– History of hereditary porphyria from the patient or close relatives?

– In porphyria attacks, drug history considering recent use of drugs which induce ALA synthase? Many drugs are porphyrinogenic, such as barbiturates, first generation quinolones, allopurinol, amiodarone, etc. A full list can be consulted on the website www.porphyrie.net. Attacks can also be triggered by alcohol or reduced calorie diets.

ASSAY METHODS

– Measurement of ALA and PBG in urine: ion exchange chromatography followed by spectrophotometric detection.

NB: the red urine fluoresces in Wood's light.

– Measurement of porphyrins in stool: reverse phase high performance liquid chromatography (HPLC) with fluorimetric detection.

– Measurement of the specific enzyme deficiency.

– Molecular diagnosis by DNA analysis is the definitive diagnosis.

NORMAL EXPECTED VALUES

Reference values vary depending on the method used and are shown for reference:

Urine:

Total porphyrins: < 30 nmol/mmol of creatinine

Coproporphyrins: < 20 nmol/mmol of creatinine

Uroporphyrins: < 10 nmol/mmol of creatinine

Delta-aminolevulinic acid (ALAU): < 3.4 µmol/mmol of creatinine

Porphobilinogen (PBG): ≤ 1.10 µmol/mmol of creatinine.

Stool:

Total Porphyrins: < 200 nmol/g dry weight of stool

Coproporphyrins: < 30 nmol/g dry weight of stool

Protoporphyrins: < 100 nmol/g dry weight of stool

Uroporphyrins: trace

Erythrocytes:

Coproporphyrins: < 150 nmol/l of red cells

Protoporphyrins: < 1900 nmol/l of red cells.

PATHOLOGICAL VARIATIONS

■ ACUTE HEPATIC PORPHYRIAS

The positive diagnosis of an attack of acute porphyria is based on a large rise in ALA and particularly PBG in urine (ALA 10 x N, PBG 50 x N). All positive results must be confirmed in a specialist centre.

These measurements can be used to diagnose an acute attack but do not provide a differential diagnosis between the three hepatic porphyrias which is only obtained from combined analysis of stool, urine and possibly blood.

Laboratory diagnosis of the acute hepatic porphyrias *(from Deybach J.C. www.porphyrie.net)*

	Urine			Stool			Enzyme / expected deficiency
	Precursors	Uro	Copro	Uro	Copro	Proto	(Type of cell for measurement)
AIP 1	+++	++	+	++	+	0	PBG-deaminase/50%
2	++	-	-	-	-	0	*(Red cells)*
HC 1	+++	++	+++	+	+++	+	Copro'oxydase/50%
2	+	-	+	-	++	-	*(lymphocytes)*
PV* 1	+++	++	++	+	++	+++	Proto'oxydase/50%
2	+	-	-	-	+	++	(lymphocytes)

1: in the acute attack; 2: in remission

** Blood measurement may be useful for the diagnosis of PV: the finding of a fluorimetric peak at 620 nm provides a distinction with cutaneous porphyria.*

The type of porphyria can usually be confirmed by measuring the specific enzyme deficiency (50% reduction in activity of the enzyme responsible) together with identification of pre-symptomatic carriers in a patient's family.

Molecular biology DNA analysis can identify the specific familial mutation and asymptomatic carriers in the patient's family.

■ PORPHYRIA CUTANA TARDA

ALA and PBG do not accumulate in urine. The diagnosis is made mostly by HPLC analysis of faecal porphyrins in a stool sample from the finding of a characteristic isocoproporphyrin peak (pathognomonic).

TREATMENT

An acute attack of porphyria is a medical emergency. If increased ALA and PBG are found in urine haemarginate (Normosang®): treatment should be started in hospital as an IV infusion for 4 days. If administered early, this treatment can produce a spectacular reduction in symptoms.

FOR FURTHER INFORMATION

■ Bogard C., Deybach J.C., *Porphyrines et porphyrie*, Ann Biol Clin 1998; 56 (Spécial): 11-22.

■ Bourlière M., Boustière C., *Porphyrie cutanée tardive: le point de vue de l'hépatologue*, Hépato-Gastro, janvier-février 1997; 1 (4): 27-33.

■ www.porphyrie.net

■ www.porphyria-europe.org

PREALBUMIN

DEFINITION

Prealbumin (or transthyretin) is a protein produced mostly by the liver. It has a short half-life, in the region of 2 days. Prealbumin is a sensitive marker of malnutrition.

Synonym: transthyretine, TTR.

INTRODUCTION

Prealbumin acts mostly as a plasma transporter for:

– Thyroid hormones T4 and T3 (approximately 15%), in association with *Thyroid binding globulin* (TBG) and albumin.

– Retinol (vitamin A), in association with *Retinol binding protein* (RBP).

INDICATIONS FOR MEASUREMENT

The main indication for measurement is ***assessment of malnutrition***. As its concentrations vary rapidly (it has a short half-life), prealbumin is a very sensitive marker of acute malnutrition. It is used in the diagnosis of malnutrition and to monitor nutrition therapy.

It can be measured in isolation or together with albumin, CRP and orosomucoid, as part of a "nutritional profile", in order to take account of the inflammatory component which is often present in malnutrition. It also forms part of the calculation of the PINI *(Prognostic Inflammatory and Nutritional Index)*, the nutritional index used particularly in geriatrics.

PINI = [CRP (mg/l) x orosomucoid (mg/l)]/[albumin (g/l) x prealbumin (mg/l)].

INFORMATION

SAMPLE

Dry tube (serum) preferably collected from a fasting patient (the serum must be clear).

QUESTIONS FOR THE PATIENT

Are you taking any of the following medicines? Oestrogen may slightly reduce serum prealbumin concentrations. Long-term glucocorticoid treatment may increase concentrations (by stimulating its synthesis).

SAMPLE STORAGE AND TRANSPORT

Serum can be stored for: 1 month at +4°C and for 1 year at –20°C.

Transport: + 4°C.

ASSAY METHODS

Immunochemical: immunoturbidimetry and immunonephelometry.

NORMAL EXPECTED VALUES

These vary slightly depending on the method used. For reference:

– In adults: 0.20 to 0.40 g/l.

– In children: 0.15 to 0.35 g/l.

An international reference material has been available for immunochemical assays since 1994, CRM470 which is calibrated for 14 proteins including prealbumin.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

- **Age-related:** Values in the newborn are between 0.10 and 0,20 g/l; and rise gradually until puberty. After the age of 50 they fall slightly in both sexes.
- **Sex-related:** Between 20 and 40 years old, values are slightly higher in men (average: 0.33 ± 0.11 g/l) than in women (0.27 ± 0.10 g/l).
- **In pregnancy:** Prealbumin synthesis falls slightly in pregnancy because of hyperoestrogenaemia.

PATHOLOGICAL VARIATIONS

- **Increased serum prealbumin concentration:**

  Renal insufficiency (inconstantly).

  Hypothyroidism.

  Hyperandrogenism.

  Endogenous or drug-induced cortisol excess (long term corticosteroid therapy).

- **Reduced serum prealbumin concentration:**

  Serum prealbumin falls rapidly in malnutrition proportionally to its severity.

  In anorexia nervosa concentrations may be normal as the fall associated with malnutrition may be masked by the associated hyperandrogenism.

  Assessment of nutritional state using the PINI
  (From Ingelbleek Y. et al., Int J Vit Nutr Res 1985; 55: 91-101):

  PINI < 1: no risk. PINI > 30: life threatening risk.

  Prealbumin is not however, a specific marker for malnutrition and concentrations also fall in inflammatory states, hepatocellular insufficiency, massive urinary protein loss and hyperthyroidism.

FOR FURTHER INFORMATION

- Alexandre J.A., *Préalbumine*, Encyl Med Biol, Elsevier, Paris, 2004.
- Thérond P., *Évaluation de l'état nutritionnel*, Cahier de formation Biochimie, tome II. Bioforma, Paris, 1994: 205-8.

PREGNANETRIOL

DEFINITION - INTRODUCTION

Pregnanetriol is the main metabolite of 17-hydroxyprogesterone (17-OHP), a steroid produced both in the adrenocortical glands and in the gonads *(cf. 17-hydroxyprogesterone)*. Like all the steroids, 17-OHP is produced from cholesterol, which is firstly converted into pregnenolone. 17-OHP is formed from pregnenolone by two pathways, the first via progesterone and the second via 17-hydroxypregnenolone. In the adrenal glands, 17-hydroxyprogesterone can then be converted to cortisol by the action of 21-hydroxylase and then 11-hydroxylase or into androgens after cleaving the side chain. In the gonads, 17-OHP is the precursor of androgens and oestrogens *(cf. 17-hydroxyprogesterone)*.

17-OHP is metabolised in the liver by successive reductions. Two isomers may be formed at each stage, although the quantitatively predominant isomer is 5β-pregnane- 3α, 17α, 20α- triol, commonly called pregnanetriol (PGT). This is then conjugated with glucuronic acid before being removed in urine as the glucosiduronate.

INDICATIONS FOR MEASUREMENT

PGT is a good reflection of 17-hydroxyprogesterone production and is measured to diagnose congenital adrenal gland hyperplasia due to 21-hydroxylase deficiency and particularly to monitor its treatment. Measurements however are not widely used for diagnosis, particularly in children, as these have long been replaced by 17-OHP. On the other hand, some clinicians still recommend urinary PGT measurement to monitor the effects of corticosteroid therapy. PGT is not sensitive to stress and is a good reflection of 17-OHP production throughout the 24 hour cycle (whereas 17-OHP is a "snapshot" indicator).

INFORMATION

■ SAMPLE

A 24 hour urine collection should be obtained, without preservative and stored at + 4°C. Urine volume should be recorded accurately and an aliquot removed and sent to the laboratory at + 4°C. Under these conditions, PGT is stable for one week. Beyond this time it is recommended that the urine be frozen at -20°C.

■ NECESSARY INFORMATION

In addition to sex and age, the phase of the menstrual cycle must be reported for women with ovarian activity. Treatment with contraceptives, corticosteroids, meprobamate and penicillins must also be reported. The request should also state if a measurement is part of a functional investigation, such as Synacthen® or hCG stimulation test.

ASSAY METHODS

After the urine aliquot has been hydrolysed and extracted using an appropriate solvent, PGT is separated and quantified by gas phase chromatography which may or may not be linked to mass spectrometry.

Creatinine is measured as a measurement of the quality of the urine collection.

NORMAL EXPECTED VALUES

Children (regardless of sex): < 0.5 mg/24 hours.

Men: < 2.5 mg/24 hours.

Women with ovarian activity: luteal phase < 1.5 mg/24 hours; follicular phase: 1.0 mg/24 hours.

Post-menopausal women: < 1.0 mg/24 hours.

mg/24 hours are converted to µmol/24 hours by multiplying mg/24 hours by 2.97.

PHYSIOLOGICAL VARIATIONS

As the main urinary metabolite of 17-OHP, physiological changes in PGT parallel those of 17-OHP in plasma *(cf. 17-hydroxyprogesterone)*. PGT concentrations are therefore lower in children than in adults in both sexes. Concentrations in women with ovarian activity are lower in the follicular phase than in the luteal phase, becoming similar after the menopause to those seen in the follicular phase.

Concentrations are generally higher in adult men than women.

PATHOLOGICAL VARIATIONS

Raised urinary pregnanetriol concentrations are seen in congenital adrenal hyperplasia due to 21-hydroxylase deficiency. This is an autosomal recessive inherited disease, the severity of which depends on the extent of the enzyme deficiency. It is characterised by accumulation of steroids proximal in the pathway to the deficiency, hence an increase in 17-OHP and therefore PGT production. In addition, 17-OHP may undergo 11β-hydroxylation, converting it into 21-deoxycortisol which is then clearly produced in excess. Downstream from the blockade, synthesis of cortisol and aldosterone is compromised resulting in increased production of ACTH causing adrenal hyperplasia.

21-hydroxylase deficiency may be complete and then usually present at birth (80% before the age of 3 years old) or incomplete, presenting later. It should be investigated for in women with signs of virilisation or hirsutism (it represents approximately 10% of cases of hirsutism) or menstrual disorders *(cf. 17-hydroxyprogesterone)*. It should be noted that 17-OHP is used rather than PGT to investigate and diagnose late presenting deficiencies.

Regardless of the type of deficiency, however, PGT measurement is currently recommended particularly to monitor corticosteroid replacement treatment.

Urinary PGT concentrations are also raised in other pathological situations (11-hydroxylase deficiency, 17-20 desmolase deficiency, hypercorticism and some cases of

Stein-Leventhal syndrome) and in some physiological situations (intensive physical activity).

On the other hand, PGT concentrations are low in 3β-hydroxysteroid dehydrogenase deficiency and in both primary and secondary adrenal insufficiency. Corticosteroid therapy naturally produces a marked fall in PGT.

Finally, meprobamate, melatonin and iodinated contrast media have been reported to be associated with a fall in urinary PGT concentrations.

FOR FURTHER INFORMATION

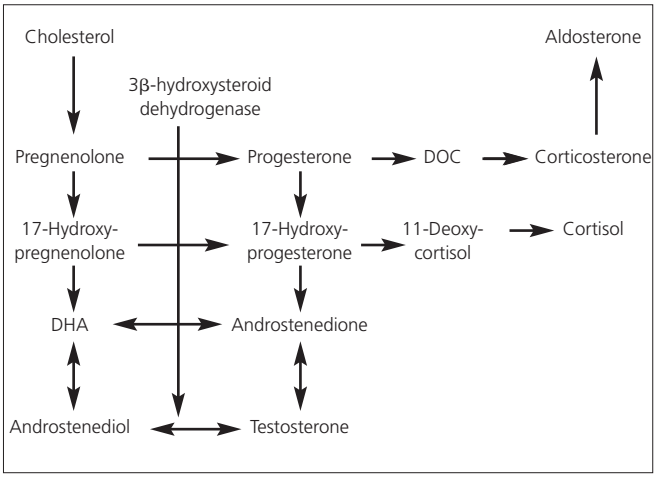
■ Faser J.S., Jansen R.P.S., Lobo R.A., *Estrogens and progestogens in clinical practice*, Whitehead M.I. (Eds), Churchill Livingstone, London UK 1998.

PREGNENOLONE

DEFINITION

Pregnenolone (5P) is a steroid synthesised in all of the steroid-producing glands, such as the ovary, testis and adrenal cortex, where it is the obligatory intermediary in the synthetic pathways for androgens, oestrogens, gluco- and mineralocorticoids.

It is formed from cholesterol by cleavage of its side chain. Pregnenolone can be metabolised in the gonads or adrenocortical glands according to the diagram below. Two metabolic pathways exist from 17-hydroxyprogesterone: one leading to cortisol and only taking place in the adrenocortical zona fasciculata and the other leading to the androgens: androstenedione and testosterone. This pathway takes place both in the testis and ovary and in the adrenal cortex.



Pregnenolone and sulphate form part of the neurosteroids as they are formed de *novo* from cholesterol in the brain, particularly in the glial cells, astrocytes and several types of neurones. This has been clearly demonstrated in mammalian (particularly rodents) and non-mammalian (birds and amphibians) vertebrates. The neurosteroids acts as potent modulators of GABAA-R receptor activity (the type A γ-aminobutyric acid receptor), the NMDA (N-methyl-D-aspartic acid receptor) and the sigma-1 receptor.

Pregnenolone is secreted particularly by the adrenocortical glands and is metabolised in the liver by successive reductions which may lead to pregnenediol, which is mostly sulpho-conjugated before being removed in urine.

Synonyms: Pregnenolone = delta 5-pregnenolone = 5P.

INDICATIONS FOR MEASUREMENT

Like 17-hydroxypregnenolone, pregnenolone is measured to identify 3β-hydroxysteroid dehydrogenase deficiency *(cf. steroid biosynthesis)*. This deficiency should be investigated in girls or women with ***hirsutism or hyperandrogenism.***

INFORMATION

SAMPLE

Pregnenolone is measured either in serum or in plasma. The anticoagulant does not interfere. Similarly, hyperlipaemic and haemolysed samples do not pose problems, as the method used involves extraction and chromatography. The sample however, must be taken in the morning before 10:00 hours in view of the 24 hour variations in pregnenolone concentrations, and should be taken under conditions avoiding stress.

NECESSARY INFORMATION

As circulating pregnenolone originates mostly from the adrenal cortex, corticosteroid treatments (systemic, topical or intra-articular) must be reported. Similarly, the request should state whether the measurement forms part of an adrenocortical Synacthen® stimulation test.

SAMPLE STORAGE AND TRANSPORT

Separated plasma or serum should be stored at + 4°C and transported at this temperature to the laboratory.

Samples can be stored for 6 months to 1 year frozen at -30°C.

ASSAY METHODS

Radio-immunological generally, with a prior purification stage involving extraction followed by chromatography. This is necessary for good specificity.

USUAL VALUES

Morning pregnenolone concentrations vary in young adult women between 0.20 and 0.60 ng/ml.

Ng/ml is converted to nmol/l by multiplying ng/ml by 3.160.

INTERPRETATION

PHYSIOLOGICAL VARIATIONS

Plasma 5P concentrations are raised at birth, both in boys and girls, and then fall regularly during the first year of life, reaching and remaining at a plateau until the age of approximately 5 years old. Concentrations then increase gradually until puberty, reaching adult values. No sex-related differences are seen.

As circulating 5P concentrations are mostly adrenocortical in origin, concentrations vary during the day following a parallel 24 hour cycle than that seen for cortisol and dehydroepiandrosterone (DHA). No variations, however, occur during the menstrual cycle.

PHARMACOLOGICAL VARIATIONS

Administration of dexamethasone or metopirone (metyrapone) produces a pronounced fall, whereas ACTH increases concentrations greatly, usually by 50 to 100%. Neither oestrogen-progestogens nor hCG change circulating 5P concentrations.

PATHOLOGICAL VARIATIONS

Changes in pregnenolone in disease are similar to those seen with 17-hydroxypregnenolone.

Circulating pregnenolone concentrations are raised in 3β-hydroxysteroid dehydrogenase deficiencies and in adrenocorticalomas *(cf. 17-hydroxypregnenolone)*. Concentrations are very low in adrenal insufficiency.

FOR FURTHER INFORMATION

■ Orth and Kovacs W.J., *The adrenal cortex*. In: Willlliam's Textbook of Endocrinology, Wilson J.D., Foster D. W., Kronenberg H.M., Larsen P.R., editors, 9th edition, 1998, W.B. Saunders Company, Philadelphia; 517-664.

■ Plassart-Schiess E., Baulieu E.E., *Neurosteroids: recent findings*, Brain Research Reviews, 2001; 37: 133 – 140.

PREKALLIKREIN

DEFINITION

Prekallikrein is a glycoprotein and a serine protease zymogen. It is bound non-covalently to high molecular weight kininogen, which itself binds to an electro-negative surface (contact factors). It is probably produced in the liver, synthesis being independent of vitamin K, and has a plasma half-life of 35 hours. Plasma concentrations are in the region of 50 mg/l (0.5 µmol/l).

Synonym: Fletcher factor.

INTRODUCTION

Prekallikrein is involved in the surface-dependent activation of coagulation, fibrinolysis, kinin generation and inflammatory reactions.

During the contact phase activation of coagulation, which is initiated by binding to an electro-negative surface, factor XII auto-activates into factor XIIa. Factor XIIa then activates the conversion of prekallikrein, complexed to high molecular weight kininogen (HMWK), into kallikrein. Kallikrein then activates factor XII and hydrolyses HMWK into several fragments, including bradykinin which has hypotensive properties. Bradykinin also stimulates the release of t-PA by the vascular endothelial cells. This is one of the pathways activating plasminogen into plasmin. In the fibrinolysis system, kallikrein also activates pro-urokinase into urokinase (another pathway for activating plasminogen into plasmin).

INDICATION FOR MEASUREMENT

Prekallikrein deficiencies increase the Activated Partial Thromboplastin Time (APTT), sometimes considerably, to variable degrees depending on the reagents (other tests to investigate coagulation, particularly the Prothrombin time, are normal). Measurement of this contact factor is indicated in isolated prolonged APTT corrected by adding control plasma, in the absence of factor VIII, IX, XI or XII deficiency. The APTT is greatly raised if the reagent used contains silica or kaolin, and less so if ellagic acid is used. The increase in the APPT is mostly corrected after incubating for 10 to 15 min compared to incubation for 1 min in prekallikrein deficiency. The diagnosis of deficiency requires specific measurement of prekallikrein.

INFORMATION

SAMPLE

Blood collected into 3.2% citrate (0.109 M) at a volume of 1/10 (0.5 ml per 4.5 ml of blood). 3.8% citrate tubes (0.129 M) are acceptable.

The sample does not have to be fasting and a light low fat snack is permitted. For further information refer to the *"General preanalytical conditions in haemostasis section"*.

QUESTIONS FOR THE PATIENT

Are you taking an anticoagulant? These treatments may increase the APTT *(cf. indications for measurement)*: unfractionated heparin, low molecular weight heparins, fondaparinux, hirudin and derivatives, dabigatran and rivaroxaban etc. (cf "Indications for measurement").

SAMPLE STORAGE AND TRANSPORT

Plasma can be stored for 2 to 4 hours at laboratory temperature, for 1 week at -20°C and beyond this time at – 80°C.

It is recommended that samples be thawed quickly in a water bath at 37°C.

Transport the frozen, centrifuged, separated sample within 2 hours of sampling.

ASSAY METHODS

Chronometric assay (coagulation method).

REFERENCE VALUES

50 to 150%.

INTERPRETATION

Prekallikrein levels are greatly reduced in severe liver disease.

Constitutional prekallikrein deficiencies are autosomal recessive in transmission and are not associated with bleeding tendencies, even with profound deficiency.

Very occasional cases of deep vein thrombosis have been reported in prekallikrein deficiency because of the involvement of this factor in the fibrinolysis system (fibrinolytic activity is reduced in prekallikrein deficiency).

If prekallikrein deficiency is found, a medical certificate must be produced and given to the patient to avoid repeated further investigations being performed if an increase in the APTT is found at a later date, and to avoid delays to any essential urgent surgery.

FOR FURTHER INFORMATION

- Samama M., *Physiologie et exploration de l'hémostase*, Doin Ed, Paris 1990.
- Abdelouahed M., Elalamy I., Samama M.M., *Physiologie de l'hémostase*, Encycl Méd Chir (Elsevier, Paris), Angéiologie, 19-0100,1997, 9p.
- Depasse F., *Un cas rare d'allongement du TCA : le déficit en prekallikrein*, Immunoanal Biol Spéc 2000; 15: 139-42.
- Samama MM et collaborateurs. *Hémorragies et thromboses. Du diagnostic au traitement*. Abrégés Masson 2e Ed, Elsevier Masson, Paris 2009: p 69.

PRIMIDONE

DEFINITION

Primidone is a first generation anti-convulsant. It is marketed as 250 mg scored tablets and it is indicated for use in the treatment of generalised epilepsy (clonic, tonic and tonic-clonic seizures) and partial epilepsy (partial seizures with or without secondary generalisation), in children and adults, as monotherapy or in association with another anti-epileptic drug.

As primidone is partly metabolised into phenobarbital, plasma measurements of both of these compounds are performed jointly.

PHARMACOKINETICS

Oral absorption	60 to 80%
Plasma peak (Tmax)	4 hours
Metabolism	Approximately 15% of primidone is metabolised into phenobarbital + active metabolite: phenyl-ethyl-malondiamide
Half-life	The half-life of primidone is 4 to 10 h. and those of its metabolites are 15 to 100 h. Steady state is reached after 2 days for primidone and after 10 to 25 days for the metabolites.
Elimination	In urine, partly in the unchanged form and partly as phenyl-ethyl-malondiamide

INDICATIONS FOR MEASUREMENT

Primidone measurements are justified because of the relationship between plasma concentrations of the anti-convulsant and reduce frequency of epileptic seizures. Measurements are indicated if treatment is ineffective or if signs of toxicity develop, particularly when primidone is associated with other drugs which may alter its metabolism *(cf. below)*.

INFORMATION

■ SAMPLE

Serum or plasma collected into EDTA or heparin; avoid tubes with separator gel.

Take the sample immediately before the next dose (trough concentration), usually before the morning dose.

■ QUESTIONS FOR THE PATIENT

Are you taking any other medical treatment?

Primidone is a potent enzyme inducer and may reduce the effectiveness of many medical drugs (oral anticoagulants, valproic acid, dihydropyridines, saquinavir and ifosfamide, etc.).

Valproic acid and valpromide also increase plasma primidone concentrations by inhibiting its hepatic metabolism, particularly in children, carrying a risk of overdose. When phenytoin is added to the treatment of a patient who is already being treated with primidone, rises in plasma primidone concentrations have been described due to competitive inhibition of hepatic metabolism, which may cause signs of toxicity.

Any request for drug measurement must include the reasons for the request (testing for efficacy or toxicity), the sampling time, treatment start date and/or any change in dosage, dosage information (amount administered, frequency and route of administration) and the age, height and weight of the person, whenever possible.

■ SAMPLE STORAGE AND TRANSPORT

Serum or plasma can be stored for a few hours at + 4°C. If the analysis is to be performed later, store at – 20°C, preferably within 4 hours of sampling. Transport frozen at - 20°C.

ASSAY METHODS

Immunoenzymatic method: EMIT.

NORMAL EXPECTED VALUES

Therapeutic range: 4 to 12 mg/l i.e. 18.3 to 55 µmol/l (conversion factor for mg/l into µmol/l = 4.58) for a sample taken immediately before the next dose of the drug at steady state, i.e. at least 20 days after starting or changing treatment.

Signs of overdose are nausea, drowsiness and ataxia. In the absence of a specific antidote, treatment is symptomatic.

FOR FURTHER INFORMATION

■ Dictionnaire Vidal®.

■ Péhourcq F., *Suivi thérapeutique des antiépileptiques : généralités*. In : Suivi thérapeutique pharmacologique pour l'adaptation de posologie des médicaments, Collection Option/Bio, Ed Elsevier, Paris. 2004: 377-82.

PROCALCITONIN

DEFINITION

Procalcitonin (PCT) is a 116 amino acid protein derived from a 141 amino acid precursor, preprocalcitonin.

Although its biological role has not been clearly established, PCT is of considerable clinical interest as an early sensitive, specific and stable marker of severe bacterial, parasitic and fungal infections. In particular, PCT enables a differential diagnosis to be made between:

– Bacterial or viral infection.

– Infectious or inflammatory processes.

– Generalised or local infection.

Concentrations correlate with severity of infection and changes can be used to monitor the effectiveness of treatment.

INTRODUCTION

The only unequivocally established physiological role of procalcitonin is as the precursor of calcitonin. PCT is synthesised by thyroid C cells and by other organs in infections, such as the liver, lungs, kidneys, pancreas and bowel, etc. Bacterial endotoxins and pro-inflammatory cytokines can stimulate PCT production. Its pathophysiological role is still poorly understood, however it may be a marker or mediator of inflammation.

PCT concentrations in healthy adults are extremely low in plasma (< 0.1 µg/l) and zero in CSF. Concentrations are raised during the first 48 hours after birth in the newborn, with a peak at around 24 to 30 hours, returning to adult values from day 3 after birth. In sepsis, PCT can be found in blood 3 to 4 hours after the onset of infection with a secretion peak at between 6 and 12 hours. It has a half-life of 25 to 30 hours in healthy people and from 30 to 45 hours in patients with severe renal insufficiency.

INDICATIONS FOR MEASUREMENT

– Marker differentiating between inflammation and infection (autoimmune diseases).

– Marker of severe bacterial infection correlating with severity of infection (nosocomial infection, septicaemia and multi-organ failure, etc.).

– Differential diagnosis of viral versus bacterial origin of a severe or systemic infection (meningitis, neonatal and paediatric pneumonia).

– Differential diagnosis between sepsis and SIRS *(Systemic Inflammatory Response Syndrome)* in intensive care patients.

– Prognostic marker and marker of effectiveness of treatment in severe infections.

– Monitoring of risk of development of infectious complications (following surgery, monitoring transplant and multiple injury patients: values which remain high on day 5 – 7 carry a poor prognosis).

PCT is a more sensitive, more specific and more stable marker of infection than CRP.

INFORMATION

SAMPLE

Blood should be collected into a dry tube (or with an anticoagulant depending on individual measurement kit recommendations). The literature does not describe any restrictions about fasting status or times to be avoided.

Repeated samples may be needed to monitor for a rise in PCT due to bacterial infection, a post-surgical peak, to monitor neonates or during treatment.

ESSENTIAL INFORMATION

The age of the patient is required. A physiological peak is seen in the newborn (0 to 48 hours). Reference values are available by time period (hours) after birth *(cf. normal expected values section)*.

Suspected disease?

Antibiotic therapy being used?

Risk of false negatives:

– Aborted meningitis.

– In patients treated with antibiotics, treatment is effective if the PCT falls by 30 to 50% per 24 hours.

– Cytokine treatment carries the risk of false positives (Muromonab CD3 (OKT3) is an anti-rejection agent and acts on the pro-inflammatory cytokine cascade).

SAMPLE STORAGE AND TRANSPORT

Storage at room temperature for 24 hours results in a 12% fall in concentration and it is recommended that serum be frozen promptly at - 20°C if measurement is to be performed more than 4 hours later.

ASSAY METHODS

Immunoanalytical methods with variants:

– Manual quantitative immunoluminometric (requires 2 hours) (LUMItest®).

– Semi-quantitative immunochromatographic (requires 30 min) (PCT-Quick®).

– Automated quantitative immunofluorescent method T.R.A.C.E. (Kryptor®).

NORMAL EXPECTED VALUES

Healthy adults	PCT < 0.5 (µg/l)

Newborn: age (hours)	PCT (µg/l) <
0 - 6	2
6 - 12	8
12 - 18	15
18 - 30	21
30 - 36	15
36 - 42	8
42 - 48	2

PATHOLOGICAL VALUES

Clinical context	PCT (µg/l)
Low probability of sepsis Inflammatory process, Autoimmune disease, Non-severe, localised viral and bacterial infections	< 0,5 µg/l
Mild to moderate bacterial infections, SIRS, multiple injuries without infection, burns Grey area which may require a later sample	0,5 - 2 µg/l
High probability of sepsis Systemic bacterial infections	> 2 µg/l
Probable multi-organ failure Septic shock	> 10 µg/l

Other causes of raised PCT:

– Thyroid C cell cancer.

– Treatment with IL2, OKT3.

FOR FURTHER INFORMATION

■ J. Bienvenu, *Procalcitonine*, Encyclopédie médico-biologique, Elsevier Paris 2003.

PROCOLLAGEN TYPE III N-TERMINAL PROPEPTIDE

DEFINITION

Procollagen type III N-terminal propeptide (PIIINP) arises from the cleaving of new procollagen type III molecules in the extracellular matrix.

Synonym: Procollagen III-peptide, PIIIP.

INTRODUCTION

The increase in concentrations of procollagen type III N-terminal propeptide (PIIINP) during chronic hepatic pathologies reflects various metabolic processes, including stimulation of collagen synthesis and a reduction in renal and hepatic clearance. Its serum concentration during liver disease, whether alcoholic, viral or autoimmune, seems to correlate with the degree of hepatic fibrosis. In effect, serum PIIINP is mainly elevated in the course of active hepatic lesions, particularly in the case of necrotic-inflammatory lesions during viral or alcoholic hepatopathies. Its sensitivity for diagnosing cirrhosis varies between 58 and 94%, while its specificity, according to studies of the aetiology of hepatopathies, ranges between 58 and 95%.

INDICATIONS FOR MEASUREMENT

PIIINP can be considered as a good marker for fibrosis, due the close pathophysiological relationship between activity and fibrosis. Its use in clinical practice remains limited, however, because of its low hepatic specificity.

INFORMATION

■ SAMPLE

Samples are collected in the morning, preferably when the subject is fasting, in a dry tube with no anticoagulant or additives.

■ QUESTIONS FOR THE PATIENT

Pathology suspected and treatment in progress?

■ SAMPLE STORAGE AND TRANSPORT

If the analysis is deferred, the sample must be centrifuged, decanted and frozen within 4 hours of collection. After freezing at -20°C, samples may be stored for several months.

ASSAY METHODS

Immunoradiometric method.

NORMAL EXPECTED VALUES

For reference: from 0.47 to 1.10 kU/l.

PATHOLOGICAL VALUES

PIIINP is elevated during numerous hepatic pathologies, including alcoholic cirrhosis, chronic viral or autoimmune hepatitis, primary biliary cirrhosis and idiopathic haemochromatosis.

PIIINP is also elevated in non-hepatic pathologies, including progressive systemic sclerosis, Paget's disease and myelofibrosis.

FOR FURTHER INFORMATION

■ Gabrielli G.B., Corrocher R., *Hepatic fibrosis and its serum markers: a review*, Dig Dis 1991; 9: 303-316.

PROGESTERONE

DEFINITION - INTRODUCTION

Progesterone is a steroid hormone synthesised in all steroid-producing glands (ovary, testis, adrenal cortex and placenta) from pregnenolone, which is obtained from cholesterol by oxidative cleavage of the side chain. Pregnenolone is converted into progesterone by the action of 3β-hydroxysteroid dehydrogenase, which takes place not only in the steroid-producing glands but also in peripheral tissues, particularly in the liver. Progesterone is one of the neurosteroids, i.e. it is formed in the central and peripheral nervous system.

The origin of circulating progesterone varies depending on age and sex. In men, pre-pubertal children, women during the follicular phase and post-menopausal women it is obtained from adrenal secretion and peripheral conversion of pregnenolone which is also produced by the adrenal gland. Conversely in women during the pre-ovulatory or luteal phase, almost all progesterone comes from the "active" ovary containing the corpus luteum.

During pregnancy, the gravid corpus luteum secretes progesterone up to week 7 of pregnancy, following which the placenta produces and secretes progesterone both into the maternal compartment and into the foetal compartment, where it is the precursor of the corticosteroids.

Progesterone synthesis by the corpus luteum is stimulated by LH, which has maximum effect at the start of the luteal phase and falls during the late luteal phase. If conception does not occur, progesterone synthesis falls, reflecting reduced sensitivity of the corpus luteum to LH.

After implantation, hCG synthesised by the syncitiotrophoblast stimulates the corpus luteum to secrete progesterone until this function is taken over by the placenta.

Regardless of its origin, progesterone circulates in plasma, mostly bound to the carrier proteins transcortin or CBG *(Corticosteroid Binding Globulin)* and serum albumin. Only a very small fraction (approximately 2.5%) is unbound. In non-pregnant women the proportions of bound and unbound fractions do not vary significantly during the menstrual cycle, although during pregnancy, because of the large increase in carrier proteins, the bound fraction becomes considerably greater than in the non-pregnant female.

Progesterone acts after binding to a specific receptor present in the myometrium, eustachian tubes, pituitary gland, hypothalamus, cerebral cortex, vagina, breasts, testes, thymus and uterine artery muscle cells. As synthesis of this receptor is stimulated by oestrogens, the effect of progesterone is only seen after that of oestrogens. The progesterone receptor is therefore a physiological product of oestrogen activity.

Progesterone has many effects. It plays a role in controlling ovulation, as it is the rise in estradiol which determines the LH peak but progesterone potentiates estradiol in the pre-ovulatory phase. It has an essential role in blastocyst implantation and maintaining pregnancy. Progesterone acts on the endometrium to enable these effects. Following the effect of estradiol which is responsible for endometrial proliferation, progesterone induces secretory transformation of the endometrium, making it receptive to possible implantation. Maintenance of pregnancy is therefore the result of its effect on uterine growth and on the myometrium, the contractility of which it reduces.

In the breast, progesterone cannot act without anterior pituitary hormones. In addition, prior oestrogen stimulation is essential. Progesterone acts synergistically with prolactin to stimulate lobulo-alveolar development and like the oestrogens, it inhibits lactation.

Progesterone also has antagonistic effects on other steroids. Its anti-oestrogen effect is caused by a fall in estradiol receptors and induction of 17β-hydroxysteroid dehydrogenase, which converts estradiol into estrone. Its anti-androgen effect is due to competition with testosterone for 5α-reductase, the enzyme required to convert testosterone into dihydrotestosterone (DHT). Finally, progesterone has anti-mineralocorticoid action as it competes with aldosterone for the tubular receptor.

Progesterone is metabolised both in the liver and in extra-hepatic tissues by successive reductions, leading to 5α and 5β-pregnandiols which are conjugated before being removed in urine. The predominant urinary metabolite is 5β-pregnane-3α, 20α-diol glucuronide, although this only represents 15 to 25% of the progesterone produced in the body. Other minor metabolic pathways exist and lead to the formation of 20α-hydroxyprogesterone and deoxycorticosterone (DOC). In the brain, progesterone is reduced firstly into 5α-pregnanedione and then into pregnanolone (5α-pregnane, 3α-ol-20-one).

It should be noted that some of these metabolites are biologically active: DOC has a mineralocorticoid action and the 5α-reduced metabolites have an anaesthetic, anxiolytic, analgesic and pyretic effect. The predominant 5α-reduced metabolite, pregnanolone, binds to the GABA receptor increasing the affinity of the receptor for GABA, a neuro-inhibitory amino acid. This produces a similar effect to the effect of barbiturates and benzodiazepines. The fall in progesterone and therefore in this metabolite after childbirth has been implicated in puerperal depression in some women.

Synonym: progesterone = P.

INDICATIONS FOR MEASUREMENT

In women with ovarian activity, progesterone measurement is used to confirm the presence of a corpus luteum and therefore ovulation if the concentration exceeds 5 ng/ml. During the luteal phase, it is used to diagnose luteal insufficiency in the investigation of female sub-fertility.

Medically-assisted pregnancy programmes use progesterone measurement to identify the pre-ovulatory peak, which appears to be more reliable than the LH peak. Similarly, measurement of progesterone is used to monitor the fate of embryo re-implantations.

Measurement of progesterone is recommended in pregnant women during the first trimester in metrorrhagia or repeated miscarriage. On the other hand, progesterone measurement is of no use in the later trimesters of pregnancy.

Finally, progesterone is measured as part of the investigation of hypertension to identify 17-α-hydroxylase deficiency.

INFORMATION

SAMPLE

Depending on the method used, measurements are performed either only on serum or on either serum or plasma (heparinised or EDTA). Haemolysed or lipaemic samples should always be discarded.

ESSENTIAL INFORMATION

For women, the day of the cycle, presence of pregnancy-related or unrelated amenorrhoea and oestrogen-progestogen contraception or progesterone treatment should be reported. In the investigation of hypertension, requests should state whether the measurement is a basal or post-Synacthen® stimulation sample.

SAMPLE STORAGE AND TRANSPORT

Separated serum is stable at + 4°C, 48 h before measurement. It can be stored for six months to one year at – 30°C.

ASSAY METHODS

Progesterone is assayed immunologically with a radio-isotopic or non-isotopic label. The antibodies now available are sufficiently specific to enable direct measurement in the serum aliquot. Many of these immunoassays can also be performed on the automated analysers which are now available.

USUAL VALUES

Values depend on the assay method used and those shown below are provided only for reference.

Results are expressed either in ng/ml or in nmol/l. Ng/ml are converted to nmol/l by multiplying ng/ml by 3.180.

Usual values are very low in children, generally below 0.5 ng/ml and very often below the limit of detection of the assay method.

In women with ovarian activity, concentrations vary during the menstrual cycle *(cf. table)*. They are low at the start of the follicular phase and increase gradually during this phase reaching a peak on the same day as the LH peak. Following ovulation, concentrations increase regularly to a peak between 5 and 10 days after the LH peak and then fall gradually until the next menstrual bleed. Secretion is pulsatile, the "pulses" correlating with those of LH.

	Follicular phase (1st week)	Follicular phase (2nd week)	Pic-ovulatory peak	Luteal phase *
Progesterone (ng/ml)	< 0.05 – 1.3	< 0.05 – 1.3	0.4 – 2.8	5.0 – 25.0

** D+4 to D+9 after the LH peak.*

During pregnancy and regardless of gestational age, progesterone concentrations are very high compared to all of the other steroids. It appears therefore to be the main steroid of pregnancy. When fertilisation occurs, average progesterone concentration reaches a plateau of 25 to 28 ng/ml, from day ten after ovulation and then begins to fall slightly between the 6th and 7th week of pregnancy before rising again, similarly to the rise in chorionic somatotropin (hCS) and development of the placenta.

Week of pregnancy	Progesterone (ng/ml)
5 - 13	15 - 50
14 - 16	30 - 75
17 - 19	40 - 80
20 - 22	40 - 90
23 - 25	50 - 110
26 - 28	50 - 150
29 - 31	70 - 150
32 - 34	73 - 170
35 - 40	95 - 200

The transient inflection of the curve undoubtedly represents the take-over of hormone production which up until that point had been provided by the corpus luteum, by the placenta.

A slight but non significant fall occurs beyond the 40th week of pregnancy.

Progesterone concentrations are very low after the menopause and are due to adrenal secretion. Progesterone concentrations in men are similar to those found in post-menopausal women.

PATHOLOGICAL VARIATIONS

Luteal insufficiency is diagnosed from reduced circulating progesterone concentrations. A threshold of 10 ng/ml has been proposed in the middle of the luteal phase but because of the large inter-individual variations in values in normal people, and the pulsatile secretion of progesterone, it emerged very quickly that a single measurement during the luteal phase was unreliable to make this diagnosis. It has therefore been proposed that three samples are taken on three different days of the temperature plateau (3rd, 6th and 9th days). Some authors believe that the samples should be mixed and only a single measurement performed and others that the samples should be measured separately and that the sum of the three concentrations be calculated, which should be over 15 ng/ml. Regardless, the diagnosis can only be made if the abnormality is repeated in several cycles.

Progesterone is greatly increased in patients with homozygous 17α-hydroxylase deficiency along with the other non 17-hydroxylated steroids (corticosterone, 11-deoxycorticosterone and 21-deoxycorticosterone). This diagnosis is suggested from hypertension with hypokalaemia and hypergonadotropic hypogonadism. Progesterone concentrations may be normal in heterozygotes, although the response to Synacthen® stimulation is exaggerated.

FOR FURTHER INFORMATION

- Perlemuter L. et Thomas J.L., *Endocrinologie.*, 5e édition, 2003, Masson, Paris.
- Casey M. L. and Mac Donald P.C., *Endocrine changes of pregnancy*. In : Willliam's Textbook of Endocrinology, Wilson J.D., Foster D. W., Kronenberg H.M., Larsen P.R., editors, 9 th edition, 1998, W.B. Saunders Company, Philadelphia; 1 259-1 271
- Carr B.R., *Disorders of the ovaries and female reproductive tract*. In : Willliam's Textbook of Endocrinology, Wilson J.D., Foster D. W., Kronenberg H.M., Larsen P.R., editors, 9 th edition, 1998, W.B. Saunders Company, Philadelphia; 751-817.
- Orth D.N. and Kovacs W.J., *The adrenal cortex*. In : Willliam's Textbook of Endocrinology, Wilson J.D., Foster D. W., Kronenberg H.M., Larsen P.R., editors, 9 th edition, 1998, W.B. Saunders Company, Philadelphia ; 517 -664.

PROINSULIN

DEFINITION

Proinsulin, a single-chain peptide precursor of insulin, is secreted in the form of preproinsulin (11500 Da) by β-cells in the islets of Langerhans in the pancreas. Preproinsulin is very rapidly converted to proinsulin in the Golgi apparatus: it comprises 86 amino acids and is formed by two chains, A and B, linked by a connection peptide or C-peptide. It is enzymatically cleaved to form insulin, a polypeptide with 51 amino acids which retains the A and B chains, linked together by two disulphide bonds, and a C-peptide in equimolar quantity. Proinsulin circulates in the blood in intact, 32 split, 33, 31, 32, 65 split, 66, 65 and 64 forms. The 31 and 32 forms, together with proinsulin, represent almost all of the circulating proinsulins. The physiological role of proinsulin is not well understood. Proinsulins bind to insulin receptors with a low affinity. They have low biological insulin activity (5 to 10%) and play little part in glycaemic control in healthy adults. Proinsulin is eliminated by the kidneys and has a half-life of approximately 30 minutes, which is considerably longer than that of insulin (4 minutes).

INDICATIONS FOR MEASUREMENT

Proinsulin is a predictive marker for the risk of type 2 diabetes: a rising level usually precedes the onset of the disease. Similarly, it is considered to be a predictive marker for coronary risk. There is in practice a relationship between proinsulinaemia and certain cardiovascular conditions.

The proinsulin/insulin ratio is sometimes used to assess the risk of cardiovascular complications in patients with type 2 diabetes.

Proinsulin measurement is also recommended when investigating hypoglycaemias in order to diagnose insulinoma and congenital hyperproinsulinaemia (rare).

INFORMATION

■ SAMPLE

Serum is preferred (EDTA or heparinised plasma may be accepted, depending on the manufacturer's recommendations).

The subject must be fasting since the previous day.

Dynamic tests in fasting patients: after 48 or even 72 hours of fasting, serial samples are collected for assay (on average every 4 hours).

Haemolysis, jaundice or hyperlipidaemia seem to have no effect on proinsulin measurement. Since, however, it is often combined with a measurement of insulin or C-peptide, precautions should be taken with regard to insulin, including rejecting haemolysed, jaundiced or hyperlipidaemic serum or plasma.

■ SAMPLE STORAGE AND TRANSPORT

The sample must be centrifuged, separated and then frozen within 4 hours of collection. Serum or plasma is conserved frozen at -20°C until the assay is carried out.

■ QUESTIONS FOR THE PATIENT

Clinical context and any current treatment?

ASSAY METHODS

Current immunometric methods can quantify intact or total proinsulin (intact and other circulating forms), as well as degraded forms. They may use isotopic (RIA) or non-isotropic (enzymatic or chemiluminescent) labels.

Depending on the technique used, varying degrees of cross-reaction with insulin and with proinsulin degradation products (including 32-33 split) are observed.

NORMAL EXPECTED VALUES

Variable according to the technique used. For reference:

Intact proinsulin: 1.2 to 4.7 pmol/l (RIA).

Total proinsulin: (RIA): 6 to 10 pmol/l; (ELISA): 3 to 28 pmol/l (average 10 pmol/l).

Intact proinsulin conversion factor: µg/l = pmol/l x 104 (with proinsulin = 9600Da).

Measurements are calibrated against the IRP 84/611 biosynthetic standard.

Proinsulin/insulin ratio: this reflects the process of conversion of proinsulin into insulin. Since the half-life of proinsulin is greater than that of insulin, it is advisable to carry out the measurement after hyperglycaemia in order to stimulate the secretion of insulin. The secretion of insulation is considerable under these conditions and its elimination can therefore be ignored.

PATHOLOGICAL VARIATIONS

High plasma concentrations of proinsulin are observed in:

– Type 2 and gestational diabetes

– Insulinoma

– Congenital hyperproinsulinaemia (very rare)

Elevated values of proinsulinaemia have also been reported in certain studies with subjects presenting glucose intolerance and close relatives of diabetic patients.

Elevated proinsulinaemia is a predictive marker for the onset of diabetes. The risk is even higher if the hyperproinsulinaemia is combined with reduced AIR (Acute Insulin Response) during an intravenous glucose tolerance test (insulin concentration in 8 to 10 minutes). In any event, proinsulin has a longer half-life than insulin and its serum concentration is thus more stable, providing better prediction.

FOR FURTHER INFORMATION

■ Sapin R., Demangeat C., *Aspects analytiques des dosages d'insuline, peptide C, proinsulines et glucagon*, Médecine nucléaire-Imagerie fonctionnelle et métabolique, 2001; 25, 73-84.

■ Clark P.M., *Assays for insulin, proinsulin(s) and C-peptide*, Ann Clin Biochem., 1999; 36, 541-564.

PROLACTIN

DEFINITION

Prolactin is a pituitary polypeptide hormone found in the bloodstream in numerous forms, most of which (> 70%) are monomeric "small" forms with a molecular weight of 70 kDa: 20% of these are glycosylated.

Other forms, of greater molecular weight, also exist physiologically. These include "big" prolactin, a polymeric (< 20%) form ranging from 50 to 70 kDa, and "big-big" macroprolactin from 150 to 170 kDa, a complex of prolactin and immunoglobulin IgG (< 10%). The big and big-big forms are biologically inactive.

PHYSIOLOGICAL ROLE

Triggers and maintains lactation.

Plays a part in fertility (action on the central nervous system and the gonads).

The regulation of prolactin secretion is dominated by the inhibiting action of hypothalamic dopamine, while TRH is one of the stimulants.

INDICATIONS FOR MEASUREMENT

In women: Menstrual disorders (oligospaniomenorrhoea), infertility, spontaneous or induced galactorrhoea.

In men: Sexual disorders (loss of libido, impotence, etc.).

Finding "true" hyperprolactinaemia, i.e. an elevated level of monomeric prolactin, is coincident with the following pathologies or situations:

Adenomas

Prolactinomas.

Mixed adenomas (growth hormone + prolactin).

Other pathologies

Lesions close to the sella turcica (intrasella miningioma and craniopharyngioma).

Endocrine pathologies (hypothyroidism, polycystic ovary syndrome and glucocorticoid adrenal failure).

Hepatic cirrhosis and renal failure.

NOTE: Hyperprolactinaemia may originate from:

– A physiological cause, such as pregnancy or breast feeding.

– An iatrogenic cause, as is the case with many psychotropic drugs (antalgics, antiemetics, opiates, neuroleptics, etc.).

INTRODUCTION

Insofar as the suspected biological stigma is hyperprolactinaemia, a check is required that no causative drugs are being taken (dopamine receptor blockers, etc.).

NOTE: contraceptives and replacement treatments during the menopause (except those with high doses of estradiol) are not considered to cause hyperprolactinaemia.

The following recommendations on sampling conditions were issued by a consensus of experts from the French Society of Endocrinologists (Médecine clinique Hors série, September 2006): "the possible very slight elevation of prolactin in response to stress suggests sample collection under resting conditions but does not necessitate the insertion of a catheter and does not justify systematic multiple sample collection. It does not in practice seem necessary to take account of the stage in the menstrual cycle, the time (although avoiding the end of the night) or meals. However, in view of the many possible causes of fluctuation, we suggest that the procedure be pursued in cases of moderate hyperprolactinaemia (less than 5 times the normal level) only after the elevated concentration has been confirmed in a second sample, in the absence of any medication which could affect the measurement and if possible using a different kit (another laboratory) in order to avoid any artefacts due to heavy forms".

■ SAMPLE

The sample may be collected using a dry tube, heparinated plasma or EDTA.

Preferably in the morning between 0800 hrs and 1000 hrs.

■ QUESTIONS FOR THE PATIENT

Date of last period?

Current treatment?

■ SAMPLE STORAGE AND TRANSPORT

Decant the serum within half a day of sample collection.

Store at +4°C if analysis is to take place with a week or at -20°C if the analysis is to be performed later than 1 week.

ASSAY METHODS

Sandwich immunoassay techniques. Monomeric "small" prolactin is recognised by all reactive systems, regardless of the binding sites of the reactive antibodies. Macroprolactin is only recognised if the reactive antibodies have binding sites distinct from that (or those) of reactive autoantibodies. In practice, all reactive systems recognise "big-big" forms in a more or less significant manner.

In certain cases, the basal measurement (static exploration) is completed by other analyses:

– Detection of a big-big prolactin (form generally associated with an absence of symptoms) by chromatography of other circulating forms of prolactin.

– TRH stimulation test if appropriate.

NORMAL EXPECTED VALUES

For reference, normal values in women range between 100 and 500 mIU/l, while those in men are between 85 and 325 mIU/l.

The "pathological" threshold is defined as 900 mIU/l. Values should no longer be expressed ng/ml, due to the fact that they vary according to the reagents used. Conversion factor (variable depending on the reagent kit): 1 ng = 22 to 36 mIU.

International reference standard: 3rd IS 84/500.

PATHOLOGICAL VALUES

Interpretation should be based on real hyperprolactinaemia. Any disagreement between clinical and biological parameters may suggest a possible analytical problem (big-big prolactin).

Any elevated value requires the elimination of causes not resulting from pituitary pathology. Values in excess of 3000 U/ml suggest pituitary adenoma and, if clinical signs are also present, should immediately trigger an investigation (MRI of the pituitary, TRH stimulation test, etc.) without waiting for chromatographic analysis of prolactin forms.

Hyperprolactinaemias with no identifiable cause are referred to as functional or idiopathic. They are treated by dopamine agonists to inhibit the secretion of prolactin.

Adenomas are treated by drugs or surgery.

FOR FURTHER INFORMATION

- Cahier BIOFORMA N° 30, *Exploration de la fonction de reproduction versant féminin*, (C. Coussieu, 2004).

PROSTATIC ACID PHOSPHATASES

DEFINITION

Prostatic acid phosphatases or PAP are ubiquitous sialoglycoproteins present predominantly in prostatic epithelial cells. They are the main constituent of seminal fluid and are normally secreted in semen and in urine and are present in low concentrations in blood.

INDICATIONS FOR MEASUREMENT

The main clinical indication for measurement of serum PAP is monitoring treatment of prostate cancer.

INFORMATION

SAMPLE

Serum only (dry tube); discard haemolysed, lipaemic or icteric samples and those containing fibrin.

A fasting sample is not necessary.

No circadian rhythm is described in healthy people, although circadian variations are described in patients with advanced prostate cancer. Because of this, it is recommended that samples always be taken at the same time.

QUESTIONS FOR THE PATIENT

Chemotherapy, radiotherapy, hormone therapy and surgery (types and date of treatment?

SAMPLE STORAGE AND TRANSPORT

Separate serum and store at between + 2 and + 8°C within 3 hours of sampling.

Can be stored (serum or aspiration fluid) for 24 hours at between + 2 and + 8°C; and at –20°C beyond this time. Transport frozen at -20°C if > 24 hours.

ASSAY METHOD

"Sandwich" immunometric method.

NORMAL EXPECTED VALUES

For reference, usual serum values are < 3.20 ng/ml (these may vary depending on the method used). Values are independent of age.

PATHOLOGICAL VARIATIONS

NON-SPECIFIC INCREASES

Moderate increase in patients with chronic active hepatitis or cirrhosis.

Moderate increase after a rectal examination, prostatic massage and biopsy. Leave at least 48 hours after these procedures before taking a sample to measure PAP.

INCREASE IN PROSTATIC DISEASES

- **Acute prostatitis:** PAP is raised, often at peak symptoms (pain, pollakiuria, etc.).
- **Distinction between prostatic adenoma and prostate cancer:** Serum PAP concentrations are raised in approximately 15% of cases of benign prostatic hyperplasia, 8 to 13% of stage A prostate cancers, 15 to 25% of stage B prostate cancers, 30 to 40% of stage C prostate cancers and approximately 70% of metastatic prostate cancers. PAP is poorly sensitive to diagnose prostate cancer (approximately 40%), although is highly specific (close to 80%).

In prostatic adenomas: A raised PAP is seen particularly in large adenomas (more than 40 g). It cannot be used for a positive diagnosis but may be useful in monitoring large adenomas when only medical treatment is given. Serum PAP concentrations return to normal after surgical excision.

- **Prostate cancer:** PAP cannot be used to diagnose prostate cancer (positive predictive value: 16.7%, less than a rectal examination, 22.2%). Concentrations do not correlate with grade of histological differentiation or with the Gleason score, although PAP concentrations are raised above all in metastatic disease; a high serum concentration suggests generalised disease and a PAP value of > 8ng/ml predicts inoperable cancer.

  PAP concentrations should return to undetectable values when monitoring patients treated by radical prostatectomy, if no metastases are present.

  After hormone therapy, PAP concentrations correlate well with response to treatment.

  A rise in serum PAP concentrations predicts bone metastases but only develops in 50% of patients who develop such metastases.

FOR FURTHER INFORMATION

- Dalifard I, Chautard D., *Phosphatase acide prostatique*, Encycl. Med. Biol. Elsevier, Paris.
- Bellet D., Bidart J.-M., *Marqueurs biologiques des cancers. Vers une utilisation sélective pour la détection et la surveillance des tumeurs malignes*, Rev Prat n° 26, 1989: 2 350-4.

PROTEIN C

DEFINITION

Protein C (PC) is a 62 kDa glycoprotein produced in the liver in the presence of vitamin K. It is formed from a 41 kDa heavy chain and a 21 kDa light chain connected by a disulphide bridge. The protein C circulates in plasma in an inactive form at a concentration of approximately 4 µg/ml.

INTRODUCTION

PC sits at the centre of a physiological coagulation inhibitor system: the physiological PC anticoagulant system. Thrombin binds to thrombomodulin, an integral protein of vascular endothelial cells and then loses its pro-coagulation properties and at the same time activated PC into activated protein C (PCa). In the presence of its co-factor, protein S, calcium and phospholipids, PCa cleaves activated factor V and activated factor VIII, which are true catalysts of coagulation and therefore blocks the thrombin generation amplification loop.

The PC system in particular controls coagulation in the microvascular network (microvessel thrombosis) and disseminated intravascular coagulation associated with septic shock.

Inherited PC deficiencies are found in approximately 3% of patients suffering from venous thrombo-embolic disease (VTED).

INDICATIONS FOR MEASUREMENT

Measurement forms part of the thrombophilia screen and is indicated in the following situations:

– In people (index cases) less than 60 years old with a first episode of spontaneous venous thrombo-embolic disease (proximal deep vein thrombosis and/or pulmonary embolism) or in a woman of childbearing age whether the episode is spontaneous or provoked. A thrombophilia screen is also indicated in recurrent proximal DVT and/or PE, whether or not provoked, following an initial episode occurring before the age of 60 years old and in the recurrence of distal unprovoked DVT when the first episode occurred before the age of 60 years old.

– Measurement is indicated for family studies in homozygous or composite heterozygous PC deficiency identified in the index case. It is also indicated before oestrogen-progestogen oral contraception is prescribed to a young woman or before pregnancy if PC deficiency is found in the index case.

PC must be measured distant to the thrombo-embolic event at least 2 to 3 weeks after oral anticoagulation has been stopped.

INFORMATION

■ SAMPLE

PC is measured in 0.109 M citrated plasma 1/10 (0.5 ml per 4.5 ml of blood). 0.129 M citrate tubes are acceptable.

A fasting sample is not required: a light low fat snack is permitted (coffee, tobacco, and alcohol must be avoided in the hour before sampling).

For further information refer to the *"General pre-analytical conditions in haemostasis"*.

■ QUESTIONS FOR THE PATIENT

Current treatments?

Drugs causing a large fall in protein C (30 to 60%):

– Anti-vitamin K agents,

– L-asparaginase, a treatment for acute lymphoblastic leukaemia and non-Hodgkin's lymphoma.

Drugs causing a (moderate) rise in protein C:

– Androgens,

– Oestrogen-progestogens

– PC measurement may be invalidated by treatment with dabigatran or rivaroxaban.

Are you pregnant?

■ SAMPLE STORAGE AND TRANSPORT

Can be stored for 2 weeks at – 20°C and for 6 months at – 70°C. It is recommended that samples be thawed promptly in a water bath at 37°C.

Transport: the sample should be centrifuged, separated and frozen within 2 hours of sampling.

ASSAY METHODS

Three types of method are conventionally used to identify and classify PC deficiency:

– Measurement of PC activity by the coagulation test.

– Immunoassay measuring plasma protein C concentration (PC Ag).

– Measurement of amidolytic activity, which assesses the functional capacity of the catalytic site.

– The recommended first line investigation is to measure the patient's protein C anticoagulant activity (French GEHT recommendations) as this is the only test which is sensitive to type IIAC deficiencies. It is however subject to more interference. For the assay per se, in this test the PC in the test sample is activated by snake venom *(Agkistrodon C. contortrix)*, Protac. The subsequent chronometric method involves measuring prolongation of the APTT due to degradation of factors Va and VIIIa by PCa, when the PC is only obtained from the test plasma when all of the other factors are provided in excess in the reagent.

NB: interferences with the chronometric method for PC anticoagulant activity:

- Activated PC is falsely reduced with increased factor VIII.

- PC activity is falsely increased in deficiency of coagulation factors responsible for prolonging the APTT: interference may also occur with circulating lupus anticoagulant or factor V Leiden, even in heterozygotes and with very high serum heparin concentrations.

- Amidolytic activity is recommended in Great Britain and the United States, as it is easier and more robust although does not diagnose IIAc variants (which are very rare). This method involves measuring PCa activity on synthetic chromogenic

substrates after Protac activation. The intensity of the colour developed by cleaving the chromogenic substrate is proportional to PC concentration.

– When a functional deficiency is identified from measurement of PC activity, PC antigen is measured by immunoassay (ELISA or ELFA) to classify the deficiency into type I (quantitative) or type II (qualitative).

NORMAL EXPECTED VALUES

Units used: % of normal values or IU/ml (1 U/ml is equivalent to 100% activity).

PC activity in adults (> 15 years old): 70 to 140%.

PC antigen: 65 to 130%.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Age:

Low levels in

- the newborn baby: approximately 35%
- children between 1 and 5 years old: approximately 65%
- children between 6 and 10 years old: approximately 70%

Very slightly increased levels in adults between 35 and 44 years old.

PATHOLOGICAL VARIATIONS

Acquired PC deficiencies are seen in the following situations:

– Hepatocellular insufficiency (hepatitis, cirrhoses),

– Vitamin K deficiencies (deficient intake or malabsorption),

– DIC and extensive thromboses,

– Anti-PC auto-Ab,

– Treatment with AVK or L-asparaginase. **NB:** it is recommended that a minimum period of 2 to 3 weeks be left after stopping AVK before measuring PC.

Constitutional protein C deficiencies:

These deficiencies are autosomal dominant in transmission and have variable penetrance. Protein C deficiency is found in approximately 3% of patients with a personal history of thrombosis. The prevalence in the general population is believed to be in the region of 0.4 to 1.5% depending on the criteria used to define the deficiency.

Classification of protein C deficiencies type I, the commonest, is defined by a parallel fall in activity and antigen, whereas type II (approximately 10% of cases) represents functional deficiencies associated with a normal antigen concentration. In type IIAM (amidolytic activity) the active site of the protein is abnormal: in type IIAC (anti-coagulant activity), the catalytic site is intact. Measurement of amidolytic activity can distinguish the two *(cf. table below)*.

	Protein C anticoagulant activity	Protein C antigen	Protein C amidolytic activity
Type I	↘	↘	↘
Type II			
A_C	↘	Normal	Normal
A_M	↘	Normal	↘

In heterozygote patients (the most common), PC levels are generally in the region of 50% although there is overlap between lower limit normal values and values in some deficient people.

Very occasional major PC deficiencies due to homozygous or composite heterozygous deficiency may also be seen from birth and are responsible for purpura fulminans.

It is recommended that a diagnosis of PC deficiency never be made from a single measurement: reduced levels must always be confirmed on a second sample taken later before making a formal conclusion.

Increased PC concentrations:

Protein C levels are increased (without clinical impact), in diabetes, inflammatory states and the nephrotic syndrome and in treatment with oestrogen, progestogens and with androgens.

PC concentrations can be increased in the premenopausal phase in women: this rise may mask a deficiency.

FOR FURTHER INFORMATION

- Le Cahier BIOFORMA N° 20, *Hémostase et thrombose*, (MM Samama, 2000)
- Samama M.M., Elalamy I., Conard J., Achkar A., Horellou M.H., *Hémorragies et thromboses : du diagnostic au traitement*, Collection «Les abrégés», Édition Masson, Paris 2004.
- Alhenc-Gelas M, Aillaud MF, Delahousse B *et al*. La recherche des facteurs biologiques de risque établis de maladie thrombo-embolique veineuse : état des connaissances et conséquences pour la pratique en biologie clinique. Sang Thrombose Vaisseaux 2009; 21, n° spécial: 12-39.

PROTEIN ELECTROPHORESIS

DEFINITION

Serum protein electrophoresis is used to assess the distribution of total proteins in blood between the different classes which it separates. The methods used are agarose gel electrophoresis and capillary electrophoresis. Agarose gel electrophoresis is a semi-automated method which migrates and separates serum proteins in an alkaline buffer on an agarose gel. The proteins are separated into 5 fractions and then stained and quantified by densitometry. Capillary electrophoresis is automated and is an electrokinetic separation method performed in a tube with a diameter of less than 100 µm, filled with an electrolyte buffer.

INTRODUCTION ET INDICATIONS

Protein electrophoresis is a routine investigation, the main current use of which is to diagnose and monitor monoclonal gammopathy (Mc Ig). In diagnostic context, it forms part of a full investigational approach alongside other biochemical investigations (measurement of immunoglobulins, immunofixation/immunoelectrophoresis and analysis of urinary proteins) and further clinical findings or information (haematology, radiology, patient's age, hospitalisation sector or reason for consultation, etc.).

Protein electrophoresis can also be used to measure Mc Ig by integrating the peak surface area by densitometry. Levels are expressed in g/l against serum protein concentrations.

It is also often used as a routine examination to assess a person's general health and is extremely useful in monitoring the change in various diseases (inflammatory diseases, liver disease, reduced immune defences, etc.).

INFORMATION

SAMPLE

Venous blood collected into a tube not containing anticoagulant.

A fasting sample is not necessary although this is recommended. The tourniquet time must not be more than 1 minute, as a longer time before the sample is taken may result in an overestimate of total serum proteins by approximately 5% (or even more with prolonged venous stasis) because of local haemoconcentration.

Avoid haemolysed samples as haemolysis causes splitting of the alpha-2 fraction.

The tests should preferably be performed on fresh serum. Beta fractions are altered in samples of old serum or those stored under poor conditions.

Do not use plasma as fibrinogen migrates in the beta-2 region.

ESSENTIAL INFORMATION

Clinical and laboratory context:

Clinical information is useful particularly when testing for monoclonal gammopathies (suspected plasmocyte proliferation, bone pain, peripheral neuropathy, etc.) together with results of other laboratory (full blood count, ESR, proteinuria, etc.) and radiological investigations.

Questions for the patient:

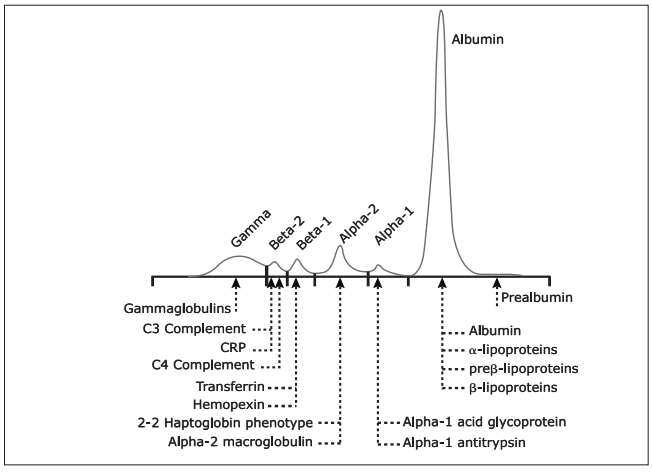
Are you taking Beta lactams (which may cause a split albumin peak), corticosteroids or immunosuppressants (reduced gamma globulins).

SAMPLE STORAGE AND TRANSPORT

Store separated serum in a closed tube for a maximum of 10 days at + 4°C and several months at - 20°C or several years at - 80°C.

NORMAL EXPECTED VALUES

Results are presented in graphical form showing the result of densitometric integration of the bands obtained after running the serum on the electrophoresis gel, together with quantitative values for each of the fractions expressed as percentages (relative values) and concentration (absolute values). A normal protein electrophoresis appearance consists of 6 fractions from the anode towards the cathode respectively: albumin, alpha-1, alpha-2, beta-1, beta-2, and gamma globulins.



As an indication, the reference values usually given are listed below:

Total protein: 60 - 80 g/l in adults; 40 - 60 g/l at birth.

Albumin: 58 ± 5%, i.e. 32 to 50 g/l

Alpha 1-globulins: 3 ± 1.5%, i.e. 1 to 4 g/l

Alpha 2-globulins: 9 ± 3%, i.e. 6 to 10 g/l

Beta-globulins: 14 ± 3%, i.e. 6 to 13 g/l

Separation of beta-1 (4.7 to 7.2%) and beta-2 (3.2 to 6.5%) fractions on capillary electrophoresis

Gamma globulins: 16 ± 4%, i.e. 7 to 15 g/l.

Values in g/l correspond to the results of the calculation from percentages of total proteins and can therefore be different to those obtained from the measurement of a specific protein or a class of proteins.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

– Age: Serum protein concentrations in newborn children are approximately 20% lower than in adults. They increase gradually during childhood reaching adult values. Serum proteins may be reduced in the elderly.

– Pregnancy: Serum proteins may be reduced by up to 10%.

– Diet and physical activity: Vegetarian diets cause a short-term fall in serum proteins, although has no long-term effect. Serum proteins are reduced in malnutrition and increase after prolonged physical exercise.

– Lying down/standing position - immobilisation: Serum proteins may vary by 8 to 10% depending on the person's position. Fluid distribution is complete and variations stabilise after 30 minutes. Serum proteins fall in a person who has been in bed for several days, by up to 5 g/l (3 g/l for albumin).

PATHOLOGICAL VARIATIONS

Agarose gel electrophoresis interpretation requires careful combined examination of the trace produced from densitometric reading and the support (gel) in order to be interpreted. Examination of the gel is essential, as only this visualises the quality of migration and can be used to recognise technical artefacts (bubbles, scratches, dust, etc.).

The interpretation of protein electrophoresis is both quantitative (in fact it is semi-quantitative as it is not calibrated) and qualitative.

SEMI QUANTITATIVE ANALYSIS OF ELECTROPHORESIS

Hyperproteinaemia

– Increased total circulating protein mass: Mono or polyclonal dysglobulinaemia.

– Reduced vascular water: Haemoconcentration due to inadequate intake or loss of fluids (heatstroke, diarrhoea and vomiting).

Hypoproteinaemia

Reduced total circulating protein mass:

– Deficient intake (malnutrition).

– Deficient absorption.

– Protein depletion (skin, renal, intestinal).

– Inadequate hepatic synthesis.

Increased vascular mass (haemodilution):

– Fluid overload.

Hypoalbuminaemia

– Inadequate intake: Severe chronic malnutrition.

– Reduced synthesis: Hepatocellular insufficiency and inflammation.

– Increased loss from the body: Urinary, skin or gastrointestinal loss

–Increased catabolism: Acquired endocrinopathy (Cushing's syndrome and thyrotoxicosis).

Alpha-1

Reduced

– Hepatocellular insufficiency, protein loss or malnutrition.

– Congenital alpha-1 antitrypsin deficiency (sometimes associated with liver disease in children and lung disease in adults).

Increased

– Inflammatory states (combined large increase in alpha-2 globulins).

Alpha-2

Split peak

– Exclude possible haemolysed samples.

– Haptoglobin of different phenotype to the commonest in France, of no pathological significance.

– Presence of free immunoglobulin light chains or a monoclonal Ig.

Reduced

– Intravascular haemolysis.

– Hepatocellular insufficiency, malnutrition or protein loss.

Increased

– Inflammatory states (increased haptoglobin and alpha 2-macroglobulin).

– Nephrotic syndrome associated with hypoalbuminaemia, proteinuria (> 3 g/l), hypogammaglobulinaemia and occasionally hyperbetaglobulinaemia (due to an increase in apo B).

β-globulins

Reduced

– By hepatocellular insufficiency, malnutrition or protein loss.

– C3 consumption hypocomplementaemia.

Increased

– In iron deficiency anaemias (raised serum transferrin).

– Occasionally, moderate increase in inflammatory states (C3 hypercomplementaemia).

– A combined increase in the gamma-globulin producing an appearance of a joint beta-gamma band suggests alcoholic cirrhosis (raised polyclonal IgA seen in this situation).

Gamma globulins

Reduced

– Physiologically in infants.

– Primary isolated or combined immunodeficiencies.

– Secondary to immunosuppressant, corticosteroid, chemo- or radiotherapy treatment.

– Light chains myeloma.

Increased

– Polyclonal in infectious (bacterial, parasitic, viral (AIDS), etc.), hepatic or autoimmune diseases.

– Monoclonal associated with malignant gammopathy (multiple myeloma or Waldenström's macroglobulinaemia, CLL and lymphoma) or "benign" in the elderly.

Some pathological syndromes can be recognised by examining changes in the different protein fractions and are useful in monitoring their progress.

The major electrophoretic abnormalities are shown in the table below.

	Total protein	Albumin	Alpha-1	Alpha-2	Beta	Gamma
Inflammation acute		↓. N	↑	↑		↓. N
subacute		↓. N	N	↑	N	N
chronic		↓. N	↑	↑	N.↑	↑
Severe hepatitis	↓. N	↓↓	↓	↓	↓	↓
Cirrhosis compensated	↓. N.↑	↓↓		↓	↓	↓
decompensated					Beta-gamma fusion	
Nephrotic syndrome	↓↓	↓↓		↑↑		↓. N
Hypo-or α-globulinaemia						↓↓↓
Monoclonal gammopathy	N. ↑	↓	↓	↓	Even band	
Hyper γ globulinaemia	N. ↑	↓				↑
Protein loss	↓↓	↓↓	N. ↑	N. ↑	↓	↓. N. ↑
α_1-AT deficiency			↓↓			

■ QUALITATIVE ANALYSIS OF ELECTROPHORESIS

■ Bisalbuminaemia

A split albumin peak may represent a genetic abnormality (with no significant clinical consequences) or an acquired abnormality (pancreatic fistula releasing proteases which degrade albumin in-vivo or during beta lactam treatment). It may also reflect high concentrations of triglycerides or bile pigments (the serum having a characteristic yellow-green colour in the latter case) in the sample, which may produce appearances of bisalbuminaemia.

■ Monoclonal gammopathy

The presence of a narrow band generally migrating in the beta or gamma globulins should suggest a monoclonal immunoglobulin, which is confirmed by immunofixation. Mc Ig however cannot be excluded by the absence of a peak on electrophoresis as it may be present at a concentration below the sensitivity of the method. Careful examination is always required and one must always be beware of hypogammaglobulinaemia or an abrupt fall in the near cathode area (far gamma) which may represent a Mc Ig.

A relative increase in the beta-2 fraction compared to the beta-1 fraction outside of the context of inflammatory disease should prompt testing for the presence of a Mc Ig.

In patient follow-up the electrophoresis is still currently the best way of quantifying the Mc Ig by integrating the peak surface areas. This is expressed in g/l against the serum protein concentration.

■ Split in alpha-2 globulin band

Some samples may contain a split in the alpha-2 band depending on haptoglobin phenotype (not pathogenic).

■ DIFFICULTIES IN INTERPRETATION AND CAUSES OF ERROR

■ Some proteins can migrate as a narrow band suggesting an Mc Ig

Fibrinogen: This is seen as a peak in the early gamma range. It may be seen if the sample is collected into anticoagulant or inadequately coagulated. In this case the investigation should be repeated on the centrifuged supernatant from the sample, precoagulated by adding thrombin (this possibility can be determined depending on whether or not the peak disappears).

Haemoglobin: In haemolysis, haemoglobin complexed to haptoglobin migrates as a narrow band generally between the alpha-2 and β-globulins or even beyond the β region.

C-reactive protein, at high concentrations (> 100 to 150 mg/l) may appear in the form of a narrow band migrating in the beta-2 position. If the CRP is very high, immunofixation should be deferred for a few days (if allowed by the clinical context).

Note that one peak may hide another. Each of these abnormalities can mask a genuine Mc Ig. Comparing the results with other further investigations can be very useful.

FOR FURTHER INFORMATION

■ Daunizeau A., *Électrophorèse des protéines du sérum*, Le Cahier de formation Biologie médicale n° 28, Bioforma, Paris, 2003: 26-46.

■ Le Carrer D., *Électrophorèse, Immunofixation des protéines sériques*, Laboratoire Sebia, Hatier, 1994.

■ Lissoir B., Wallemacq P., Maisin D. *Electrophorèse des protéines sériques: comparaison de la technique en capillaire de zone Capillarys® (Sebia) et de l'électrophorèse en gel d'agarose Hydrasis® (Sebia)*. Ann Biol Clin 2003; 61 n° 5: 557-62

PROTEIN PROFILES

DEFINITION

The protein profile (PP) is the simultaneous comparative study of proteins chosen because of their involvement in major body functions and the pathological significance of their changes. Results are expressed as percentages of standardised values for age and sex and shown in graphical form (the protein profile).

Measurement of a single protein is difficult to interpret or link to a specific disease as a protein can be involved in several opposing pathophysiological mechanisms producing a normal result.

	Mechanism 1	Mechanism 2	Result
Transferrin	Inflammation ↘	Iron deficiency ↗	Normal
Haptoglobin	Inflammation ↗	IV haemolysis ↘	Normal
Orosomucoid	Inflammation ↗	Urine loss ↘	Normal
Fibrinogen	Inflammation ↗	Fibrinolysis ↘	Normal
C3	Inflammation ↗	C3 activation ↘	Normal
CRP	Inflammation ↗	Increased catabolism (SLE) ↘	Normal

The concept of the protein profile proposed by P. Giraudet simplifies interpretation by visualising the relative changes in proteins with respect to each other. It also links together proteins which are involved in the same pathophysiological process but have different kinetics of change and to assess the magnitudes of changes in each protein measured.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

Depending on the practical assistance which is intended for the clinician, indicative PP (aid to diagnosis) should be distinguished from "targeted" PP (disease monitoring).

■ INDICATIVE PROTEIN PROFILE

This is a profile of 8 proteins: IgG, IgA, and IgM (immunoglobulins involved in the humoral immune response), complement fraction C3, orosomucoid and haptoglobin ("positive" inflammatory proteins), transferrin and albumin ("negative" inflammatory proteins and markers of nutritional status). This profile provides an indication towards diagnosis if clinical features are not characteristic, such as prolonged fever, long term fatigue, dysimmune disease, deterioration in general health, weight loss, etc. Serum protein electrophoresis is generally performed alongside the protein profile in order to identify any dysglobulinaemia.

■ TARGETED PROTEIN PROFILES

These are profiles with a smaller number of proteins used to study and monitor changes in proteins involved in particular pathological processes.

■ THE IMMUNE PROTEIN PROFILE

This includes the immunoglobulins (IgG, IgA and IgM) and is used to identify humoral immunodeficiencies, which may be either congenital or acquired and results in a greater or lesser fall in several or all classes of immunoglobulins.

The major deficiencies include:

– Transient hypogammaglobulinaemia in young children.

– Acquired deficiency of the three immunoglobulins, associated with malignant blood dyscrasias, protein loss, toxic or drug-induced disorders (chemotherapy, radiotherapy, immunosuppressants, corticosteroid therapy etc.), viral diseases (EBV, CMV and Herpes), malnutrition and auto-immune diseases.

– Congenital deficiencies: Bruton's sex-linked congenital agammaglobulinaemia (rare).

– Selective deficiencies: IgA: congenital IgA deficiency, coeliac disease, Crohn's disease; IgM: the elderly, IgG or IgA myeloma; IgG: IgA myeloma, Waldenström's disease.

Increases in immunoglobulins:

– Polyclonal rise due to over-stimulation of synthesis affecting all three classes of immunoglobulins or more selectively affecting one of the three, with or without an inflammatory reaction (bacterial, viral or parasitic infectious diseases, auto-immune diseases, cirrhosis and cancers).

– Quantitative abnormalities in monoclonal gammopathies (characterised by serum protein electrophoresis and immunofixation) and in the follow-up of malignant blood dyscrasias (CLL and lymphoma).

■ THE INFLAMMATORY PROTEIN PROFILE

Two types of proteins produced by hepatocytes are involved in inflammation:

– The "positive" proteins which rise in concentration during inflammation, such as CRP (C reactive protein), orosomucoid (alpha 1-acid glycoprotein), haptoglobin, fibrinogen, alpha 1-antitrypsin, complement C3 and C4 and ceruloplasmin.

– The "negative" proteins, synthesis of which is reduced, such as albumin, prealbumin and transferrin.

Three of these proteins constitute the inflammatory protein profile: ***CRP*** (C reactive protein) a protein with rapid, large amplitude kinetics, and ***orosomucoid*** and ***haptoglobin*** which have slower kinetics and lesser amplitude.

This profile enables the dynamics of the inflammatory reaction to be studied. CRP increases from 6 hours into the inflammatory process, reaching a peak at 24 hours and falling after 48 hours with a short half-life, returning rapidly to normal if the inflammatory process stops. Haptoglobin has slower kinetics and a half-life of 3 to 5 days. Orosomucoid rises slightly before haptoglobin, although is rapidly overtaken by haptoglobin, concentrations being linked by the ratio:

HAPT% = ORO% x (1.3 +/- 0.2)

The fall in CRP can be used for example to assess the effectiveness of antibiotic therapy. Return of haptoglobin and orosomucoid concentrations to normal is evidence of recovery.

Table 1

	CRP	Orosomucoid	Haptoglobin
Half-life	6-8 hours	72 hours	72 hours
Kinetics	Rapid	Fairly slow	Fairly slow
Start	6 hours	12 hours	18 hours
Return to normal	48 hours	8-10 days	10-12 days

Table 2

Inflammatory reaction	CRP	Orosomucoid	Haptoglobin
Acute, early	+++	+	N or +
Acute, established phase	+++	+++	+++
Resolving	N or +	+	++
Chronic	N or +	+ or ++	++

From J. Rousseaux, www.med.univ.lille2.fr

■ THE HAEMOLYTIC PROTEIN PROFILE

This consists of haptoglobin and orosomucoid and is used to investigate different haemolytic states:

– Intra-vascular haemolysis (mechanical, secondary to a prosthetic heart valve) causing a large fall in haptoglobin.

– Extra-vascular or tissue haemolysis (haemoglobinopathy), which only produces a fall in haptoglobin in severe haemolysis.

– Interstitial haemolysis (haematoma) which is both intra- and extra-vascular and only produces a modest fall in haptoglobin.

Haptoglobin is an inflammatory protein which binds plasma haemoglobin released during haemolysis to form a macromolecular complex, preventing the renal excretion of haemoglobin, protecting the nephron and avoiding iron loss. The complex is catabolised in the liver. Because of its small plasma pool, haptoglobin is a very sensitive indicator of haemolysis and it is estimated that haemolysis of 20 ml of blood can reduce haptoglobin concentration by 1 g/l.

Haptoglobin must not be interpreted in isolation but along with orosomucoid. If no inflammation is present the fall in haptoglobin is not pathologically significant unless it falls below 50% of its median value. In the presence of inflammation, haptoglobin concentrations may be falsely normal and only a concentration below 100% of the orosomucoid concentration (haptoglobin/orosomucoid ratio < 1) is a significant indicator of haemolysis.

■ NUTRITIONAL PROFILE

This profile consists of four proteins: albumin, prealbumin (PALB), retinol binding protein (RBP) and orosomucoid.

The first three of these are used to assess nutritional status and orosomucoid to assess inflammation and any effects on the fall in nutritional proteins (excessive catabolism caused by inflammation).

Albumin has a half-life of 20 days and is a marker of chronic severe malnutrition, whereas prealbumin has a half-life of 2 days and is an earlier marker which is very useful to assess early or even subclinical malnutrition states. It falls proportionally to the extent of malnutrition and can be used to monitor the effectiveness of nutrition therapy.

RBP which has a shorter half-life than that of prealbumin (approximately 12 hours) is even more sensitive in early malnutrition. PALB and RBP are increased in haemodialysis patients because of the renal insufficiency, and in this situation albumin is the only marker of malnutrition. The profile must be interpreted taking account of the orosomucoid level, which is used to establish whether or not a concomitant inflammatory reaction is present. If no inflammation is present and only prealbumin is reduced the malnutrition is recent: if albumin and prealbumin are both reduced the malnutrition is chronic.

The benefit of the nutritional protein profile is therefore that it can be used to investigate for early malnutrition or to assess how long the malnutrition has been present, but also to monitor the effectiveness of nutrition therapy (oral, enteral or parenteral). Prealbumin rises from the second day, followed by albumin (in 7-10 days). The nutritional protein profile can also be used prognostically as malnutrition increases the morbidity and mortality of some diseases. Ingelbleek and Carpentier have proposed an index, the PINI (pronostic inflammatory and nutritional index) which combines two nutritional proteins, albumin (ALB) and prealbumin (PALB) with two inflammatory proteins, orosomucoid (ORO) and C reactive protein (CRP).

PINI =CRP (mg/l) x orosomucoid (mg/l)/albumin (g/l) x prealbumin (mg/l).

Interpretation:

< 1: no risk

1 to 10: low risk

11 to 20: medium risk

21 to 30: high risk

> 30: life-threatening.

INFORMATION

■ SAMPLE

Serum sample, preferably taken from a fasting patient.

Discard hyperlipaemic and/or haemolysed samples.

Patient age and sex are essential for graphical interpretation.

■ SAMPLE STORAGE AND TRANSPORT

Serum storage: 1 week at + 4°C, several months at – 20°C.

ASSAY METHODSS

Immunochemistry: immunonephelometry and immuno-turbidimetry.

NORMAL EXPECTED VALUES

Results are expressed as percentages of values standardised for age and sex and displayed graphically.

FOR FURTHER INFORMATION

■ Giraudet P., *Concept et intérêt symptoms des profils protéiques*, Feuilllets Biol 1992; 33/188: 61-69.

PROTEIN S

DEFINITION

Protein S (PS), discovered in Seattle, hence its name is a 69 kDa vitamin K-dependent, glycoprotein produced by hepatocytes, endothelial cells, megakariocytes and osteoblasts. Its synthesis requires the presence of vitamin K. It has a half-life of 42 hours and a plasma concentration of 25 mg/l (0.33 µm).

INTRODUCTION

Protein S is a physiological coagulation inhibitor, which acts as a cofactor for activated protein C (PCa), which promotes inactivation of factors Va and VIIIa by proteolysis. It inhibits the activation of prothrombin and formation of the prothrombinase complex on phospholipids together with the activation of factor X.

Protein S circulates in the blood in two forms: 40% as a biologically active free form and 60% bound to a complement system transport protein C_{4b}BP (C4b *Binding Protein*), which is inactive.

INDICATIONS FOR MEASUREMENT

Measurement forms part of the thrombophilia screen and is indicated in the following situations:

– In people (index cases) less than 60 years old with a first episode of spontaneous venous thrombo-embolic disease (proximal deep vein thrombosis and/or pulmonary embolism) or in a woman of childbearing age, whether the episode is spontaneous or provoked. A thrombophilia screen is also indicated in recurrent proximal DVT and/or PE whether or not provoked following an initial episode occurring before the age of 60 years old and in the recurrence of distal unprovoked DVT when the first episode occurred before the age of 60 years old.

– Measurement is indicated for family studies in homozygous or composite heterozygous PS deficiency identified in the index case. It is also indicated before oestrogen-progestogen oral contraception is prescribed to a young woman or before pregnancy if PS deficiency is found in the index case.

PS must be performed distant to the thrombo-embolic event at least 2 to 3 weeks after oral anticoagulation has been stopped and outside of pregnancy (two months after childbirth).

PS deficiency should never be diagnosed from a single measurement: low titres must always be confirmed on a second sample taken later before drawing a formal conclusion.

INFORMATION

SAMPLE

PS is measured in 0.109 M citrated plasma 1/10 (0.5 ml per 4.5 ml of blood). 0.129 M citrate tubes are acceptable.

A fasting sample is not required: a light low fat snack is permitted (coffee, tobacco, and alcohol must be avoided in the hour before sampling).

For further information refer to the *"General pre-analytical conditions in haemostasis"*.

NB: PS measurement requires strict pre-analytical conditions to be followed.

QUESTIONS FOR THE PATIENT

Current treatments?

Drugs causing a large fall in protein S:

– Anti-vitamin K agents.

– L-asparaginase prescribed to treat acute lymphoblastic leukaemias and non-Hodgkin's lymphomas …,

– Oestrogen-progestogens (oral contraception and hormone replacement therapy for the menopause),

– PC measurement may be invalidated by dabigatran or rivaroxaban.

Are you pregnant?

SAMPLE STORAGE AND TRANSPORT

Can be stored for 2 weeks at – 20°C and for 6 months at – 70°C.

It is recommended that samples be thawed promptly in water bath at 37°C.

Transport: the sample should be centrifuged, separated and frozen within 2 hours of sampling.

ASSAY METHODS AVAILABLE

Measurement of protein S activity (co-factor for activated protein C): chronometric method.

Assay of free protein S antigen or total protein S (free + bound to C_{4b}BP) antigen: immunological methods.

Only measurement of functional protein S activity tests for all types of deficiencies and for this reason is recommended for first line use. Historically this was not the case because of its poor specificity, although the reagents have improved.

Activity measurements involve measuring the prolongation of the coagulation time of a mixture of the test plasma and a PS-depletes reagent plasma in the presence of PCA (exogenous or produced in the mixture with Protac). Coagulation is studied in a QT or APTT test or after activation with added FXa or FXa produced by endogenous activation of FX by Russel viper venom.

The assay is subject to interference from factor V Leiden, very high levels of factor VIII, heparins and some lupus anticoagulants. The influence of these factors varies depending on reagent used, although can produce false diagnoses.

If activity is reduced (even if only slightly) immunological measurements of free PS and total PS must be performed in parallel. Commercially available methods use Elisa or immunoturbidimetry.

Further investigations:

Measurement of C_{4b}BP: this may be useful if reduced free protein S is suspected to be due to an increase in C_{4b}BP (mostly inflammatory states), although the test must not be used systematically.

Molecular biology: study of the protein S gene (PROS1) is not performed routinely. This gene, which contains an inactive pseudogene (PROS2), stems over 80Kb and contains 15 exons and 14 introns: in addition, mutations identified up until now have been distributed over the entire gene. Only a few specialist hospital laboratories analyse the gene for family studies.

UNITS AND REFERENCE VALUES

UNITS

Units used: % compared to normal values. Values are expressed in IU/ml (100% is equivalent to 1 IU/ml) are also reported in the literature.

NORMAL EXPECTED VALUES

– PS anticoagulant (activity): 65 to 140%.

– PS free antigen: 65 to 140%.

– PS total antigen: 65 to 140%.

It should be noted that regardless of the method used to assay protein S an overlap exists between normal people and people suffering from protein S deficiency. The results should be interpreted according to age and sex and in the clinical and therapeutic context *(see below)*.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Age:

Levels are lowered in "term" neonates (12 to 60%). Adult values are only reached after six months to one year old. Values are also low in premature infants in whom they should be interpreted according to gestational age.

Up to 3 months old almost all protein S is in the free form because of low levels of C_{4b}BP due to neonatal hepatic immaturity.

Protein S concentrations increase gradually with age in adults.

Sex:

Protein S concentrations are slightly higher in men than in women (hormonal influence); values in post-menopausal women not taking hormone replacement therapy are similar to those of men of the same age.

Pregnancy:

Protein S concentrations fall from the 10th week of pregnancy. Protein S activity and free protein S antigen levels in pregnancy and post-partum are shown below (from Conard J., *Manuel d'hémostase*, Elsevier, Paris, 1995). Following pregnancy, it is recommended that measurements be taken 6 to 8 weeks after child-birth.

Changes in protein S levels during pregnancy and post-partum.

	10 to 20 weeks pregnant	20 to 30 weeks pregnant	30 to 40 weeks pregnant	6 hours to 4 days after childbirth	2 weeks to 12 months after childbirth
PS free antigen	67 - 102%	52 - 96%	41 - 59%	55 - 79%	65 - 95%
PS activity	62 - 112%	43 - 70%	34 - 60%	22 - 45%	80- 93%

A rise in PS levels has no clinical impact.

PATHOLOGICAL VARIATIONS

Several situations can be seen in inflammatory states depending on the inflammatory mediators involved. Increased total PS, free PS and PS activity or reduced free PS and PS activity may result in incorrect diagnoses.

Acquired protein S deficiencies are seen in the following situations:

– hepatocellular insufficiency,

– vitamin K deficiencies (deficient intake or malabsorption),

– some inflammatory states (due to a rise in C_{4b}BP concentration causing a fall in the free protein S fraction),

– anti-protein S antibodies, in varicella or systemic lupus erythematosus,

– treatments with anti-vitamin K agents, L-asparaginase, oestrogen-progestogens (oral contraception and hormone replacement therapy for the menopause). NB: it is recommended that a minimum period of 2 to 3 weeks be left after stopping AVK before measuring PS. For oestrogen-progestogens, the hormone therapy should be stopped for two cycles before performing measurements.

Constitutional protein S deficiencies:

The prevalence of heterozygous protein S deficiency in patients with a personal history of thrombosis is between 4 and 8% depending on the study. The risk of thrombosis is increased by a factor of two in protein S deficiency.

Very occasional major congenital homozygous or composite heterozygous protein S deficiencies may present from birth and cause purpura *fulminans*.

Classification of constitutional protein S deficiencies:

Quantitative deficiencies (type I) are the most common and are characterised by a parallel reduction in free antigen and activity. Qualitative congenital deficiencies (type II) are rare.

Classification of congenital protein S deficiencies:

	Protein S Activity (%)	Free protein S antigen (%)	Total Protein S (%)
Type I: quantitative deficiency	< 65	< 65	< 65
Type II: qualitative deficiency			
IIa: free fraction deficiency (or "selector" deficiency)	< 60	< 60	65 - 130
IIb: functional deficiency	< 60	65 - 130	65 - 130

PS deficiency should never be diagnosed from a single measurement. Reduced concentrations must always be checked on the second sample taken later before drawing a formal conclusion.

FOR FURTHER INFORMATION

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PROTHROMBIN FRAGMENTS 1 + 2

DEFINITION

Prothrombin is a polypeptide chain, whose C terminal portion consists of thrombin and whose N6 terminal contains ten gamma-carboxyglutamic groups. These are called prothrombin "fragments 1 + 2". During clotting the conversion of prothrombin to thrombin releases several fragments including the "fragments 1 + 2". This is then split into its two components (fragment 1 and fragment 2) by the thrombin already generated in the process. Fragment 1 contains the 10 gamma-carboxyglutamic groups and plays a role in phospholipid binding. Fragment 2 appears to be necessary for the action of factor V during the conversion of prothrombin to thrombin. Thus, F 1 + 2 liberated into the plasma reflect the action of factor Xa on prothrombin. They are markers of thrombin generation and, therefore, indirect markers of the activation of coagulation. Their plasma half-life is approximately 90 minutes.

Synonym: F1 + 2

INTRODUCTION

The generation of thrombin is responsible for the amplification of the clotting process and for consolidation of the thrombus. It results from a series of reactions involving numerous agents such as F1 + 2, thrombin-antithrombin complexes (TAT) and fibrinopeptide A, whose measurement can be employed to identify patients at high risk of vascular thrombosis. These markers of hypercoagulability reflect activation of coagulation and, therefore, disturbance of the haemostatic equilibrium.

INDICATIONS FOR MEASUREMENT

As yet no indication has been validated in current practice. The assay is employed in basic and clinical research to follow thrombin generation. It is used notably in the development of new anticoagulant drugs to monitor their effectiveness in reducing thrombin generation.

INFORMATION

SAMPLE

Use a citrated tube at a concentration of 3.2% (0.109 M) in dilution ratio of 1/10 (0.5 ml for 4.5 ml of blood). Citrated tubes at 3.8% (0.129 M) are also acceptable. Blood can also be collected into a CTAD tube (citrate, theophylline, adenine, dipyridamole). No other anticoagulants are allowed.

A fasting sample is not necessary. A low fat breakfast is allowed. For further information, refer to the leaflet "general conditions for sampling in haemostasis".

QUESTIONS FOR THE PATIENT

Are you taking an anticoagulant? These drugs cause a significant reduction in plasma concentrations of F 1+ 2 below the normal expected values within hours of administration.

SAMPLE STORAGE AND TRANSPORT

Centrifuge shortly after sampling and separate the plasma. Plasma can be kept at room temperature or between 2 and 8°C for 4 hours, for 1 month at – 20°C and for several months at – 70°C. If the assay is to be performed off-site, then transport the sample in the frozen state.

ASSAY METHODS

Immunoenzymatic ELISA method.

NORMAL EXPECTED VALUES

Example: 0.4 to 1.1 nmol/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

F 1 + 2 increase with age from 45 years.

PATHOLOGICAL VARIATIONS

- **Elevation of the plasma concentration of F 1 + 2** is seen in situations where clotting is activated, such as deep venous thrombosis, pulmonary embolus, disseminated intravascular coagulation, myocardial infarction, multiple trauma and septicaemia. It is also raised in patients whose thrombotic risk is increased because of hereditary deficiency of physiological inhibitors of coagulation. These include antithrombin (AT), protein C and protein S. In patients with AT deficiency the plasma concentration of F 1 + 2 can be normalised by infusing concentrated AT.

  In general, elevation of plasma F 1 + 2 is suggestive of early activation of clotting and is an indication of increased thrombogenic risk. It is not, however, employed other than in specific studies. For example, a significant correlation has been demonstrated between increased plasma F 1 + 2 four hours after coronary artery bypass surgery and the later development of post-operative complications (multi-organ failure).

- **Normal plasma concentration** argues against the existence of recent venous thrombosis or pulmonary embolus, but this test is less useful in this situation than measurement of D-dimers.

- **During the development of new anticoagulants**

FOR FURTHER INFORMATION

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PROTHROMBIN GENE MUTATION 20210

DEFINITION

Polymorphism of the thrombin gene associated with moderate hereditary thrombophilia, corresponds to the replacement of guanine by adenine at position 20210 i.e. in the non-coding region 3' of the gene for prothrombin (or Factor II).

Synonyms: Mutation or variant or polymorphism 20210G > A of Factor II; mutation G20210A, mutation or variant 20210G > A of prothrombin.

INTRODUCTION

The polymorphism has no effect in a specific biological functional test. It manifests itself by a level of plasma prothrombin which is higher on average (values overlap, however, between subjects who are carriers of the polymorphism and those who are not).

INDICATIONS FOR MEASUREMENT

A search for mutation 20210 in the prothrombin gene, as a first or second line step, is indicated when investigating deep vein thrombosis or pulmonary embolism.

INFORMATION

■ SAMPLE

Whole blood collected on EDTA (5 ml).

This test is not affected by anticoagulation treatments.

■ ESSENTIAL INFORMATION

The prescription must be legally issued in the context of an individual medical consultation with a doctor working in a multidisciplinary group. In France, a consultation certificate, confirming that the patient gave their informed consent, must be attached to the sample. A copy is added to the patient's medical records.

■ SAMPLE STORAGE AND TRANSPORT

The sample can be stored for 24 hours at room temperature (15 - 20°C).

Transport at room temperature or at +4°C if duration of transport > 24 hours.

Extracted and purified DNA can be stored for several months at -70°C.

ASSAY METHOD

Searching for variant 20210G > A of Factor II by molecular biology

After an extraction of genomic DNA, a search for variant 20210G > A in prothrombin, is based on PCR (polymerase chain reaction) amplification of a region of the prothrombin gene, targeting the molecular abnormality. Various operating procedures are able to reveal the mutation through PCR results.

The PCR-RFLP method *(Restriction Fragment Length Polymorphism)*

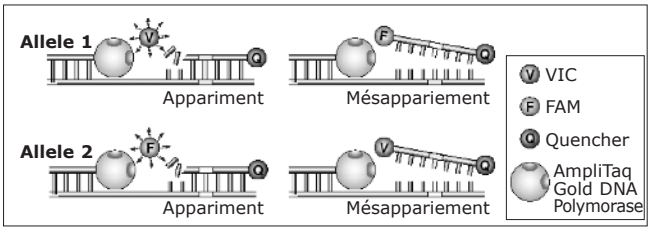
The amplification products are subjected to digestion by a restriction enzyme whose action depends on the presence or absence of the targeted mutation. Electrophoresis is used to separate the DNA fragments obtained by enzymatic digestion and amplification on the basis of their molecular weight and therefore their length.

The real-time PCR method

Amplification of the target sequences of the prothrombin gene can be performed using two TaqMan R-MGB probes, one of which is specific to the normal (or wild-type) allele and the other to the mutated allele. The extremity 5' of each probe carries a specific fluorochrome of which the fluorescence is inhibited by a "quencher" fixed to its extremity 3'.

The 5'-nucleotidase activity of the Taq-polymerase allows cleavage of the fluorochrome solely from the hybridised probe and the emission of fluorescence from the fluorochrome thus released from the influence of the quencher.

Real-time PCR is performed with a reading at the final point of the fluorescence emitted by the FAM and VIC fluorochromes, specifically of the wild-type allele and the mutated allele.



– Treatment of the amplification products using restriction endonucleases (the PCR-Restriction Fragment Length Polymorphism or **PCR-RFLP** method)

– Amplification of the wild-type and mutated alleles using specific primers (**SSP-PCR** or Sequence-Specific Primer PCR or modified primers (**ARMS-PCR** or Amplification-Refractory Mutation System PCR)

– Revealing of the amplification product by means of specific probes for the wild-type and mutated allele forms (ASO-PCR or Allele-Specific Oligonucleotide-PCR)

– PCR in a homogeneous medium (or **Real-Time PCR**), based on the detection of a fluorescence signal (hydrolysis probes, tandem probes, molecular markers, scorpion probes and fluorescent primers)

– Treatment of amplification products by primer extension (**minisequencing** or SNaPshot, Single Base Extension ELISA)

– "Multiplex" methods are available for simultaneously searching for mutation 1691G > A of Factor V Leiden and variant 20210G > A of prothrombin.

A recent development is a molecular hybridisation method with amplification of a fluorescent signal (absence of DNA amplification by PCR), based on Invader® technology, which is applicable for the genotyping of variant 20210G > A of prothrombin.

RESULTS

Mutation 20210G > A of prothrombin is found to be absent or present, in the heterozygous or homozygous states.

PATHOLOGICAL VARIATIONS

The frequency of this variation in the gene is 2% in the general population of Caucasian origin and 7% in patients with a history of venous thromboembolism. A North/South distribution gradient is observed, with a higher frequency in Spain than in Northern Europe. There is a moderate risk of thrombosis associated with the genetic variation. When present in the heterozygous state, the polymorphism brings a risk of venous thromboembolic disease of the order of 3. In homozygous patients, the risk is multiplied by a factor ranging between 5 and 10.

Clinical features, such as deep vein thrombosis and/or pulmonary embolism, occur spontaneously or when triggered by certain factors (prolonged bed rest, oestrogen-progestin contraception, pregnancy, etc.) in young adults (adolescence onwards) and in older subjects (after the age of 40). The mutation has also been suggested as a risk factor for arterial thrombosis, but the results of various studies undertaken are not in agreement. Numerous associations of mutation 20210G > A of prothrombin with other congenital abnormalities have been described, particularly with the Factor V Leiden mutation. In all cases, the risk of thrombosis is higher in patients presenting combined deficits.

FOR FURTHER INFORMATION

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PSA

DEFINITION

Prostatic-specific antigen (PSA) is a glycoprotein, with a molecular weight of 34 kDa, secreted by the epithelial cells of the prostate gland. It is present in the bloodstream in the free form (FPSA, approximately 10 to 15%) and in a form linked to inhibitors (85 to 90%): PSA linked to alpha1-antichymotrypsin (PSA-ACT), sometimes referred to complexed PSA (CPSA), and PSA linked to alpha2-macroglobulin (PSA-AMG), not susceptible to measurement (the alpha2 macroglobulin masks the PSA epitopes recognised in the measurement process). Total PSA (TPSA) is therefore the sum of FPSA and CPSA.

Free PSA	• Predominant in normal prostate tissue and in cases of benign hypertrophy of the prostate (20 – 90%) • Approximately 10 % in cancer of the prostate • ½-life in plasma: 1 to 2 hours.
PSA-ACT (complexed PSA)	• Minority in normal prostate tissue and in cases of benign hypertrophy of the prostate • Approximately 90 % in cancer of the prostate • ½-life in plasma: 2 to 3 days.
PSA-AMG (hidden PSA)	• Not susceptible to measurement • Very short ½-life in plasma: 2 to 3 minutes.

Quantity of PSA secreted by prostatic tissue
- 0.3 µg/l/g of hyperplasic tissue: 80 % CPSA
- 0.1 µg/l/g of normal tissue : 80 % CPSA
- 3 µg/l/g of cancerous tissue: 95 % CPSA

(according to Stenman UH, Urology 1997)

INTRODUCTION

PSA is mainly secreted by the prostatic epithelium and excreted in seminal fluid, where it is thought to be involved in the hydrolysis of seminogelins I and II, causing liquefaction of the seminal coagulum formed after ejaculation.

INDICATIONS FOR MEASUREMENT

PSA is used as a marker for prostate cancer. There are 3 indications for measurement:

– Screening and early diagnosis for prostate cancer

An elevation of TPSA allows earlier detection of tumours than digital rectal examination (DRE). However, the most sensitive method for detecting early cancer combines DRE and PSA.

Measurement of FPSA is indicated for all patients whose total PSA is between 2 and 10 ng/ml. It is only of interest in this group of patients and when combined with total PSA measurement, due to the fact that FPSA is depressed in cancers and elevated in prostatic adenomas. The ratio FPSA/TPSA is thus a useful parameter in the differential diagnosis of malignant and benign pathologies. It avoids unnecessary biopsies in patients with benign prostatic hypertrophy.

– Pre-treatment determination of the stage reached in prostate cancer

Serum PSA before treatment is predictive of the response to local therapy.

– Post-therapeutic monitoring of prostate cancer

Regular PSA measurements are suggested in evaluating the response to treatment (surgery, radiotherapy or hormone therapy) and detecting recurrence of the disease. Elevation of total PSA in cases of recurrence or metastasis appears 6 to 18 months earlier than clinical symptoms.

INFORMATION

SAMPLE

Serum (dry tube) or EDTA or heparinated plasma. Collection when the patient is fasting is preferable (measurements on hyperlipaemic samples are not advisable).

It is essential to collect the sample before any prostatic manipulation (prostatic massage or, more particularly, transurethral ultrasound examination or prostatic biopsy), which causes a rise in the serum concentration of PSA, or otherwise to wait for 2 to 3 weeks. The effect of rectal examination on PSA is debated, but believed to be slight. It is prudent to defer PSA for a few days after DRE.

Avoid taking the sample after an ejaculation (average increase of 0.8 ng/ml one hour afterwards and returns to normal in 48 hours).

QUESTIONS FOR THE PATIENT

Current treatment: Finasteride (Chibro-Prosca®, Propecia®) and Dutasteride (Avodart®) prescribed for the treatment of benign prostatic hyperplasia, result in a reduced serum concentration of TPSA during the first months of treatment, then, after 6 months, PSA stabilises at around 50% of the initial value measured prior to treatment. Take care not to overlook an early cancer in a patient taking one of these drugs (you should double the measured PSA to estimate the true value).

SAMPLE STORAGE AND TRANSPORT

Total PSA: 24 hours at +4°C (or even 5 days according to certain authors); 6 months to 12 years at –20 °C.

Free PSA: 8 hours at +4°C (freeze for longer periods). Repeated freezing and thawing must be avoided.

Transport frozen at – 20 °C.

ASSAY METHODS

Total PSA: Immunometric measurements.

Until relatively recently, the principal measurement difficulties were linked to the absence of a common calibration standard (old Yang or Hybritech standards). The differences in results between the various kits available diminished considerably after the adoption in September 1994 of the Stanford PSA Standard (international standard) composed of 90% of PSA-ACT and 10% of free PSA, these percentages reflecting the average proportions of molecular forms encountered in prostate cancer.

Other measurement difficulties remain, linked to the heterogeneous nature of the circulating forms of PSA and the specificity of antibodies with regard to these different forms.

Free PSA: Measurements are direct or indirect (FPSA calculated from the difference between measurements of total PSA and PSA-ACT) or from non-complexed PSA (FPSA obtained after blocking PSA-ACT).

NORMAL EXPECTED VALUES

Total PSA: Variations according to age *(see below)*.

Ratio of free to total PSA > 15 - 25 % for benign pathologies. This threshold varies according to the laboratory and the method used.

PATHOPHYSIOLOGICAL VARIATIONS

■ VARIATIONS TO BE TAKEN INTO ACCOUNT

■ **Increase in TPSA:**

– With age: 0.04 ng/ml/year increase in the over 60's.

– With prostatic volume:.

Age (years)	"Normal" PSA values (ng/ml)	Prostatic volume (ml)
40 - 49	< 2.5	13 - 51
50 - 59	< 3.5	15 - 60
60 - 69	< 4.5	17 - 70
70 - 79	< 6.5	20 - 82

According to Oesterling et al. JAMA 1993; 270/8604.

■ **A decrease of TPSA** has been observed (18 to 50%) in hospitalised patients 24 hours after admission.

■ PATHOLOGICAL VARIATIONS

PSA is a specific marker of prostatic tissue, not of cancer. This means that its serum level rises (sometimes significantly) during:

– Other prostatic pathologies, such as in benign adenoma, obstruction, prostatitis (acute prostatitis +++).

– Nearby pathologies, such as in renal failure or acute urinary retention.

■ **PSA and prostate cancer**

Results are interpreted according to the PSA measurement indication:

1- Early detection of prostate cancer

A biopsy of the prostate is generally recommended *(The American Urological Association Practice Parameters Guidelines And Standards Committee, PPGSC, Feb 2000)*:

– In a patient whose life expectancy is 10 years or more and whose TPSA is 4 ng/ml or more.

– When there is a significant rise in TPSA from one measurement to the next.

– If a DRE is abnormal.

NB: The majority of biopsies performed because of elevated TPSA give a negative result, which leads to repeated biopsies.

If the serum concentration of TPSA is between 4 (or even 2) and 10 ng/ml, a measurement of free PSA is recommended and allows the FPSA/TPSA ratio to be calculated. If this ratio is higher than 25%, the probability of absence of prostatic cancer is greater than 95%. If prostatic cancer is present, the ratio is less than 15% and the reduction of the FPSA/TPSA ratio is greater in the more advanced forms. If the value is between 15 and 25%, monitoring is essential.

In patients with TPSA between 2.5 and 4 ng/ml, complexed PSA (PSA-ACT) is more specific than the FPSA/TPSA ratio. The discrimination threshold for complexed PSA is at 2.3 ng/ml (Okihara, Babaian et al, J., Urol 2001; 165: 1930).

2- Disease staging before treatment for prostate cancer

In asymptomatic patients, a systematic bone scan to determine the stage of a clinically localised prostate cancer is not necessary if PSA is less than or equal to 20 ng/ml.

A scan and MRI are not generally indicated to determine the stage of a clinically localised prostate cancer if PSA is less than or equal to 25 ng/ml.

Pelvic lymph node dissection in the case of a clinically localised prostate cancer may not be necessary if PSA is < 10 ng/ml, or if it is < 20 ng/ml and the Gleason score is less than or equal to 6.

3- Post-therapeutic monitoring of prostate cancer

– After radical prostatectomy

Serum PSA should decrease and become undetectable in 3 to 4 weeks. Clinical monitoring and regular measurements (every 4 months approximately) can thus be used to verify patient remission. A concentration in excess of 0.1 ng/ml nearly always reflects the presence of residual disease or an early recurrence.

If recurrence occurs, the PSA velocity is a useful parameter. In benign hypertrophy of the prostate, the annual rise in TPSA is approximately 0.7 ng/ml. A velocity > 0.75 ng/ml per year suggests cancer.

A related prognostic parameter when PSA rises again after radical prostatectomy is the PSA doubling time: a value of less than 11 months is believed to indicate visceral metastasis.

– After radiotherapy and cryotherapy, or after hormone therapy (androgen deprivation)

Serum PSA should descend to a low level and then not rise again. Serum PSA concentrations are not, however, undetectable, due to persistence in prostatic tissue.

The profile of rising PSA after local therapy for prostate cancer may be of assistance in distinguishing between local and remote recurrence.

The lowest point of serum PSA and the percentage drop in PSA at 3 and 6 months are predictive of survival without progression in men with metastatic prostate cancer after treatment by hormone therapy. The magnitude of the PSA reduction after second-intention treatment of metastasis is related to the residual disease.

A renewed rise in PSA after radiotherapy or during hormone therapy is a precursor sign of therapeutic escape and recurrence, which should be the subject of investigation.

FOR FURTHER INFORMATION

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PTH-rP

DEFINITION

Parathyroid Hormone-Related Protein (PTH-rP) is a precursor polyhormone of several active peptides, which sometimes have conflicting actions. The PTH-rP gene codes in fact for three proteins of 139, 141 and 173 amino acids, differing in their C-terminal extremity and expressed by alternative splicing, in proportions which vary according to the tissue concerned. PTH-rP only shares 11 amino acids with parathyroid hormone (PTH), including 8 of the first 13 of the N-terminal section of the two molecules. This homologous domain activates the receptor for PTH and PTH-rP and explains their common action in bone resorption, hypercalcaemia, hyperphosphataemia and the production of cyclic AMP.

INTRODUCTION

PTH-rP is produced and expressed by numerous foetal and adult tissues, whether endocrine or not. It is found in cells of epithelial, mesenchymal, endodermal or neuroectodermal origin. After birth, it evolves within those tissues and disappears from certain organs during adult life. This observation has given rise to a suggested local role of PTH-rP in cell differentiation and/or cell growth.

The three above-mentioned isoforms of PTH-rP can be cleaved by various enzymes, producing different fragments with specific actions. In addition to the N-terminal fragment interacting with the PTH/PTH-rP receptor, responsible for mimicry with PTH and hypercalcaemic effects, there is a 37-94 fragment, whose role is to transport calcium through the placenta to allow mineralisation of the foetal skeleton, and a 107-139 (C-terminal) fragment, also known as osteostatin, which is able to inhibit osseous resorption.

In addition, PTH-rP is involved in the growth of hair and the development of teeth, the maturation of chondrocytes, mammary gland development and the vascular response in atherosclerosis. Unlike PTH, its synthesis is not regulated by calcium, but probably by various cytokines and 1,25-dihydroxy vitamin D.

PTHrP acts at local level and its plasma concentration is much lower than that of PTH, which corroborates the fact that it plays a very small part (compared with PTH) in the physiological regulation of calcaemia.

PTHrP was initially identified as the molecule responsible for malignant humoral hypercalcaemia (MHH), a major complication in many cancers, in which the biochemical scenario is close to that of primitive hyperthyroidism, although it differs from the latter by its low PTH concentrations.

In cancer pathology, apart from its role as a mediator in malignant hypercalcaemia, PTH-rP stimulates the proliferation of several tumoural lines in-vitro and the growth of tumours in-vivo. It may be a predictive factor in the occurrence of osseous metastases in cancers of the breast and prostate.

INDICATIONS FOR MEASUREMENT

For the aetiological investigation in hypercalcaemia, as a measurement of second intention, after PTH measurement (except in particular cases), during:

– Solid tumours, such as cancer of the lung, breast, kidney, bladder, oesophagus or prostate.

– Haematological tumours: lymphoma or myeloma.

– Other conditions, such as phaeochromocytoma and sarcoidosis.

INFORMATION

SAMPLE

Plasma collected in a tube containing EDTA and a protease inhibitor such as aprotinin. The sample must be centrifuged at +4°C very rapidly after collection, decanted and frozen at -20°C within an hour of collection. PTH-rP is in fact a fragile molecule which breaks down rapidly in whole blood, falling by 20% in 3 hours.

SAMPLE STORAGE AND TRANSPORT

Store and transport the plasma, frozen at -20°C.

ASSAY METHODS

Sandwich immunoassay techniques. Several kits are commercially available, recognising different circulating fragments (regions 1-74, 1-86 or C-terminal fragments).

NORMAL EXPECTED VALUES

PTH-rP levels in plasma are undetectable in more than 50% of healthy individuals. The threshold of normality depends on the technique used. An indicative normal value is < 1.3 pmol/l (radioimmunology).

There is no international measurement standard.

PATHOLOGICAL VARIATIONS

PTHrP is produced in large quantities in MHH, which is characterised by hypercalcaemia, hypophasphataemia, increased urinary secretion of AMPc and a reduction in PTH.

High PTH-rP concentrations in cancer are generally measured in patients with hypercalcaemia (without obvious osseous metastasis), particularly in those patients with solid tumours (breast, kidney, lung, bladder or oesophagus), haematological tumours (lymphoma or myeloma), and in patients suffering from phaeochromocytoma or sarcoidosis with hypercalcaemia.

Women with breast cancer, or men affected by prostate cancer with hypercalcaemia and osseous metastases also frequently show elevated PTH-rP concentrations in the plasma (a factor in a poor prognosis).

NB: a raised PTH-rP level in the blood does not necessarily mean cancer. Commercial kits using antibodies which recognise C-terminal fragments give falsely high results in patients suffering from chronic renal failure, in which the fragments accumulate. More rarely, circulating PTH-rP can rise in hypercalcaemic patients, in connection with mammary hypertrophy or lymphoadenoma.

Furthermore, 20% of patients with an authentic malignant hypercalcaemic tumour do not have elevated PTH-rP levels in the blood (in these cases, the hypercalcaemia probably results from tumoural production of cytokines with PTH-rP-like effects).

FOR FURTHER INFORMATION

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PTH

DEFINITION

Parathyroid hormone (PTH) is the main hormone involved in calcium and phosphate homeostasis. It is synthesised and secreted by the parathyroid glands.

Biologically active intact PHN is formed from a single 84 amino acid peptide chain. It is produced after cleavage of pro-PTH (1-90) and pre-pro-PTH (1-115). The parathyroid glands also secrete a (7-84) fragment which inhibits intact PTH and inactive C terminal and (44-68) Mid-region fragments.

The half-life of PTH (1-84) in the circulation is no more than 4 minutes. It is degraded almost instantaneously by the liver into non-(1-84) fragments, mostly C-terminal (53-84) and (7-84). Non-(1-84) PTH fragments are excreted by the kidney with a long half-life. They accumulate in the blood in renal insufficiency.

In healthy people, circulating immunoreactive forms of PTH consist of 10% PTH (1-84) and 90% non-(1-84) fragments. Non-(1-84) fragments may account for up to 99% of immunoreactive forms of PTH in chronic renal insufficiency.

INTRODUCTION

In the kidney, PTH increases distal tubular reabsorption of calcium (causing hypocalcuria and hypercalcaemia) and inhibits proximal tubular phosphate reabsorption (causing a fall in serum phosphate). In the proximal tubules it increases the enzyme activity of 1-alpha-hydroxylase which converts 25-hydroxyvitamin D into 1,25-dihydroxyvitamin D (calcitriol). The hypercalcaemic effect is seen both short-term (mobilisation of rapidly available bone calcium) and long-term, by stimulating bone resorption (release of calcium from the bone matrix), reabsorption of urinary calcium and gastro-intestinal absorption of calcium (via calcitriol).

PTH stimulates bone remodelling by directly activating osteoblasts (anabolic effect) and by local secretion of cytokines it activates osteoclasts (osteolysis). PTH is used in osteoporosis in a recombinant form, rhPTH (1-34) or teriparatide, which reduces the number of bone fractures.

PTH secretion is stimulated by hypocalcaemia and increased serum phosphate. Conversely, it is inhibited by hypercalcaemia and reduced serum phosphate. PTH secretion is also inhibited by calcitriol, the (7-84) fragment and magnesium deficiency.

Calcimimetics (for example cinacalcet) suppress PTH secretion and are used in primary hyperparathyroidism (hyperplasia) and in renal osteodystrophy.

PRIMARY HYPERPARATHYROIDISM

The adeonoma is a monoclonal proliferation which independently secretes excessive amounts of PTH. It is single in 90% of cases and occasionally dual: multiple or eptopic adenomas are rare. Parathyroid hyperplasia is an uncommon form of hyperparathyroidism but responds to pharmacological treatments.

Familial benign hypocalciuric hypocalcaemia involves resistance to the calcium-sensitive receptors expressed in the kidneys and parathyroids. The hypocalciuria is due to excess urine calcium reabsorption.

Primary hyperparathyroidism in multiple endocrine neoplasia or MEN types I and II is very rare.

SECONDARY HYPERPARATHYROIDISM

Secondary hyperparathyroidism is seen above all in chronic renal insufficiency and in vitamin D deficiency. In chronic renal insufficiency, defective urine phosphate excretion results in phosphate accumulation in the blood and in tissues. Tissue precipitation of calcium phosphate salts and calcitriol deficiency (due to a loss of renal mass) cause hypocalcaemia and PTH secretion is stimulated both by the raised serum phosphate and reduced serum calcium.

HYPOPARATHYROIDISM

Defective secretion

The post-operative form (thyroid surgery) is rare.

Congenital primary hypoparathyroidism or the Di George syndrome is caused by hypoplasia of the branchial pouches which contribute to the development of the parathyroids and thymus.

Primary autoimmune hypoparathyroidism is seen either in isolation or in a group of autoimmune disorders (Hashimoto's thyroiditis, pernicious anaemia, type I diabetes and lupus). Anti-parathyroid cell antibodies are usually directed against the calcium sensitive receptors and more rarely against PTH or its receptors. Their pathophysiological contribution is uncertain.

Suppressed secretion

Hypoparathyroidism due to inhibition of PTH secretion is usually due to hypercalcaemia. In the paraneoplastic form, secretion of PTH-rP causes hypercalcaemia of malignancy with very low PTH levels. Hypercalcaemia is also seen in vitamin D poisoning when, excessive endogenous production of 1,25-dihydroxyvitamin D (granulomatous diseases) and 25-hydroxyvitamin D (Williams' syndrome).

Inhibition of PTH secretion due to magnesium deficiency is usually due to chronic alcohol abuse.

Resistance to the action of PTH

This is "pseudo-hypoparathyroidism". Constitutional forms (mostly Albright's syndrome) are due to mutations in the PTH receptor genes expressed in the renal tubules and in their signalling proteins. These mutations cause resistance to the tubular action of PTH. Acquired forms involve adynamic osteopathy seen in chronic renal insufficiency despite high PTH (1-84) concentrations. The low level bone remodelling is then explained by resistance to the action of PTH as a result of increased levels of (7-84) fragment, a potent PTH (1-84) inhibitor or by the presence of anti-PTH and anti-PTH receptor antibodies.

INDICATIONS FOR MEASUREMENT

Investigation of hypercalcaemia, assessment of renal stones, differential diagnosis with paraneoplastic hypocalcaemia, monitoring patients with renal insufficiency on dialysis, diagnostic indicator in subclinical vitamin D deficiency.

Assessment of hypocalcaemia, autoimmune polyendocrinopathy and other causes of hypoparathyroidism, mostly chronic alcohol abuse and haemochromatosis.

INFORMATION

SAMPLE

1 ml of non-haemolysed serum or EDTA plasma.

Centrifuge blood as soon possible at +4°C.

For serum, leave the sample to coagulate.

ESSENTIAL INFORMATION

Clinical context (malignant or endocrine disease….)?

Current or past treatment? Thyroid or gastro-intestinal surgery, chest and neck irradiation, drugs (calcium, furosemide or thiazide diuretics, vitamin D, vitamin A, levothyroxine, lithium, corticosteroids, ulcer treatments, rhPTH, osteocalcin, bisphosphonates).

SAMPLE STORAGE AND TRANSPORT

Transport the sample frozen at –20°C.

Samples can be stored for 3 months at –20°C, for 6 months to one year at –80°C.

ASSAY METHODS

Tests must be both specific for PTH (1-84) and sensitive to detect low concentrations. The reliability of the tests available is limited, however, by the existence of a very large number of circulating forms of PTH. Tests suitable for automated immunoanalysers use cold tracers in a sandwich method. The antibodies separately recognise the N-terminal and C-terminal end sequences. In the "intact PTH" tests (2nd generation), a set of antibodies recognises the N-terminal (1-37) sequence and another recognises the (38-84) sequence and do not differentiate PTH (1-840) from PTH (7-84). "Biointact PTH" tests (Nichols) or "Whole PTH (1-84) specific" (Scantibodies Laboratory) (3rd generation) are immuno-chemoluminescence (ICMA) tests which use sets of antibodies specific to the N-terminal (1-6) end and antibodies against the C-terminal (39-84) end for "Whole PTH". They measure PTH (1-84) without interference from PTH (7-84) and non-(1-84) fragments.

The 3rd generation tests are no longer relevant in 2009 because:

– the Nichols company has disappeared (only the Scantibodies method remains available on the market and cannot be used routinely as it is an RIA and 2 measurements are required when only 1 tariff payment is offered in France).

– At the same time, doubts were raised about the interpretation of 3rd generation PTH results. In dialysis patients (in whom only the 7-84 fragment is present in large amounts because of defective filtration), the PTH reference ranges were set (KDOQI guidelines: 150 to 300 ng/l) from a study comparing PTH levels with bone biopsies. These PTH levels were obtained with the former Nichols kit (Allegro, RIA), a 2nd generation kit. There are no reference ranges for dialysis patients using the 3rd generation kits. As a result, it is recommended that 2nd generation kits be used.

NORMAL EXPECTED VALUES

Serum PTH = 6-50 pg/ml (Biointact PTH test).

INTERPRETATION

PATHOPHYSIOLOGICAL VARIATIONS

Variations in PTH are seen during the 24-hour cycle, by sex, exposure to sun (season, geography, clothing, degree of skin pigmentation, etc.), diet (calcium and vitamin D daily intake should be estimated) and physical activity. Drug-related variations are also seen: diuretics (thiazides), hormone treatments (corticosteroids, sex steroids), bone remodelling treatments (bisphosphonates, vitamin D, oestrogens and androgens). Similarly, PTH secretion is altered in endocrine (hyperthyroidism), gastro-intestinal and renal disease.

CALCIUM AND PHOSPHATE PATHOLOGY

In general, the specificity of the tests used must always be checked before interpreting a PTH result. In addition, a minimum of a calcium/phosphate profile, possibly combined with measurement of blood ionised calcium and 24-hour urinary calcium should also be done. The diagnostic distinction between primary and secondary forms requires measurement of 25-hydroxyvitamin D and calcitriol concentrations and renal function.

HYPERPARATHYROIDISM

Primary hyperparathyroidism

The association of "hypercalcaemia and high PTH" provides a diagnosis of primary hyperparathyroidism in most cases. The serum calcium is occasionally normal, although this is very rare with measurement of ionised calcium. In 10 to 20% of cases the PTH concentration is normal or at the upper limit of the physiological range, although this nevertheless indicates inappropriate PTH secretion. It is nevertheless, essential to exclude hyperthyroidism and sarcoidosis as these are associated with inhibition of PTH secretion and underlying hyperparathyroidism may only be unmasked after these have been treated.

Primary hyperparathyroidism is rare (prevalence of 4/1 000 in women over 60 years old). The hypercalcaemia is generally moderate, asymptomatic and changes slowly, in women over 45 years old. In the usual presentation (80-90%), the parathyroid adenoma is single and surgery produces excellent results. PTH concentrations should fall within minutes of removal of the adenoma (intra-operative measurements – at induction – and 15 minutes after excision) and should be confirmed within 24 hours. If not, and the levels fall by less than 50% intra-operatively, a second adenoma, ectopic adenoma or parathyroid hyperplasia must be considered. Under the age of 40 years old, hyperparathyroidism suggests multiple endocrine neoplasia (MEN).

Lithium and tertiary hyperparathyroidism also cause hypercalcaemia with raised PTH. Finally, the diagnosis of benign familial hypocalciuric hypercalcaemia is one of exclusion. PTH is occasionally raised but in principle is sub-normal. This must be recognised in order to avoid unnecessary surgery.

Secondary hyperparathyroidism

The association of "hypocalcaemia with raised PTH" gives a diagnosis of secondary hyperparathyroidism, although the serum calcium may be within physiological limits and compensated by the effect of PTH. The two main causes are 25-hydroxyvitamin D deficiency and renal insufficiency.

Schematically, in vitamin D deficiency, 25-hydroxyvitamin D concentrations are reduced and calcitriol concentrations are increased. In chronic renal insufficiency, 25-hydroxyvitamin D is normal or reduced and calcitriol is reduced.

Tertiary hyperparathyroidism

This is chronic secondary hyperparathyroidism which has become autonomous.

HYPOPARATHYROIDISM

Defective PTH secretion

The biochemical picture is an association of "hypocalcaemia, hyperphosphataemia, hypocalciuria, with reduced or normal PTH".

Primary hypoparathyroidism after neck surgery is rare (mostly total and sub-total thyroid removal). Apart from idiopathic and autoimmune forms, the causes of primary hypoparathyroidism include hemochromatosis, systemic infections and inflammatory diseases. The Di George syndrome is suggested at birth from an association of velocardiofacial abnormalities and hypocalcaemia. Immunodeficiency is also seen due to thymic hypoplasia. A micro-deletion on 22q11 has been found and has enabled genetic counselling to be developed.

Suppressed PTH secretion

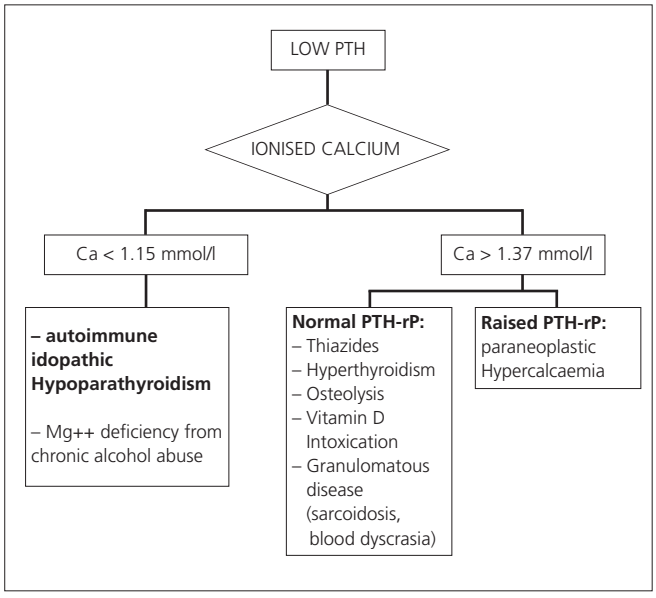
When associated with hypercalcaemia patients should be investigated for thiazide treatment, bone lysis, para-neoplastic disease with raised PTH-rP, granulomatous disease (sarcoidosis, blood dyscrasia), hyperthyroidism and vitamin D or vitamin A intoxication.

When the serum calcium is low, hypoparathyroidism due to tissue magnesium deficiency should be considered. This is seen particularly in chronic alcohol abuse and involves an association of "hypocalcaemia with low PTH" and usually normal blood magnesium concentrations. Hypomagnesaemia only develops when tissue magnesium stores are extremely low. The hypoparathyroidism resolves with magnesium replacement.

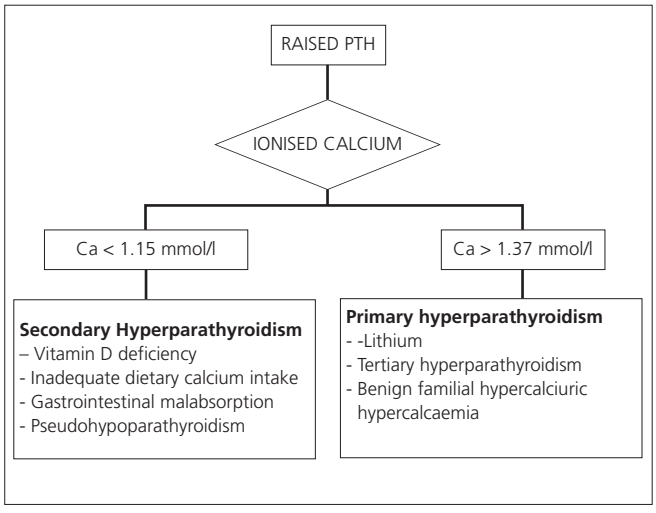
PSEUDOHYPOPARATHYROIDISM

The Albright syndrome is the usual form of pseudo-hypoparathyroidism. This is an inherited form of tubular PTH resistance which is associated with a biochemical picture of hypoparathyroidism: hypocalcaemia, raised serum phosphate, hypocalciuria, but with normal or raised PTH (1-84). Nephrogenic cAMP is low even after administration of PTH. Pseudohypoparathyroidism is not associated with mental retardation or osteodystropy.

Diagnostic flow chart: Low PTH



Diagnostic flow chart: Raised PTH



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PYRUVATE KINASE

DEFINITION

Pyruvate kinase (PK) is an enzyme which catalyses the conversion of phosphoenolpyruvate into pyruvate, also producing ATP. This reaction occurs in the regulation of anaerobic glycolysis. PK is present in a large number of human cells.

Two human genes code for PK:

– The M gene, which codes for muscle PK-M1 and ubiquitous PK-M2

– The L gene, which codes for hepatocyte PK-L and erythrocyte PK-R .

INTRODUCTION

Erythrocyte PK deficiency is a disease communicated by autosomal recessive transmission, linked to a mutation of the gene which codes for PK, resulting in a reduction in the activity or stability of the enzyme. It is the most common of the enzymopathies involving glycolysis abnormalities. It affects subjects of either sex, although only homozygotes are symptomatic. Heterozygotes are clinically and haematologically normal. Erythrocyte PK deficiency causes a chronic non-spherocytic haemolytic anaemia, which sometimes occurs very early. In effect, it causes a reduction in intracellular ATP, which gives rise to abnormalities in the renewal of membrane phospholipids, particularly in the erythrocytic membrane. The seriousness of the haemolytic anaemia varies, ranging from very severe forms which necessitate repeated transfusions and even a splenectomy, to less serious forms where the haemolysis is well compensated. Most cases exhibit moderate haemolytic anaemia with variable degrees of splenomegaly, which may be complicated by bilirubin lithiasis and haemochromatosis.

MEASUREMENT INDICATIONS

Searching for erythrocyte pyruvate kinase deficiency.

INFORMATION

SAMPLE

Full blood collected using EDTA or heparin. Sampling on ACD is recommended if analysis is to be deferred by more than 24 hours.

SAMPLE STORAGE AND TRANSPORT

The sample is kept at ambient temperature prior to analysis.

Care must be taken to collect the sample before a transfusion, otherwise an interval of 1 month must be allowed after transfusion.

ASSAY METHODS

Measurement of erythrocyte PK enzyme activity is based on the conversion of phosphoenolpyruvate into pyruvate by the PK present in the sample. The reaction is coupled with a reduction of the pyruvate to lactate by the LDH in presence of NADH. A spectrophotometric reading allows monitoring of the reduced absorption at 340 nm proportional to the PK activity.

NORMAL EXPECTED VALUES

For reference: erythrocyte PK = 60 to 220 Mu/10^9 GR.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Erythrocyte PK activity is physiologically raised in new-born babies and subjects with reticulocytosis.

PATHOLOGICAL VARIATIONS

Erythrocyte PK activity is variable in subjects who are homozygous for this deficit and ranges from 0 to 30% of the normal activity. It is very low in the typical form of the disease. In heterozygous subjects, erythrocyte PK activity is approximately 50% of the normal enzyme activity.

Reduced PK activity has been described in certain cases of acquired haemopathy, refractory anaemia, dyserythropoiesis and acute myeloid leukaemia.

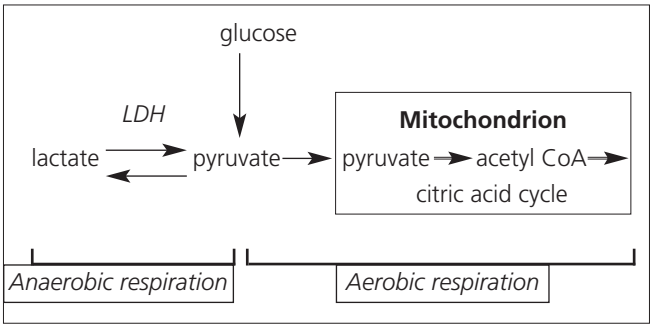
FOR FURTHER INFORMATION

Dreyfus B., *Anémies hémolytiques et déficit en PK*. In : l'Hématologie, Flammarion Medecine Sciences, 3e Ed. 1992, Paris : 416-19.

PYRUVIC ACID

DEFINITION

Pyruvic acid and lactic acid are both organic acids that are important in metabolic exchanges occurring in the cytoplasm of the cell.

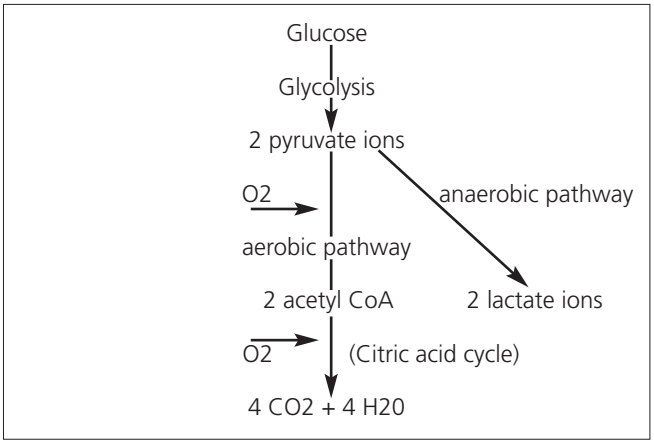


Pyruvic acid is generated by the breakdown of glucose (glycolysis). It is converted into lactate in a reaction catalysed by lactate dehydrogenase which mainly proceeds in muscle tissue, the brain, red blood cells, the gut and the skin. Only pyruvate can enter mitochondria to drive the citric acid cycle.

Synonym: pyruvate

INTRODUCTION

Glycolysis corresponds to the series of reactions in which glucose is oxidised to generate pyruvate. These reactions occur at cytosolic membranes and can proceed in both anaerobic as well as aerobic conditions.



The oxidative decarboxylation of pyruvate occurs inside the mitochondrion. Pyruvate is the precursor for many synthetic products, including alanine, oxaloacetate and fatty acids.

In anaerobic conditions, pyruvate is converted into lactate by lactate dehydrogenase. This is something of a chemical dead-end in that the resultant lactic acid has to be stored until oxygen becomes available again and it can be reconverted back into pyruvate. Lactic acid is normally sequestered in the liver (to produce glycogen), in the heart (where it can be used as an energy substrate), and in the kidneys. When these sequestration systems are saturated, lactic acid builds up and compromises cellular functions.

INDICATIONS FOR MEASUREMENT

The circumstances in which this test is ordered are similar to those for ordering a lactic acid test, i.e. to investigate certain metabolic diseases, to diagnose and monitor pathologies that lead to tissue hypoxia (shock, severe acute asthma, etc.) and/or lactic acidosis, and to evaluate exertion in sports medicine.

INFORMATION

SAMPLE

To be strictly adhered to:

– Patient fasting and at rest; draw the blood without using a tourniquet whenever possible.

– Draw 5 ml of whole blood into a heparinised tube. Immediately after blood drawing, the protein must be precipitated using perchloric acid (1 N or 6%) with the correct ratio of perchloric acid to whole blood, which will depend on the technique. Vortex-mix the tube vigorously for 30 seconds and then incubate the sample at +4°C for 5 minutes to ensure precipitation of all the protein. Vortex-mix again and centrifuge for 10 minutes at 1,500 g. The resultant supernatant should be clear and colourless; if not, centrifuge again. Transfer the supernatant into a dry tube (2 ml minimum) and freeze straight away.

QUESTIONS FOR THE PATIENT

– ***Have you ensured that you have fasted properly?*** The blood pyruvate concentration rises during digestion.

– ***Do you play any sport?*** Have you exerted yourself recently?

SAMPLE STORAGE AND TRANSPORT

Store and transport samples frozen at –20 °C.

ASSAY METHODS

Spectrophotometric chemical analysis.

NORMAL EXPECTED VALUES

For reference: 3-7 mg/l.

Conversion factor: 1 mg/l = 11.40 µmol/l.

In a healthy subject, the normal lactate/pyruvate ratio is about 10.

PATHOPHSIOYLOGICAL VARIATIONS

Increased pyruvate concentrations are found in the blood:

– During digestion.

– Following physical exertion.

– In vitamin B1 deficiency, usually secondary to gut malabsorption disorders, heavy vomiting or prolonged diarrhoea. The symptoms are systemic (lassitude, anorexia, gastric disorders) coupled with peripheral neuropathy, psychiatric manifestations (irritability and depression) and/or impaired cardiac function (with ECG changes).

– In unstable type-1 diabetes and diabetic acidosis.

– In tissue hypoxia, notably in asthmatics.

– In children with febrile convulsions.

– In arsenic or mercury poisoning.

– Moderate elevations are seen in certain forms of cancer (of the stomach, colon, lung, breast, testicle and kidney), in Hodgkin's disease, and in non-Hodgkin's lymphoma.

FOR FURTHER INFORMATION

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■ Friedman R.B., Young D.S., *Effects of disease on clinical laboratory tests*, 3rd Ed. AACC Press, Washington, 1997.

Q FEVER

DEFINITION

Q fever is a ubiquitous zoonosis caused by *Coxiella (C) burnetii*, a member of the *Rickettsia* family and of the genus *Coxiella*, of which it is the sole species.

C. burnetii is a very small, strictly intracellular Gram negative bacillus, which is able to survive for long periods in the environment. It is characterised by a variation of its antigenic phase. Thus, there is a phase I during which it is virulent and an attenuated phase II.

Synonyms: Q fever, Derrick-Burnet disease, *Coxiella burnetii* and *Rickettsia burnetii*.

INTRODUCTION

EPIDEMIOLOGY

Q fever has a worldwide distribution; it occurs in small epidemics and also as sporadic cases. It is usually transmitted by sheep, goats and cows. These animals excrete the organism in faeces, urine and in the placenta. The illness particularly affects those who work with cattle (farmers, veterinary workers, butchers and staff of abattoirs, etc.) and is listed as an occupational disease. Human infection occurs through inhalation of infected particles or by direct contact with animals, but it can also be contracted via the digestive tract through the ingestion of non-pasteurised milk and dairy products.

SYMPTOMS

- **Incubation** is 2 to 3 weeks on average.
- **The illness** is asymptomatic in around half of cases. Clinical manifestations can be acute or chronic.

The acute form is more common and includes a variety of symptoms, such as flu like symptoms, high fever alone, atypical febrile pneumonia, febrile hepatitis and meningo-encephalitis.

The chronic form involves individuals with heart valve abnormalities or an artificial valve, pregnant women and patients with neoplasia and associated impaired immunity. If untreated, it can cause fatal endocarditis and, in pregnancy, it can lead to abortion, premature labour and foetal infection.

SEARCH INDICATIONS

Diagnosis of Q fever if the symptoms are compatible with the disease and if there is a possibility of infection.

Diagnosis of Q fever in individuals who are exposed by virtue of their work.

Differential diagnosis serologically between the acute and chronic forms of the disease.

INFORMATION

SAMPLE

Blood in a dry tube (serum) for serological testing.

Blood in an EDTA tube (whole blood) for diagnosis with molecular biological techniques.

Blood in a heparinised or citrated tube for cell culture diagnosis.

Tissue samples (biopsies and heart valve) into a dry sterile container for cell culture.

Other possible human samples, such as urine and sputum may also be indicated.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

Is the patient exposed to infection at work or is he/she a person at risk of developing chronic infection?

On antibiotic treatment?

SAMPLE STORAGE AND TRANSPORT

Serum can be stored for a week at + 4°C and then frozen for 1 year at – 30°C. Serum samples can be transported at + 4°C or frozen.

Other specimens can be transported at ambient temperature within 24 hours; if later they should be frozen at – 20°C.

BIOLOGICAL DIAGNOSTIC METHODS

GUIDING DIAGNOSIS

Initial neutropaenia.

Elevation of transaminases and gamma GT.

DIRECT BACTERIOLOGICAL DIAGNOSIS

- **Culture:** Isolation of the organism in cell culture of eukaryocytes from blood or biopsy specimens must only be performed in specialised laboratories with bio-safety facilities, as cell cultures present a high infective risk for laboratory staff. It must be noted that the diagnostic relevance of this method is limited by its low sensitivity.
- **Molecular biology:** The technique of gene amplification confers diagnostic certainty. *C. Burnetii* can be detected directly in samples.

INDIRECT DIAGNOSIS

In practice this is serological. The reference method is **indirect immunofluorescence**, which uses phase I and phase II antigens. Screening serological testing gives total Ig and IgM titres in phase II. Positively screened sera in phase II are then tested with respect to both antigenic phases, I and II, to detect and titrate antibodies of types IgA, IgM and IgG. Acute and chronic infections can be differentiated by the use of antigens of both phases, where the detection of anti-phase II antibodies at high titre suggests a recent or acute infection, whereas the presence of anti-phase I and II antibodies at high titre favours chronic disease.

TREATMENT

CURATIVE

Tetracycline (doxycycline) or fluoroquinolones are used to treat acute forms. Other antibiotics are also active, such as rifampicin, cotrimoxazole and chloramphenicol. Chronic disease is treated with doxycycline alone or in combination with another antibiotic (fluoroquinolone or rifampicin).

PREVENTIVE

Consumption of pasteurised dairy products.

Protective measures to prevent infection in staff who have direct contact with animals at work.

There is no effective vaccine.

FOR FURTHER INFORMATION

- *Référentiel en microbiologie : Rickettsies et bactéries apparentées*, 2000: p 111-113
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QUININE

DEFINITION

Quinine is a quinquina alkaloid which has exclusively schizonticide antimalarial activity. It is indicated for use in the treatment of benign or complicated acute malaria, particularly in patients who are resistant to the 4-aminoquinolines. The usual dosage is 24 mg/kg/d of quinine as 3 daily 8 mg/kg doses for 5 to 7 days.

The parenteral form should be used for patients with vomiting, severe or cerebral malaria, at a dosage of 3 daily injections of 8 mg/kg of base alkaloids by slow intravenous infusion, each lasting 4 hours or as a continuous infusion by an electric syringe pump. In severe disease, a loading dose of 17 mg/kg of quinine may be administered over 4 hours followed by maintenance therapy at the above dosage.

PHARMACOKINETICS

Bioavailability (oral)	Approximately 95%
Plasma peak	1 to 3 hours
Time to steady state	5 half-lives, i.e. approximately 3 days.
Protein binding	70% in healthy people; at least 90% in malaria patients
Metabolism	80% hepatic, into an inactive metabolite
Elimination	80% in bile; 20% in urine as the unchanged form
Half-life of elimination	10 hours in healthy people, rising up to 18 hours in malaria patients.

INDICATIONS FOR MEASUREMENT

Therapeutic monitoring of quinine is indicated to monitor patients suffering from severe malaria. It is used to confirm that plasma concentrations are effective but not within the toxic range, as quinine has a narrow therapeutic margin. Monitoring is recommended particularly in patients with renal or hepatic insufficiency (increased half-life of elimination), to avoid any risk of overdose and in both children and pregnant women. The initial signs of overdose are hypoglycaemia and "cinchonism", which present as nausea, tinnitus, dizziness, headache, visual and auditory disturbances.

Quinine is also measured in blood in suspected acute poisoning. Poisoning may be serious or even fatal at and above 3 g of quinine base as a single oral dose in an adult (this may occur at lower doses in children). Toxicity is mostly cardiac (arrhythmias, hypotension and cardiac arrest) and ocular (blindness).

INFORMATION

■ SAMPLE

Serum or heparinised plasma. Do not use tubes with separator gel.

Sampling methods for therapeutic monitoring:

– Sample taken immediately before the infusion or next dose of the drug: trough concentration (C0).

– Sample taken at the end of the infusion for a slow discontinuous 4 hour infusion or during the infusion for a continuous infusion (Cmax).

– When doses are changed (treatment of severe disease), the sample may be taken at the end of dose in order to confirm the initial dosage.

■ QUESTIONS FOR THE PATIENT

Are you taking any other medical treatment?

The following therapeutic interactions should be considered when interpreting blood quinine concentrations:

– Rifampicin reduces the half-life of elimination of quinine in healthy volunteers (risk of quinine overdose);

– Cimetidine reduces the half-life of elimination of quinine in healthy volunteers.

Any request for drug measurement must include: the reasons for the request (testing for efficacy or toxicity), the sampling time, treatment start date and/or any change in dosage, dosage information (amount administered, frequency and the route of administration) and the age, height and weight of the person whenever possible.

■ SAMPLE STORAGE AND TRANSPORT

Serum or plasma can be stored for 48 hours at room temperature, for at least a week at +4°C and for seven months at –20°C. Transport at +4°C.

ASSAY METHOD

HLPC method.

NORMAL EXPECTED VALUES

The therapeutic range for plasma quinine (Cmax) is between 2 and 10 mg/l These plasma concentrations must be achieved as early as possible and maintained throughout treatment in severe malaria. This range is well tolerated in patients with malaria but may be associated with signs of poisoning in health people (different pharmacokinetics: plasma concentrations are approximately 50% higher in malaria patients than in convalescing patients).

Risk of toxicity develops at and above 15 mg/l in people treated for acute malaria, although only very few cases of toxicity have been reported between 15 and 20 mg/l: the plasma concentration threshold which should not be exceeded during treatment may therefore be considered to be 20 mg/l.

Conversion factor: 1 mg/l = 0.324 µmol/l.

FOR FURTHER INFORMATION

■ Dictionnaire Vidal®.

■ Compagnon P., Diquet B., *Suivi thérapeutique de la quinine*. In : Suivi thérapeutique pharmacologique pour l'adaptation de posologie des médicaments, Collection Option/Bio, Ed Elsevier, Paris, 2004: 129635.

RABIES

DEFINITION

Rabies is an anthropozoonosis found widely throughout the world and is caused by a virus which belongs to the *Rhabdoviridae* family and *Lyssavirus* genus. The genus contains 7 different genotypes and an 8th which is currently being classified. The classical rabies or carnivore rabies virus is genotype 1. Rabies is still one of the 10 leading causes of deaths throughout the world, killing approximately 55,000 people annually, particularly in developing countries. It is transmitted accidentally to human beings and causes a fatal encephalomyelitis.

Under the electron microscope the rabies virus has a cylindrical morphology measuring between 130 and 300 nm long and 75 nm in diameter. It is surrounded by a double phospholipid envelope and contains a nucleocapsid consisting of a single strand linear genomic RNA of negative polarity and 5 viral proteins.

INTRODUCTION

EPIDEMIOLOGY

Animal rabies occurs on all continents: Asia, America and South Africa but also the Middle East and Central Europe. Certain mammals form the reservoir for the disease but stray dogs are the main reservoir and vector for rabies throughout the world, although other vectors including wild animals (foxes, wolves, mongoose, etc.) or chiroptera (vampire bats and common bats). Wild animals infect human beings directly or indirectly through infecting domestic animals.

The principal mode of transmission is through saliva from infected animals, following a bite, scratch or lick to the mucosal membranes or damaged skin (the virus does not pass through healthy skin). Cases of accidental infection of laboratory staff or human-human transfer after transplantation of a cornea infected with the rabies virus and aerial infections have been reported.

SYMPTOMS

The rabies virus has tropism for the nervous system and it spreads from its inoculation site to the central nervous system where it actively replicates and then reaches different peripheral organs through the nerves, notably the salivary glands, eye and hair follicles, where it continues to multiply.

– ***The incubation period*** is long and depends on the severity and site of the bite, varying between 1 and 3 months.

– ***The prodrome period*** is short and involves local pain and paraesthesia at the site of the bite, and various non-specific symptoms (headache, asthenia, fever, etc.).

– ***The established disease or encephalitis*** phase involves neurological and psychiatric signs. Animal rabies has two clinical forms: a paralytic form in rodents and a furious form in carnivores.

There are 2 forms of human rabies:

- **The furious or spastic form** is the most common and involves symptoms of encephalitis such as contractures and facial paralysis, seizures, agitation and periods of aggression, hallucinations, characteristic hydrophobia and aerophobia sometimes associated with a high fever, heavy sweating, respiratory disorders and cardiac conduction disorders. The disease progresses over 4 to 8 days after the onset of symptoms to coma and death from cardiac arrest.
- **Paralytic rabies** or tranquil rabies is an ascending paralysis which begins in the lower limbs reaching the respiratory system and progressing inexorably to death from cardio-respiratory arrest.

SEARCH INDICATIONS

Diagnosis of rabies following a bite from a suspect animal.

Diagnosis of overt rabies.

Unequivocal autopsy diagnosis in human beings and animals.

Testing for post-vaccination immunity.

INFORMATION

SAMPLES

Post-mortem animal samples: brain and salivary glands.

Samples from human beings: saliva obtained by aspiration, CSF, urine, skin biopsies to test for the virus and rabies antigens. Whole blood and CSF to test for antibodies. Post-mortem samples of brain or salivary glands for unequivocal diagnosis.

QUESTIONS FOR THE PATIENT

All samples are transported in France to the national reference centre (CNR, Pasteur Institute, Paris) accompanied by a model questionnaire.

SAMPLE STORAGE AND TRANSPORT

The CNR describes the optimal packaging and refrigeration conditions consistent with safety requirements.

Serum samples are stored at + 4 °C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

- **Identification of rabies antigens in cells** is performed by indirect immunofluorescence (reference method) using anti-nuclear capsid antibodies coupled to fluorescein, with a very rapid response (less than 2 hours) or by a fast, sensitive and specific ELISA method (3 to 4 hours).
- **Isolation of the virus on cell culture** is replacing the mouse inoculation technique which is dangerous and less reliable. Culture enables a rapid diagnosis to be made (within 24 hours) if the sample has been transported under optimal storage conditions. The cells used are continuous mouse neuroblastoma lines. Confirmation of viral antigens in inoculated cells uses IIF or EIA methods.

- **Detection of viral RNA** is performed using PCR amplification methods from samples of saliva and CSF, with an answer in 48 hours.

INDIRECT DIAGNOSIS

Titration of anti-rabies antibodies is used to assess the level of immunity in people who have previously been vaccinated or are being treated for rabies.

This uses an EIA or seroneutralisation method. Testing for anti-rabies antibodies in blood is not particularly useful in the diagnosis of rabies as serology is only positive at the end stage of the disease. On the other hand, testing for antibodies in CSF is of more use, particularly to differentiate active infection from immunisation following vaccination or recent injection of immunoglobulins.

TREATMENT

CURATIVE OR POST-EXPOSURE VACCINATION

This is administered after infection and is possible because of the long incubation period of the disease, allowing the vaccine to be effective before the rabies virus has reached the central nervous system. Vaccination is given as several injections which may or may not be combined with serotherapy depending on the case.

Several vaccines for human use are commercially available; these are produced on Vero continuous cell lines or on purified chick embryo cells. All of these are inactivated.

PREVENTATIVE OR PRE-EXPOSURE VACCINATION

This is recommended specifically in exposed people, such as veterinary practitioners, abattoir staff, laboratory staff, forest workers, game wardens and travellers visiting a rabies endemic area. Vaccination against animal rabies has also been shown to be very effective over recent years.

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RENAL STONES

DEFINITION

A renal stone is defined as any solid complex body obtained through a biological process in the kidney or in the urinary tract. It excludes any foreign body and urine sand or sediment.

FORMATION OF A RENAL STONE

THE DIFFERENT MECHANISMS AND HYPOTHESES

Hypotheses

Lithogenesis is not always fully understood although several hypotheses are advanced:

– Crystallisation of a chemical substance due to its excessive concentration in the kidney, or even to saturation (above the solubility limit)

– Deficiency of crystallisation inhibitors

– The presence of crystallisation inducers

– The presence of a matrix, a protein skeleton which may enable or promote crystallisation of other chemical substances

– The presence of calcified papillary corpuscles which may enable or promote crystallisation of other chemical substances

– The presence of foreign bodies which may enable or promote crystallisation of other chemical substances.

Mechanisms

These involve general mechanisms such as inadequate urine output, specific mechanisms such as gout and specific genetic mechanisms such as primary oxalosis.

General mechanisms

– Inadequate urine output often due to inadequate daily water intake.

– Diet is often responsible for or promotes stone formation in some patients.

– Urine pH: Excessively acid urine pH promotes the formation of uric acid whereas excessively alkaline urine pH promotes the formation or urate stones.

– Urine infections can cause formation of stones such as struvite or carbapatite stones in urease-positive bacteria.

Specific mechanisms

Uric acid stones are formed due to high concentrations of urinary uric acid in diseases such as gout and also in the metabolic syndrome and type II diabetes. Cystine stones are found in patients suffering from cystinuria, a disease involving a defect of the transmembrane cystine and lysine transporter, which lowers blood concentrations and concentrates the two amino acids in urine.

THE DIFFERENT STAGES

Several stages precede the formation of the renal stone:

– Nucleation

– Growth

– Aggregation

– Retention

– Agglomeration.

OTHER POSSIBLE SITES FOR STONES

Apart from some stones being encrusted within the structure of the kidney, stones are also found in the gallbladder (gallstones), salivary glands, joints and gastro-intestinal tract, etc.

INFORMATION

SAMPLE

The sample is a solid structure and two rules should be observed regardless of origin:

– Optimal isolation from other biological materials.

– A dry specimen submitted for analysis.

Obtaining a renal stone

This may be passed spontaneously either with or without prior lithotripsy and occasionally following renal colic. It may also be obtained from urological or renal surgery particularly for large stones.

Drying the stone

It is recommended that the stone be isolated promptly from other biological media (urine, surgical material, etc,). Drying is an important stage as water prevents the components of the stone being read by infra-red techniques. The stone is dried at room temperature as any rise in temperature can produce crystalline conversion and therefore changes in the compounds.

ANALYTICAL METHODS FOR RENAL STONES

These establish the different components of the stone and their relative quantities to explain the mechanism of stone formation and the triggering factors for the mechanisms resulting in the development of the stone.

DIFFERENT ANALYTICAL METHODS FOR RENAL STONES

Morphology

Morphological examination

Examination with an x 10 binocular magnifying glass is used to describe the number of stones present, measure their size(s) in mm, consistency, appearance, shape, surface colour (mamillated, brown, etc.). After breaking open the stone, its appearance, shape, and colour of the section and then centre (the structure of which is sometimes different from the rest of the stone) are noted. This detailed examination is reported in writing.

Morphological typing

Results of the examination are compared to known reference standard classifications. The morphological type of a pure crystalline stone, which is possible for renal stones, is described by a Roman digit followed by a lower case letter or even number.

Chemical examination

Kits are available to determine the chemical components of a renal stone, using specific chemical reactions for the compounds present. These methods occasionally lack sensitivity (minor components) and even specificity.

Infra-red spectrum

An infra-red absorption spectrum is obtained and the infra-red absorption peaks and occasionally wider bands, shoulders, peak broadening and slope changes are described. The different components are described either by letters followed by a figure indicating the number of water molecules such as C1 (monohydrated calcium oxalate) or C2 (dehydrated calcium oxalate) or AU0 (anhydrous uric acid) or AU2 (dehydrated uric acid), or by a specific name such as carbapatite (carbonated apatite).

X ray diffraction

This is specific but has difficulty in detecting carbonates and requires larger stones than the above method.

ANALYTICAL METHODS USED

In both France and other countries, two combined methods are used to analyse stones. The first is analysis and morphological typing and the second is reading the infra-red spectrum.

Morphology

Morphological typing provides information about the history of the stone and can also be used to identify very specific diseases. Two morphological presentations exist for example for carbapatite stones: IV (or IV a1) and IV a2. The latter has a very specific morphology and represents very specific diseases (tubule acidosis).

Similarly, whewellite or C1 (monohydrated calcium oxalate) stones often have, an "Ia" morphology or occasionally even more rarely "Id" or "Ib". "Ic" types in young children indicate primary oxaloses, which are serious inherited diseases. "Active I" types which have recently been described, represent diseases associated with gastro-intestinal absorption problems in some bowel diseases in which oxalic acid is concentrated.

Reading methods: The stone must be clean and free from any blood or tissue contamination (particularly following surgical excision). It should preferably be rinsed with distilled water. Be aware that containers such as pill boxes could result in contamination from the active substances or excipients. The stone must be dry and should be dried at normal temperature.

Morphological examination is performed using a binocular magnifying glass. The number of stones, dimensions, appearance and surface colour are recorded after which the stone is broken open and the section and then centre are examined. Unusual or minor appearances or colours must not be omitted.

Classification and morphological typing: The stone is typed based on the criteria described above from standard photographic plates. Only trained and fully competent staff perform morphological examination and typing.

Morphological classification of stones

Type I *Whewellite or monohydrated calcium oxalate*	**Ia**	• Smooth or mamillated or "budding" surface, occasionally finely lined appearance. • Concentric compact section with radial crystallisation. • More or less dark brown colour. • Often small stones. A simple umbilication is often seen representing an attachment point to the renal papilla.
	Ib	• Rough mamillated surface. • Compact, disorganised crystalline section occasionally with lacunae. • Dark brown to brown-black colour.
	Ic	• Smooth or budding surface clear in colour • Finely granular disorganised crystalline section. • Light brown to cream colour.
	Id	• Smooth surface. • Compact microcrystalline concentric section with no radial organisation. • Light brown-yellow to oatmeal colour.
Type II *Weddelite or dehydrated calcium oxalate*	**IIa**	• Spiculated surface (shiny crystals, translucent at angles with sharp edges). • Radial crystalline section, more or less loosely packed with diffuse convergence area. • Light brown-yellow to cream colour.
	IIb	• Spiculated surface (dull, opaque crystals, thick at the angles, more or less blunt). • Compact, disorganised crystalline section. • Oatmeal to cream colour.
	IIC	• Finely rough microcrystalline surface with diffuse peripheral concentricity. • Beige to dark oatmeal in colour.
Type III *Uric acid and urates*	**IIIa**	• Smooth homogeneous or very slightly lumpy surface, oatmeal, yellowish, ocre or orange in colour. • Compact concentric section with radial crystallisation, ocre, orange or brick red in colour. = Anhydrous uric acid.
	IIIb	• Heterogeneous crystalline and/or rough microcrystalline surface, locally porous. • Cream to brown-red. • Section more or less compact, crystalline, disorganised, orange or brick red. = Dehydrated uric acid ± anhydrous uric acid.
	IIIc	• Homogeneous rough microcrystalline surface, more or less porous. • Compact microcrystalline section generally disorganised. • Cream to grey-brown colour. = Various urates.
	IIId	• Heterogeneous, microcrystalline, rough, very porous surface. • Concentric, porous, more or less loosely packed microcrystalline section. • Greyish, oatmeal or brownish colour. = Acid ammonium urate.
Type IV *Phosphates*	**IVa1**	• Whitish, oatmeal or brown-yellow homogeneous crystalline surface, rough or lumpy. • More or less distinct concentric section, whitish to beige. = Carbapatite ± struvite.
	IVa2	• Homogeneous crystalline surface, lacquered in appearance, more or less smooth with irregularities sometimes suggestive of a piece of silex; the colour is homogeneous and brown-yellow. • The superficial layer may contain fine small cracks. • More or less leaved concentric section in thick brown-yellow crystalline and finer beige micro-crystalline layers. = Carbapatite + small amount of protein (± whewellite, small amount)
	IVb	• Heterogeneous, lumpy rough surface, even porous, whitish and oatmeal to brown. • Concentric section with alternating thick whitish and oatmeal, fine crystalline or micro-crystalline layers. = Carbapatite + struvite whewellite acid ammonium urate
	IVc	• Homogeneous granular crystalline surface with large crystals, few angles, more or less fixed to each other or simply rough. • Radial or simultaneous concentric and loosely packed radial crystalline section. • Whitish colour. = Struvite (as struvite is rarely pure in human beings the internal structure is often heterogeneous)
	IVd	• Homogeneous rough or dappled crystalline surface. • Compact concentric section with radial crystallisation. • Cream to oatmeal colour. = Brushite
Type V *Cystine*	**Va**	• Homogeneous granular, crystalline surface with crystals containing few angles or simply lumpy with a more or less translucent waxy appearance • Homogeneous diffuse radial crystalline section. • Yellowish to light brown-yellow colour.
	Vb	• More or less smooth cream to yellowish homogeneous micro-crystalline surface. • Compact heterogeneous section, concentric, peripheral cream micro-crystalline with a disorganised central yellowish crystalline structure.
Type VI *Proteins*	**VIa**	• Unstructured more or less translucent homogeneous whitish to light brown soft stones.
	VIb	• Heterogeneous only slightly soft or hard stones more or less structured, of rough and scaling surface. • Loosely packed, rough, more or less leaf structured section. • Brown-black colour. = Large amounts of protein in stones containing predominantly various constituents (drugs– whewellite – uric acid– etc.).

Interpretation of stone morphology

Morphology	Composition	Specific features	Predisposing factor
I a	*Whewellite (C1)* *= lithiasis with oxalate-dependent structure*	Randall Plate	• High output or concentration hyperoxaluria. • Cacchi-Ricci Disease. • Oxalate and animal protein-rich diet. • Inadequate urine output. • Heterogeneous nucleation on Randall plate in the renal papilla.
I b	*Whewellite*		• High output or concentration hyperoxaluria. • Oxalate and animal protein-rich diet. • Stasis due to urological abnormality. • Inadequate urine output.
I c	*Whewellite*		• Oxalosis, primary hyperoxaluria.
I d	*Whewellite*		• High output or concentration hyperoxaluria. • Oxalate and animal protein-rich diet. • Inadequate urine output. • Stasis, multiple lithiases, anatomical confinement, urological abnormality.
I actif	*Whewellite*		• Malabsorption syndromes (Crohn's disease, coeliac disease).
II a	*Weddelite (C2)* *= lithiasis with calcium-dependent structure*		• Hypercalciuria regardless of origin.
II b	*C2 + C1* *due to H2O loss or mixed crystallisation*		• Hypercalciuria associated with moderate or intermittent hyperoxaluria. • Urinary stasis. • Diet rich in animal proteins, dairy products or oxalate. • Inadequate urine output.
II c	*Weddelite (C2)*		• Hypercalciuria. • Stasis, anatomical confinement.
II Ia	*Anhydrous uric acid (AU0)* *= lithiasis with urate-dependent structure*		• Acid urine pH. • Intermittent hyperuricuria. • Stasis.
III b **III ab**	*Dehydrated uric acid (AU2)* *+/- anhydrous uric acid (AU0)*	If bladder stone uric stasis stone	• Acid urine Ph. • Hyperuricuria + hyperuricaemia. • Gout diathesis. • Consumption of purine rich foods. • Defective renal ammoniogenesis. • Urinary candidiasis. • Water and electrolyte disturbances.
III c	*Various urates*		• Hyperuricuria with either therapeutic or infectious alkalinisation of urine.
III d	*Acid ammonium urate*		• Renal or urinary hyperammoniogenesis. • Urinary infection with ammoniogenic organisms. • Malnutrition. • Anorexia nervosa • Gastro-intestinal base loss (infectious diarrhoea, laxative abuse).
		Children from 0 to 3 years old	• Renal or urinary hyperammoniogenesis. • Urinary infection with ammoniogenic organisms. • Malnutrition. • Gastro-intestinal base loss (infectious diarrhoea).
IV a 1	*Carbapatite (CA) +/- oxalate* *= lithiasis with phosphate structure*	Without struvite	• Urine infection with urease-negative organisms. • Hypercalciuria. • Phosphate diabetes. • Tubular acidification disorder. • Primary hyperparathyroidism.
IV a 2	*Carbapatite*		• Congenital or acquired tubular acidosis (Sjögren's syndrome, Albright syndrome, chronic parenchymal urinary infections, chronic active hepatitis). • Focal renal acidification disorder (Cacchi-Ricci disease).
IV b	*Carbapatite*	Without struvite	• Primary hyperparathyroidism.
	Carbapatite	with struvite	• Primary hyperparathyroidism. • Chronic urine infection with urease-positive organisms.
IV c	*Struvite (PAM)*		• Urinary tract infection with urease-positive organisms. • Inadequate urine output.
		Animal	• Urine infection with urease-positive organisms.
IVd	*Brushite*		• Primary hyperparathyroidism. • Hypercalciuria. • Phosphate diabetes. • Urological abnormality. • Sarcoidosis.
V	Cystine		• Cystinuria – Lysinuria.
VI a	Proteins		• Chronic pyelonephritis.

Infra-red spectrum

A representative part of the stone (or even the entire stone if it is small), or better still, part of the surface, section and centre of the stone are powdered by scratching with a needle. It is crushed, diluted, mixed, and homogenised in a small mortar with a very small pestle in potassium bromide powder. The mixture obtained is passed through a special press to obtain a hard pellet of defined diameter and thickness. Potassium bromide is relatively transparent to infra-red rays and enables the powdered stone to be diluted and its spectrum to be recorded.

Physical reading method: Measurement involves passing an infra-red beam through the pellet which is placed on a suitable support and measuring absorbency. A power system is used to rapidly vary wavelengths and frequencies from 4000 cm^{-1} to 400 cm^{-1}. The absorbance is measured at each frequency and is recorded and stored on computer. Several spectra are recorded for each pellet and the mean spectral result is used.

Measurement interpretation method: The absorption peaks (their wavelength) are identified from this spectrum, together with their widths, presence or absence or shoulders, deformities in slopes and changes in slopes. Peak differences (a few cm^{-1}) may be important. The presence or absence of supernumerary peaks compared to another spectrum indicates a difference in crystals (for example the number of water molecules bound). Changes in slopes and shoulders can now be identified other than visually by calculating derivatives and secondary derivatives. Even minor constituents with low absorbency supernumerary peaks must be included. Finally each constituent is quantified in detail (or even approximately) and expressed as a percentage.

LABORATORY INTERPRETATION OF MORPHOLOGICAL DATA AND INFRA-RED SPECTRA

A mechanism explaining the lithiasis is then proposed from these data.

Combining the two methods enables a detailed analysis of the constituents of the stone and their distribution to be described together with retracing its history (centre). This also allows detailed discrimination between hydration rates which are essential for interpretation for molecules such as calcium oxalate. When monohydrated, the lithiasis is oxalate-dependent and when di-hydrated it is calcium-dependent.

EPIDEMIOLOGY

A number of the diseases are genetic in origin. The epidemiology of stones involves a Male/Female ratio of approximately 2.6 and an average age of developing stones of 47 years old or older, in both men and women.

The most common stones in France contain calcium oxalate (≥70%), with a high proportion of whewellite or monohydrated calcium oxalate (50%) and less commonly, weddelite or dehydrated calcium oxalate (21%). Following these, are calcium phosphates (12%), the majority of which are orthophosphates. Only approximately 10% of stones involve uric acid.

Regional disparities are found, whewellite being present in a greater extent in the South-East of France and less in the north and north-east. More weddelite is found in the north-west quarter of France. There are large disparities with carbapatite which predominates in the north-east quarter with far lower levels in the east, south-east and south-west.

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RENIN

DEFINITION – INTRODUCTION

Renin is a 44 kDa glycoprotein enzyme. It is an aspartyl-protease which specifically cleaves angiotensinogen, an α-2 globulin produced by the liver, producing angiotensin I, a decapeptide. This is then converted into angiotensin II, an octopeptide, by the effect of angiotensin converting enzyme which is present in the lungs and other tissues. Angiotensin II is then cleaved by removal of asparagine to produce angiotensin III, a heptapeptide. Whereas angiotensin I has no known biological action, angiotensin II and angiotensin III have an equivalent action in stimulating secretion of aldosterone by the zona glomerulosa, although angiotensin II is also a more potent vasoconstrictor.

Renin is produced by the juxta-glomerular cells of the renal cortex which are endocrine cells derived from vascular smooth muscle cells. Renin has two secretory and release pathways. One unregulated constitutional pathway in which an inactive precursor, prorenin, is secreted and released into the plasma and a second, regulated, pathway by which renin is stored in secretory granules and then secreted in response to changes in urinary sodium concentration in the macula densa, changes in afferent artery perfusion pressure, sympathetic stimulation and plasma angiotensin II concentrations.

Renin concentrations are also increased by anything which stimulates sympathetic nervous system activity through the juxta-glomerular cell β-adrenergic receptors, such as standing up, hypovolaemia, fear and pain. Conversely, renin is reduced by lying down, hypervolaemia, β-blockers and drugs which interfere in sympathetic transmission such as clonidine or α-methyl-DOPA.

INDICATIONS FOR MEASUREMENT

Measurement of renin simultaneously with aldosterone is recommended in the investigation of hypertension. Used in combination with aldosterone it can identify renin-angiotensin-aldosterone system (RAAS) abnormalities and particularly a Conn's adenoma, removal of which may cure the hypertension.

INFORMATION

SAMPLE

The blood sample is collected into an EDTA tube. The sample ***must then be processed at room temperature*** to avoid cryoactivation of prorenin. After the sample has been centrifuged at room temperature the plasma is separated from the cells and frozen immediately at - 20°C or below. It is kept at this temperature until assay. The use of serum, heparinised plasma or citrated plasma produces lower renal values and is therefore not recommended.

Patients must be following a normal sodium diet (6 to 9 g of NaCl/day), demonstrated by a urine sodium output of between 100 and 200 mmol/24 h. They must be normokalaemic (minimum ≥ 3 mmol/l) and not subject to any drug interference (cf table below). The blood sample must be taken approximately 2 hours after the patient has got out of bed in the morning (whether or not the patient has been walking), between 0800 and 1000 hours (samples may be taken up until midday after the patient has been seated in the laboratory for 15 to 30 minutes.

Recommendations: time for which drugs should be stopped before samples are taken for measurement

Drug class	Discontinuation time (weeks)
Spironolactone	6
Non-spironolactone diuretics	3
CEI, ARA 2, beta-blockers	2
Aliskiren	2

If anti-hypertensive treatment cannot be stopped it may be replaced with hydralazine, beta-blockers vasodilators or centrally acting anti-hypertensives.

QUESTIONS FOR THE PATIENT

Information about age, position (standing or lying at the time of the sample), sampling time, phase of the menstrual cycle for women of childbearing age, dietary salt and treatment must be provided.

SAMPLE STORAGE AND TRANSPORT

Plasma samples may be stored frozen at – 20°C for approximately 6 months.

ASSAY METHOD

It is important to note that samples should be thawed at room temperature (and never in a refrigerator) and then mixed before assay.

It is now far preferable to measure active renin itself rather than renin activity. Active renin is measured by radio-immunoassay, although immunoassays with non-isotopic tracers have recently become available and can be performed on auto-analysers. These are chemoluminescence assays using two specific monoclonal antibodies, one labelled with biotin for capture and the other labelled with acridinium ester for detection.

DYNAMIC TESTS

Stimulation tests rely on RAAS stimulation by effective hypovolaemia and/or sodium depletion. These are the same methods as are used to investigate aldosterone (standing and sodium depletion). The same applies to the salt loading and the captopril suppression tests *(cf. Aldosterone)*.

Failure of blood aldosterone or renin to change would appear to be evidence in favour of primary hyperaldosteronism due to an adenoma, whereas hyperaldosteronism secondary to aldosterone production from zona glomerulosa hyperplasia responds to stimuli which activate the RAAS.

ALDOSTERONE/RENIN RATIO

As in primary hyperaldosteronism, aldosterone and renin vary reciprocally; the simplest way to identify independent aldosterone production in this syndrome is to calculate the aldosterone/renin ratio (or plasma renin activity = PRA / renin).

The threshold value for this ratio, however, must be defined. This varies with the assay method and units in which aldosterone and renin (or PRA) results are expressed.

The thresholds proposed, therefore, differ between studies.

– If aldosterone is expressed in ng/dl and PRA in ng/ml/h, normal values currently range from < 20 to < 40.

– If aldosterone is expressed in pmol/l and PRA in ng/ml/h, normal values are < 555 to < 750, depending on the author.

– If aldosterone is expressed in pmol/l and renin in mIU/l, the normal value is < 64.

– Finally, if aldosterone concentration is expressed in ng/dl and renin in µU/ml, the threshold value is 3.

USUAL VALUES

Usual values vary depending on the assay method. For reference, those found by chemoluminescence in adults are shown in the table below.

Reference values for plasma active renin in adults on a normal sodium diet

Renin	pg/ml	µU/ml
Lying	1.5 – 17.0	2.5 – 28.4
Standing	2.0 – 25.0	3.3 – 41.8

Pg/ml is converted into µU/ml by multiplying pg/ml by 1.67.

Values vary with age in children.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

RAAS activity is affected by age, sex, circadian rhythm, hormone status and salt intake. Renin concentrations vary in parallel to those of aldosterone *(cf. Aldosterone)*.

Renin is reduced in people over 65 years old (slightly), Africans, diabetics and in people who take excessive sodium or are on beta-blockers. Renin secretion is also pulsatile. Because of these limitations the aldosterone / renin (or PRA) ratio must be measured on two occasions.

■ PATHOLOGICAL VARIATIONS

(cf. Aldosterone)

■ Increase to concentrations

Renin secreting tumours

Renin excess produces an increase in angiotensin II, causing vasoconstriction and secondary hyperaldosteronism which results clinically in hypertension with hypokalaemia. There are different types of renin secreting tumours.

– Reninomas

The hypokalaemia associated with hyperaldosteronism is generally severe. Renin concentrations are very high, often more than five times the upper limit of normal. Prorenin is also raised, as are plasma and urinary aldosterone. Dynamic tests, standing up and salt loading show renin secretion to be independent.

The diagnosis is based on finding raised renin concentrations associated with a renal tumour in the absence of other causes of renin excess, such as renal artery stenosis or renal infarction. The most sensitive imaging method is CT scan.

Angiography is relatively insensitive but must be performed to exclude renal artery stenosis or renal infarction.

– Renal renin-secreting tumours other than reninomas

These are renal tumours derived from cells other than juxtaglomerular epithelial cells, which secrete renin. Neuroblastomas and Wilms tumours cause hypertension in 60% of cases and renin concentrations may be raised, although these tumours secrete mostly prorenin. Prorenine is therefore used as a marker for this type of tumour as it appears to be more sensitive than NSE *(Neuron Specific Enolase)* which is generally recommended.

Other types of renal tumours can secrete renin including mesoblastic nephroma and clear cell carcinoma though these tumours mostly secrete prorenin and aldosterone is not raised.

– Extra-renal tumours

These are extremely rare tumours including hepatocellular carcinomas, and tumours in the lung, ovary, Eustachian tube and colon. They rarely present as renin excess.

Secondary hyperaldosteronism

Renin secretion increases in all pathological states associated with oedema and ascites (heart failure, cirrhosis and nephrotic syndrome), because of the reduced effect of circulating blood volume secondary to reduced cardiac output or transudation of intravascular volume into the extravascular space. The increased renin causes a rise in aldosterone, producing secondary hyperaldosteronism.

Secondary hyperaldosteronism is also seen in renal artery stenosis and in salt-losing nephropathy.

Primary adrenocortical insufficiency (Addison's Disease)

In primary adrenocortical insufficiency (Addison's Disease), all three areas of the adrenal cortex are affected by the disease and secretion of all adrenal steroids, glucocorticoids, mineralocorticoids and androgens, is defective (cf. aldosterone). The laboratory diagnosis is based on finding low concentrations of cortisol, aldosterone and DHA sulphate. ACTH and renin concentrations, however, are raised.

It should be noted that in adreno-cortical insufficiency secondary to corticotrophin deficiency, mineralocorticoid function is not affected as the renin-angiotensin system is not involved in this disease.

Primary hypoaldosteronism

Hypoaldosteronism is suggested by hyperkalaemia without an apparent cause and may be primary or secondary to renin angiotensin system deficiency. Primary hypoaldosteronism is not associated with hyperreninaemia and may be congenital or acquired.

– Congenital forms (cf. Aldosterone)

CYP11B2 (Aldosterone synthase) deficiency

This is a rare disease due to deficiency of an enzyme in the aldosterone biosynthesis pathway, aldosterone synthase (CYP11B2). This enzyme has two different activities: CMO I (corticosterone 18-methyloxydase I) responsible for hydroxylation of corticosterone and CMO II which converts the 18-hydroxyl group into an aldehyde.

In COM I deficiency, aldosterone is undetectable and 18-hydroxycorticosterone (18OHB) is very low. In COM II deficiency, 18-OHB is greatly raised, although aldosterone

is detectable. Calculation of the 18-OHB/aldosterone ratio is used to differentiate between these two types. The ratio is less than 10 in COM I deficiency and more than 100 in COM II deficiency.

CYP21A2 (21-hydroxylase) deficiency

Classical forms present in two ways, one characterised by isolated virilisation and the other with virilisation associated with mineralocorticoid deficiency. In the second, salt-loosing form, hyponatraemia, hyperkalaemia, hypovolaemia and increased plasma renin due to the hypoaldosteronism are seen.

– Acquired forms

Heparin may have a direct toxic effect on the zona glomerulosa and cause hypoaldosteronism. Similarly, primary acquired hypoaldosteronism may develop in some severe diseases and metastatic adrenal disease with an isolated effect on mineralocorticoid function.

Pseudohypoaldosteronism

This is a rare salt losing syndrome in childhood. It has been attributed to renal tubule insensitivity to the mineralocorticoid action of aldosterone. It may be transmitted either as an autosomal dominant or recessive condition. In the dominant form, resistance to aldosterone activity is limited to the renal tubule, whereas in the more severe recessive form, the salivary glands, sweat glands and colon are also affected.

The syndrome may be asymptomatic or present as hypoaldosteronism with hyponatraemia, hyperkalaemia and renal sodium loss. Aldosterone and renin concentrations are always greatly raised. It does of course not respond to mineralocorticoid administration.

Bartter's Syndrome

Bartter's syndrome is rare and is characterised by hyperreninaemia, hyperaldosteronism, hypokalaemia and alkalosis without hypertension or oedema. It begins in childhood with muscle weakness, cramps, polyurea, growth retardation and delayed intellectual development. Juxtaglomerular cell hyperplasia and increased urinary prostaglandin E2 excretion are found. Nephrocalcinosis and nephrolithiasis are relatively common. Serum ionised calcium is reduced and serum parathyroid hormone increased, with osteopaenia.

■ **Reduced concentrations *(cf. Aldosterone)***

Active renin concentrations are reduced or may be very low in several circumstances.

Primary hyperaldosteronism

Regardless of cause: Conn syndrome, idiopathic hyperaldosteronism due to zona glomerulosa hyperplasia or dexamethasone sensitive hyperaldosteronism. Primary hyperaldosteronism is characterised by raised aldosterone associated with low or very low renin concentrations.

Tumour related hypermineralocorticoidism

Secretion of mineralocorticoids other than aldosterone by tumours, such as deoxycorticosterone (DOC) or corticosterone, is associated with hypertension and hypokalaemia, although both aldosterone and renin concentrations are low.

Hypermineralocorticoidism due to enzyme deficiency

In 11β-hydroxylase and 17α-hydroxylase deficiencies, the aldosterone precursors, DOC (11β-hydroxylase) and DOC and corticosterone (17α-hydroxylase) deficiency accumulate. These steroids, particularly DOC, have mineralocorticoid activity and when produced in excess cause hypertension, hypokalaemia, salt retention and inhibition of both renin and aldosterone.

Secondary hypoaldosteronism or hyporeninaemic hypoaldosteronism

Hyporeninaemic hypoaldosteronism is characterised by chronic unexplained asymptomatic hyperkalaemia associated with moderate renal insufficiency and renal tubular acidosis. The diagnosis is considered in the presence of chronic unexplained hyperkalaemia and is confirmed by the very low concentrations of plasma and urinary aldosterone associated with low renin concentrations.

Pseudohypermineralocorticoidism

Deficiency of 11β-hydroxysteroid dehydrogenase (11HSD) activity, which converts cortisol to cortisone causes this syndrome, which is characterised by hypertension, hypokalaemia and low aldosterone and renin concentrations. It may either be congenital (apparent excess of mineralocorticoids, AME type I or type II), or secondary to administration of glycyrrhizinic acid, the active substance contained in liquorice *(cf. Aldosterone)*.

In Cushing's syndromes due to ectopic ACTH production (small cell lung cancer), excess cortisol production produces a relative deficiency of 11HSD and therefore hypermineralocorticoidism. Similarly, in adreno-cortical tumours (adrenocorticalomas), over-secretion of cortisol causes a relative deficiency of 11HSD.

Liddle's Syndrome

This is a familial disease characterised by hypertension, hypokalaemia, hyporeninism and hypoaldosteronism associated with hyperpermeability of the amiloride-sensitive sodium channel.

■ **In conclusion,** investigation of the RAAS must always include measurement of aldosterone linked to measurement of renin. Laboratory diagnosis of an RAAS abnormality is firstly established by comparing the results of analyses.

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REPTILASE TIME

DEFINITION

The reptilase time is the time taken from platelet-poor citrated plasma (PPP) to coagulate in the presence of reptilase (an enzyme extracted from the venom of the snake *Bothrops atrox*). This investigates fibrin formation, i.e. production of fibrin from fibrinogen through the proteolytic action of thrombin and polymerisation. Unlike the thrombin time this is not sensitive to heparin, hirudin or any thrombin inhibitor treatments.

INDICATION FOR MEASUREMENT

This is a second line test used for a prolonged thrombin time to confirm an abnormality of fibrin formation and exclude the presence of heparin (or hirudin or other anti-thrombin) in the sample.

It can reveal the presence of FDP (in DIC), even if the patient has been started on anticoagulation.

INFORMATION

■ SAMPLE

The sample should be taken into 3.2% citrate (0.109 M), volume 1/10 (0.5 ml per 4.5 ml of blood). Tubes containing 3.8% citrate (0.129 M) are acceptable. Blood can also be collected into CTAD tubes (citrate, theophyllin, adenine, dipyridamole). No other anticoagulant may be used.

A fasting sample is not necessary and a light low fat snack is permitted.

For further information refer to the *"General preanalytical conditions in haemostasis"* section.

■ QUESTIONS FOR THE PATIENT

Are you taking anticoagulation? Unfractionated heparin, low molecular weight heparin, hirudin and derivatives or dabigatran prolong the thrombin time. The new anticoagulants (dabigatran, rivaroxaban) do not prolong the reptilase time.

Have you received thrombolytic treatment, such as streptokinase, urokinase, rt-PA or tenecteplase? These treatments lyse fibrinogen into fibrin degradation products and fibrin (FDP and D-dimers) and prolong the reptilase time.

■ SAMPLE STORAGE AND TRANSPORT

For 4 hours at laboratory temperature, 1 month at - 20°C; and – 80°C beyond this time.

It is recommended that samples be rapidly thawed in a water bath at 37°C.

Transport: the sample should be centrifuged twice and then separated and frozen within 2 hours of sampling.

ASSAY METHODS

By measurement of the coagulation time at 37°C of a platelet poor plasma in the presence of a defined amount of reptilase.

NORMAL EXPECTED VALUES

"Normal" reptilase time: < 20 seconds or patient time/control time < 1.20.

PATHOLOGICAL VARIATIONS

Prolongation of the reptilase time reflects constitutional or acquired abnormalities:

■ CONSTITUTIONAL FIBRINOGEN DEFECTS

– Afibrinogenaemia, hypofibrinogenaemia, (quantitative deficiency): risk of bleeding.

– Dysfibrinogenaemia (qualitative defect: normal amounts of non-functional fibrinogen). The constitutional dysfibrinogenaemias are usually asymptomatic and occasionally present with moderate bleeding and in approximately 10% of cases are associated with venous or arterial thromboses.

■ ACQUIRED ABNORMALITIES

– Defibrination syndrome: disseminated intra-vascular coagulation or, far more rarely, primary fibrin (ogen) lysis.

– The presence of a specific thrombin inhibitor (anti-thrombin) in plasma: circulating anticoagulant, dysglobulinaemia, and high levels of PDF or D-dimers.

– Inflammatory states with pronounced hyperfibrinogenaemia (> 10 g/l).

– Acquired dysfibrinogenaemias (cirrhosis, etc.).

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RESPIRATORY SYNCYTIAL VIRUS

DEFINITION

Human respiratory syncytial virus (RSV) causes respiratory viral infections, particularly infant bronchiolitis, a worrying child public health problem because of its incidence and severity.

It more rarely causes adult pneumonias particularly in immunosuppressed patients and the elderly.

RSV belongs to the *Paramyxoviridae* family and *Pneumovirus*. genus. It is an RNA enveloped virus measuring between 80 and 350 nm. Two groups of RSV are distinguished on antigenic and genetic criteria: RSV A and RSV B, which are themselves subdivided into sub-groups.

INTRODUCTION

EPIDEMIOLOGY

RSV infection occurs every winter as epidemics (from October to March with a peak in December or January) and is constantly increasing in incidence. It is usually caused by a combination of the 2 groups of RSV A and B, although one group occasionally predominates compared to the other.

Primary childhood RSV infection occurs very early (before the age of 2 years old in 90% of cases) and is usually mild. RSV respiratory diseases mostly affect young children. Transmission is directly from respiratory secretions or more rarely, by hands.

SYMPTOMS

After a short incubation period of 2 to 4 days the virus replicates in the respiratory tract ciliated cylindrical cells causing a nasal illness (rhinitis), which extends in infants to the bronchi and bronchioles.

It also causes otitis, laryngitis, and bronchitis.

The 3 clinical forms of the disease usually seen are:

- **Infant bronchiolitis:** this is the typical form of RSV infection and occurs as a result of a combination of pulmonary immaturity in the infant and tissue damage causing bronchiolar obstruction.

  It presents as mild rhinitis followed by bronchiolitis in 20% of cases with a combination of cough, obstructive dyspnoea, variable low grade fever, otitis and frequent gastro-intestinal problems.

  RSV infection recovers spontaneously over around ten days, although some children develop recurrent respiratory problems for several years.

  Serious forms of the disease occur in 0.5 to 2% of infants with acute respiratory failure leading to hospitalisation: in premature infants, 2-3 month old infants and in the presence of concomitant disease (cystic fibrosis, heart disease, bronchodysplasia or immunosuppression).

- **Childhood RSV infections:** otitis, bronchitis and pneumonia. RSV also appears to be involved in triggering asthma attacks in children (25% of cases).

- **Adult RSV infections:** pneumonia in the elderly and immunosuppressed.

INDICATIONS FOR MEASUREMENT

Diagnosis of bronchiolitis or pneumonia in infants.

Diagnosis of acute otitis media in children.

Diagnosis of pneumonia in children, immunosuppressed adults or in the elderly.

Investigation of the cause triggering an asthma attack.

INFORMATION

SAMPLE

Respiratory samples: nasal or tracheobronchial secretions.

– The nasal sample is obtained by swabbing with a cotton bud, or aspirating through a probe connected to a collection bottle. Nasal lavage is preferable if no exudates are present.

– Bronchial secretions are recovered during respiratory physiotherapy procedures in infants; tracheo-bronchial secretions are used in preference in the elderly and immunosuppressed; a BAL sample may be used.

– In acute otitis media a small amount of exudate may be obtained during the ENT examination.

– Blood sample for serology: 2 ml of serum (blood collected into a dry tube).

QUESTIONS FOR THE PATIENT

Age of infant?

Prematurity?

Concomitant diseases (asthma, heart disease, etc.)?

SAMPLE STORAGE AND TRANSPORT

The quality of the result of direct diagnostic methods depends on observing sample collection and transport conditions. RSV is fragile and specific transport media must be used.

Secretions collected to test for viral antigens are sent directly to the laboratory if they are to be kept for no more than 2 hours at room temperature. If the sample is not processed within 2 hours a transport medium provided by the laboratory must be used and the sample stored at + 4°C. Slide smears must be dried, fixed with acetone for 10 minutes and then transported at room temperature.

Secretions intended for cell culture require a transport medium: the sample in transport medium should be stored at + 4°C and transported at + 4°C.

Samples for PCR can be stored for a few days at + 4°C. Swab samples require a viral transport medium.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

- **Detection of viral antigens by immunofluorescence (IF):**

  – Direct testing of infected cells by IF produces a rapid response and may be combined with concomitant testing for other respiratory viruses. If necessary, monoclonal antibodies can be used to distinguish between RSV A and B subgroups. The method offers good sensitivity similar to that of culture.

– Commercially available reagents revealed by immuno-enzymatic methods are available and have a similar sensitivity to IF.

- **Cell culture:**

  This is the reference method. The RSV is isolated on several types of cells: continuous human cell lines (HeLa, Hep2), diploid human cells (MRC5) or continuous animal cell lines (Vero).

  The CPE (cytopathic effect), which develops between days 5 and 10 of culture is characterised by syncytial appearances on continuous cells lines (hence its name). In view of the response time this is mostly reserved for research laboratories.

- **Molecular biology:**

  RT-PCR method, which is the most sensitive.

INDIRECT DIAGNOSIS

The diagnosis is made by demonstrating seroconversion in primary infection or from a significant rise in antibody concentrations in reinfection, mostly using ELISA techniques.

IgM and IgG appear early in primary infection. IgM disappears after a few weeks although IgG persists in the long-term. Reinfections may be characterised by a further rise in IgG but not IgM.

INTERPRETATION

Detection of viral antigens in respiratory secretions by IF or ELISA is the simplest and fastest method but is very dependent on sample quality. It indicates active respiratory tract viral infection and is particularly suitable for the diagnosis of RSV in an infant.

Gene amplification methods provide an unequivocal diagnosis as they confirm the presence of viral replication. They can detect low viral loads unlike immunological detection of viral antigens, although are difficult to perform.

In the absence of epidemiological evidence, detection of viral antigens should be combined with isolation by cell culture.

Serology: this is not well suited to the diagnosis in infants who secrete small amounts of antibodies. Its use is also limited by the delay before the serological response develops. The presence of IgM antibodies may indicate primary RSV infection, although specificity problems exist. Serology is mostly of retrospective use in epidemiological studies.

TREATMENT

RSV infection can be prevented in frail patients by injection of specific immunoglobulins. A vaccine is currently being studied.

The treatment of infant bronchiolitis is above all symptomatic.

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RETINOL-BINDING PROTEIN

DEFINITION

Retinol-binding protein (RBP) is a protein produced by the liver which migrates on serum electrophoresis in the alpha-2 globulin band and has a short half-life of 12 hours. It has a molecular mass of 21 kDa and is formed from a single 182 amino acid polypeptide chain which is folded to form a specific site for one molecule of retinol.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

The role of RBP is to transport vitamin A in the circulation in its physiological form, retinol, from the liver to its target tissues. Synthesis in the liver is highly dependent on energy and nitrogen (tryptophan) intake and on zinc. In the hepatocyte, RBP binds to retinol to form holoRBP (which is soluble) and which once released into plasma, complexes with prealbumin (or transthyretin, TTR) to form a stable trimolecular complex which transports vitamin A to its target tissues of skin, mucosa and retina. This non-ultrafiltrable complex which is not removed by the kidney protects RBP and retinol from urinary excretion. Only retinol enters the cell in the target tissues. RBP loses its affinity for TTR and is promptly filtered by the glomerulus and then reabsorbed and metabolised by the proximal tubule. RBP enables retinol to be dissolved and protected but also protects cells from the toxic lytic effects of free retinol. Any fall in RBP synthesis causes a fall in vitamin A and conversely, retinol deficiency results in a fall in circulating RBP.

The short half-life of RBP (12 hours) makes it a sensitive marker of acute ***malnutrition*** and it is used to assess nutrition replacement. Despite its dependency on glomerular filtration, it offers equivalent sensitivity to that of prealbumin.

Urinary RBP is a marker of tubular nephropathy in the same way as alpha-1microglobulin, beta-2 microglobulin, urinary transferrin and microalbuminuria. Measurement of these analytes can be combined to assess the level of tubular damage. Incomplete or transient only alpha-1 microglobulin is raised and complete if it is associated with a rise in urinary RBP. Urinary RBP measurement is indicated in the assessment of renal tubular damage, particularly secondary to toxin exposure, notably lead.

INFORMATION

SAMPLE

Serum (dry tube). Discard hyperlipaemic and/or haemolysed samples.

A second pass morning urine sample.

SAMPLE STORAGE AND TRANSPORT

Serum can be stored for 1 week at + 4°C and for several months at – 20°C. Urine should be stored and transported at + 4°C.

ASSAY METHODS

Immunochemistry: Immunonephelometry, immunoturbidimetry and less commonly, radial immunodiffusion.

ELISA.

NORMAL EXPECTED VALUES

Reference values in adults in serum are between 30 and 60 mg/l. They are lower at birth and in children (between 20 and 45 mg/l) and reach adult values at around 15 years old.

In urine: 10 to 540 µg/l (ELISA).

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Serum RBP concentrations are reduced in response to oestrogens, particularly in pregnancy.

PATHOLOGICAL VARIATIONS

Reduced serum RBP

Malnutrition

RBP is a very sensitive marker of malnutrition, particularly latent and subclinical forms. Together with TTR it is used to monitor oral or parenteral nutrition replacement. TTR however appears to be more used as its serum concentrations are higher and are independent of renal function.

Hypovitaminosis A

Serum RBP concentrations are reduced as a result of reduced synthesis, whether the deficiency is due to inadequate intake or malabsorption (Crohn's disease, coeliac disease).

Acute or severe chronic Inflammation

RBP (and TTR) fall rapidly, whereas inflammatory proteins (CRP, haptoglobin and orosomucoid) increase.

Hepatocellular insufficiency

Due to reduced synthesis.

Hyperthyroidism

Moderate fall in RBP concentrations.

Zinc deficiency

Hepatic synthesis is zinc-dependent.

Tubular nephropathies

RBP is not re-absorbed and not metabolised by the tubule and is excreted to a large extent in urine.

■ **Raised serum RBP**

This occurs mostly in renal insufficiency of glomerular origin. Free RBP is no longer filtered by the glomerulus and accumulates in serum. Increased values parallel those of creatinine.

Raised RBP in urine indicates proximal tubular damage.

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REVERSE T3

DEFINITION

3-3'-5'tri-iodothyronine (rT3 = Reverse T3) is an iodinated amino acid (RMM = 651) mostly obtained (95%) from peripheral deiodination of approximately 40% of T4 (3-5-3'-5'tetra-iodothyronine) by the action of type I (liver, kidney, brain and muscle) or type III (all tissues) 5-deiodinase. A small amount (5%) is produced directly by the thyroid gland. It circulates in blood almost entirely bound to the thyroid hormone carrier proteins: *Thyroxine Binding Globulin* (TBG), *Thyroxine Binding Prealbumin* (TBPA) and Albumin (A). rT3 production is closely linked to regulation of thyroid hormone secretion, and particularly to the body's needs for the active T3 hormone (3-5-3'tri-iodothyronine). Reverse T3 is the inactive form of the hormone.

Its production is influenced to various degrees by numerous pathological (thyroid or other disease), physiological (age) or drug-induced interactions. It is removed mostly in bile and faeces after deiodination, conjugation and deamination. The daily secretion rate is: 10 to 25 µg and is almost stable over the 24-hour cycle. Its plasma half-life (T1/2) is approximately 20 hours.

INTRODUCTION

Compared to T3, rT3 is almost inactive and has no direct physiological role. However, as it is produced from T4 at the expense of T3 it modulates T3 activity to meet the body's requirements. Levels can be adjusted in very short periods of time (approximately 48 hours) and it regulates hormonal homeostasis in euthyroid people. Levels are very high in the foetus (cord blood) and blood concentrations fall rapidly after birth (in 8 days) reaching values similar to those in adults.

High rT3 values are also found in amniotic fluid (2nd and 3rd trimester).

INDICATIONS FOR MEASUREMENT

rT3 is of limited discriminatory value in common thyroid diseases, although measurement is useful to confirm and classify some of the following disturbances:

- **Non-thyroidal**

  – "Low T3" syndromes or *Non-Thyroidal Illness* (NTI). These are caused by reduced peripheral 5'-deiodinase activity and are characterised by raised rT3 in the presence of a normal TSH, reduced total T3 and free T3 and normal or high total T4 and Ft4. This is seen in clinically and laboratory euthyroid elderly patients suffering from serious diseases (cancer, cirrhosis, renal insufficiency, acute fever, myocardial infarction, post-operatively, etc.) or in patients treated with some drugs (amiodarone, glucocorticoids, dexamethasone, etc.). It is also relatively often seen in hospitalised patients, particularly in intensive care and in malnourished patients.

  – "Low" T4 syndromes.

- **Thyroidal**

  – Hyperthyroidism with low TSH and discordant FT4.

- **Genetic**

  – A profound reduction in serum rT3 is seen in trisomy 21 subjects.

  Measurement is not widely used in practice as paradoxical results (normal or low) are sometimes seen, associated with renal function or serum transport protein concentrations. Measurement is also only available in a small number of laboratories.

INFORMATION

SAMPLE

The sample should preferably be taken by venepuncture into a dry tube without additive.

Depending on the methods used, assays may be performed on plasma obtained by collecting blood into EDTA or other anti-coagulants (confirm with the manufacturer).

Avoid haemolysed, hyperlipaemic and jaundiced serum.

QUESTIONS FOR THE PATIENT

Age?

Suspected diseases and clinical features?

Results of thyroid profiles?.

SAMPLE STORAGE AND TRANSPORT

rT3 is relatively stable. After centrifugation and separating, serum can be stored for 4 days at room temperature for 2 weeks at + 4°C and for 1 year frozen at– 20°C.

Avoid repeated freeze-thaw cycles.

Current treatments? Many compounds interfere, notably: T3 and derivatives, amiodarone, aspirin, non-steroidal anti-inflammatory drugs and radioactive iodine.

Recent radiological functional investigations (iodinated contrast media).

ASSAY METHODS

These measurements are mostly realised by competitive immunoassay (IRMA method with ^{125}I label). Because of very high antibody specificity (monoclonal or polyclonal) no cross-reactions occur particularly with T3. All methods are associated with numerous causes of error, the incidence of which varies between each method.

The most well known are due to:

– changes in circulating carrier protein concentrations

– cross-reactions with some drugs

– the patient's physiological status (liver, kidney, etc.).

Specific assay methods (dialysis) are used in research to measure free rT3.

REFERENCE VALUES

Values vary with age and the patient's physiological state (pregnancy). Total rT3 = nmol/l or µg/l → (rT3 nmol/l x 0.651 = rT3 µg/l).

Age (years)	rT3 nmol/l
0 – 8 days	2 to 3
Over 8 days	0.12 – 0.60
Pregnancy 2nd trimester	3 – 7.5
Pregnancy 3rd trimester	0.75 - 2

Comment: rT3 values are higher in summer (25 to 40%) than in winter in euthyroid subjects.

■ PATHOLOGICAL VALUES

Low rT3 values are seen in:

– Hypothyroidism

– Trisomy 21

High rT3 values are seen in patients suffering from:

– Hyperthyroidism

– Low T3 syndromes of disease or drug-induced origin.

– Malnutrition.

FOR FURTHER INFORMATION

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■ Google, puis, chopra et rT3.

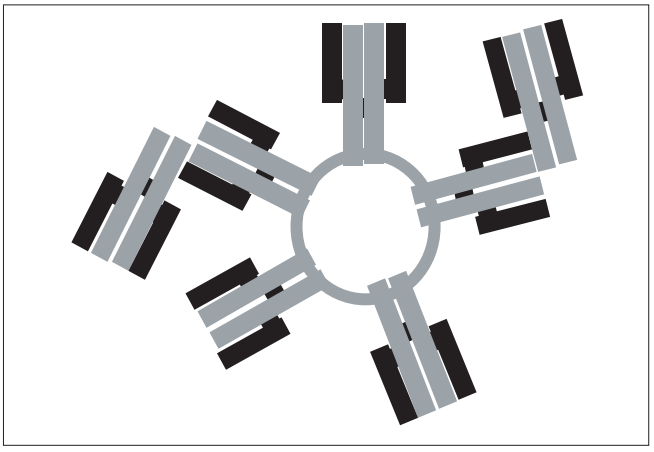
RHEUMATOID FACTOR

DEFINITION

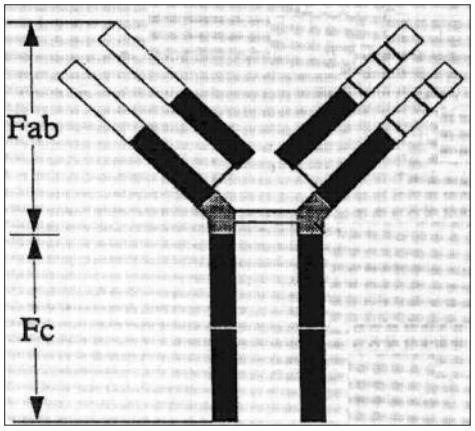
This is a heterogeneous family of auto-antibodies reacting with the Fc fragment of human and animal IgG.

Classically they are IgM isotypes but there are also some which are of the IgA and IgG isotypes (in the absence of details on the request or the report, it is IgM rheumatoid factor which applies).

Rheumatoid factors are present in the course of numerous rheumatological and infectious diseases. They are much used for the diagnosis of rheumatoid arthritis (RA) and are, together with anti-CCP antibodies, the only blood markers adopted by the *American Rheumatism Association* for their classification of this disease.



IgM class rheumatoid factors



Diagrammatic representation of immunoglobulin structure

In small amounts they facilitate the elimination of immune complexes by the reticulo-endothelial system and form part of the first line of defence against infection. They have an immuno-regulatory function through their role in the idiotype system.

In the auto-immune diseases, they prevent the capture of immune complexes by receptors in the renal glomeruli, thus reducing the risk of renal damage. In RA they stimulate inflammation by activating complement in synovial tissue.

There are **physiological RF's** (natural polyreactive low affinity antibodies) and **pathological RF's** (monoreactive and of high affinity). The pathological RF's include the monoclonal RF present in Waldenström's macroglobulinaemia and chronic lymphocytic leukaemia (they are associated with cryoglobulins) and polyclonal RF present in inflammatory and infectious disease.

INDICATIONS FOR MEASUREMENT

RF IgM is (together with inflammatory markers and anti-CCP) the blood marker of choice for the diagnosis of RA. Unfortunately, sensitivity is low (50% of early and 25% of well-established RA are seronegative) and specificity is poor *(see table below)*.

A positive RF must, therefore, always be subject to careful interpretation.

Prevalence of positive rheumatoid factor (%)

Rheumatoid arthritis	60 - 80
Sjögren's syndrome	70 - 90
Systemic lupus erythematosus	25 - 40
Scleroderma	20 - 30
Polyarteritis nodosa	10 - 20
Lymphoproliferative syndrome	10 - 20
Pulmonary fibrosis	10 - 30
Autoimmune liver disease	10 - 50
Infective endocarditis	30 - 50
Leishmaniasis	50 - 80
Chronic Hepatitis C	50 - 75
Syphilis	15 - 25
Viral infections (EBV...)	20 - 60
"Normal" subjects < 30 years	1
30-65 years	5
> 65 years	15

INFORMATION

■ SAMPLE

Serum (Dry tube). Haemolysed or lipaemic serum must be discarded. A fasting sample is not necessary.

Separate the serum within 12 hours of taking the specimen.

■ SAMPLE STORAGE AND TRANSPORT

Store and transport at + 4°C or frozen at – 20°C. Avoid repeated freezing and thawing.

ASSAY METHODS

There are many:

Rose – Waaler

This is a passive haemagglutination method using sheep red blood cells coated with rabbit IgG anti-sheep red cell antibodies. This requires the use of a control with non-sensitised red cells to avoid false positives resulting from the presence of heterophile antibodies. The titre obtained by dilution on slide or microplate (preferred) is converted to IU/ml by employing a WHO standard.

Latex

This agglutination test employs particles coated with human IgG. It is easily performed on a slide but has to be combined with another test because of the high false positive rate.

Nephelometry

This measures the intensity of light scattered by the complex formed by sensitised polystyrene particles and rheumatoid factor. The immunoglobulins bound to the polystyrene can be human, animal or a mixture. The method can be automated and it is reproducible, linear and sensitive. Because of the considerable dilution of the serum, it is not subject to certain interfering factors.

Turbidimetry

This depends on a principle similar to that for nephelometry, but transmitted rather than scattered light is measured. It is slightly less sensitive but has the advantage of being adaptable to certain automated biochemical analysers.

ELISA

Immunoglobulin G (human or animal) is fixed on a micro-plate. The presence of rheumatoid factor translates into a colorimetric reaction proportional to its level. ELISA has the same virtues as nephelometry with a small advantage in terms of sensitivity and specificity. Isotypes can also be measured.

IgM class rheumatoid factor is best correlated with RA.

IgA class rheumatoid factor is noted by some authors to be associated with more aggressive RA. Specificity is poor.

IgG class rheumatoid factor has not been shown to have significance.

Dot blot

This is an ELISA method transposed to a membrane. Reading is qualitative and any positive serum has to be retested to determine the titre.

Test performance characteristics:

	Sensitivity	Specificity
Rose Waaler	65%	90%
Latex	75%	75%
Nephelometry	80%	90%
ELISA, human Ig	85%	80%
ELISA, animal Ig	75%	90%

UNITS AND REFERENCE VALUES

Whatever method is used, the threshold of positivity is approximately 20 IU/ml.

A concentration < 50 IU/ml is weakly positive and > 100 IU/ml is elevated.

In 10% of cases there is a discrepancy, with positive anti-human Ig RF positive and anti-animal Ig RF negative. Such discrepant results need to be considered in the clinical context.

In RA, we expect positivity in 2 tests with concentrations above 50 IU/ml.

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RICKETTSIOSIS

DEFINITION

The rickettsioses are zoonoses found widely throughout the entire world caused by bacteria called Rickettsiae which belong to the *Rickettsiaceae* family and are transmitted by arthropods. Rickettsiae are very small immobile bacilli, with a wall structure similar to that of Gram-negative bacteria. They are obligate intracellular organisms and are very fragile in the external environment.

There are 2 groups of rickettsioses which are classified depending on the vector involved:

– The typhus group transmitted by fleas and lice which include: *R. prowasekii*, the exanthematous typhus agent, *R. typhi* which causes murine typhus and *R. canada* the new typhus agent.

– The spotted fever group transmitted by ticks, which includes the following major species: *R. conorii*, the Mediterranean spotted fever (boutonneuse fever), *R. rickettsii* responsible for Rocky Mountain spotted fever, *R. africae*, the agent which causes African tick-borne fever, *R. akari*, the agent responsible for Indiana fever, *R. australis*, which causes a tick-borne rickettsiosis similar to classical Mediterranean spotted fever.

INTRODUCTION

EPIDEMIOLOGY

The Rickettsiae are transmitted to human beings from the bite of an infected arthropod which acts as the vector for the disease (ticks, fleas, lice and mites). They have a clearly defined geographical distribution and method of epidemiological spread, depending on the species *(see table)*. In France, *R. conorii* is seen in the South-East, *R. slovaca* and other species imported accidentally.

Disease	Bacterium	Vector	Reservoir	Geographical location
Mediterranean spotted fever	*R. conorii*	Dog ticks	Dogs and rodents	Mediterranean basin, Black Africa, Middle-East, Europe
Rocky Mountain spotted fever	*R. rickettsii*	Wood and other ticks	Dogs, rodents	North and South America
Vesicular fever	*R. akari*	Rat ticks	Rat	Africa and the United States
Trench fever	*R. quintana*	Lice	Human beings	Europe, Mexico
Other tick-borne fevers	*R. siberica* *R. australis* *R. pijperi*	Ticks	Rodents, dogs, marsupials	Siberia, Australia Austral Africa
Q fever	*Coxiella burnetii*	Transmitted through the respiratory or gastro-intestinal tract, ticks	Wild and domestic mammals, but also birds	Cosmopolitan distribution
Exanthematous typhus	*R. prowazeki*	Lice	Human beings	Cosmopolitan
Murine typhus	*R. typhi*	Rat ticks	Rat	Cosmopolitan
Scrub typhus	*Orientia tsutsugamushi*	Trombicula larvae	Wild rodents	South-East Asia

In: Gentilini M. Rickettsioses. Médecine tropicale (Flammarion Paris) 6th Edition, 2001: p377.

PATHOPHYSIOLOGY

The Rickettsiae often multiply at the inoculation site, causing a sore. They have trophism for the small blood vessel endothelial cells and cause vasculitis and thrombosis. Perivascular inflammation and perivasculitis causes visceral damage and features of encephalitis.

SYMPTOMS

The rickettsioses take on many varied clinical forms. After an average incubation period of one to two weeks it presents with an acute flu-like syndrome with high fever followed by a skin rash, occasionally associated with an inoculation sore (black spot). Visceral, neurological, pulmonary and gastro-intestinal features are often present.

- **Exanthematous typhus** develops endemically due to adverse hygiene conditions. It starts with a fever of 40°C and headaches. The established disease phase is an association of typhus (stupor and prostration) and macular or maculopetechial exanthema.
- **Murine typhus** is endemic and sporadic. Clinical features are similar to those of exanthemous typhus with a combination of a macular rash and lesser degree of typhus.
- **Mediterranean spotted fever** is contracted by human beings during the summer period following a bite from an infested tick. Symptoms involve sudden onset high fever, diffuse pains (headaches, myalgia, and arthralgia) which may or may not be associated with an inoculation sore, still called a "black spot", as it contains a necrotic black centre and an erythematous edge. The maculo-papular erythema then becomes generalised in flares progressing to cover the whole body including the face, palms and plantar surfaces of the feet. It generally resolves spontaneously. Complications may occur in the elderly, malnourished or immunosuppressed.
- **Rocky Mountain spotted fever** has a sudden onset with high fever, headaches and myalgia. The established disease phase is a combination of severe infection and exanthema becoming maculo-petechial and progressing in successive flares from the wrists to the whole body. Complications are rare as a result of antibiotic therapy.

SEARCH INDICATIONS

Diagnosis of rickettsiosis in the presence of a skin rash, particularly if associated with an inoculation sore following an arthropod bite in an endemic area.

Diagnosis of rickettsiosis from clinical and, particularly, epidemiological features.

Differential laboratory diagnosis between other species of Rickettsiae.

INFORMATION

SAMPLE

Two serum samples taken 15 days apart for serological diagnosis.

Plasma samples or blood cultures for direct diagnosis by isolation of the organism or detection by gene amplification.

Skin biopsies for diagnosis by gene amplification or isolation of the organism.

■ QUESTIONS FOR THE PATIENT

Clinical symptoms (looking for an inoculation skin sore)?

Place of residence or stay in endemic area?

History of arthropod sting or bite?

Current antibiotic treatment?

■ SAMPLE STORAGE AND TRANSPORT

Samples can be transported at room temperature for a period not exceeding 24 hours. Beyond this time they should be frozen at -20°C. Serum samples can be stored at + 4°C for 1 week and then frozen at – 30°C for 1 year.

DIAGNOSTIC METHODS

■ GUIDING DIAGNOSIS

– Thrombocytopaenia.

– Initial leukopaenia then leukocytosis with lymphocytosis.

– Increased ESR.

– Mild rise in transaminases.

■ DIRECT BACTERIOLOGICAL DIAGNOSIS

This is performed in France by the National Reference Centre.

- **Immunodetection:** Rickettsiae may be identified directly by immunodetection on a skin biopsy, and *R. conorii* identified on circulating endothelial cells after immunoseparation. This is an immunofluorescence or immunoperoxidase method using monoclonal antibodies.
- **Isolation of the organism:** This can be performed in specialist laboratories from blood or skin biopsies using a centrifugation method on the pathological material. Strains of Rickettsia belonging to the typhus or spotted fever group can be identified by immunofluorescence using monoclonal antibodies.
- **Molecular biology:** Rickettsia DNA is identified by gene amplification from the leukocyte layer isolated from blood samples or from skin biopsies. Most of the strains of spotted fever or typhus groups of Rickettsiae are identified by analysing the RNA 16 S gene sequences.

■ SEROLOGICAL DIAGNOSIS

The reference method is indirect immunofluorescence which establishes total Ig and IgM titres. Other methods are available including the complement fixation reaction, haemagglutination inhibition and ELISA. Many cross-reactions occur between spotted fever and typhus group species and therefore IIF methods are needed after cross-absorption of serum, or alternatively Western blot to identify the species responsible.

In practice, the confirmatory diagnosis is obtained on 2 serological samples taken 2 weeks apart.

TREATMENT

Rickettsiae are obligate intracellular bacteria. As a result they are only sensitive to antibiotics which offer good intracellular penetration, such as the tetracyclines, fluoroquinolones, macrolides (josamycin), azithromycin and chloramphenicol. The usual treatment is with doxycy¬cline, dose 200 mg/day for 7 days.

Prophylaxis involves the use of repellents and long clothing to protect against arthropod bites.

FOR FURTHER INFORMATION

■ Gentilini M. Rickettsioses. Médecine tropicale, Flammarion Paris 6e tirage, 2001: p. 376 à 383.

■ *Référentiel en microbiologie : rickettsies et bactéries apparentées*, 2000: p111 à 113.

RIFAMPICIN

DEFINITION

Rifampicin is an anti-tuberculous antibiotic belonging to the rifamycin family. It is indicated for use in multiple drug therapy for the curative treatment of all forms of tuberculosis and as mono or dual therapy for chemoprophylaxis. It is also used to treat other infections due to sensitive mycobacteria, leprosy, brucellosis and some serious hospital acquired infections and for the chemoprophylaxis of meningococcal meningitis.

Treatment of tuberculosis

Two treatment regimens are conventionally used:

Isoniazid (INH), rifampicin and ethambutol for 2 months followed by INH and rifampicin for 7 months.

INH, rifampicin, ethambutol and pyrazinamide for 2 months followed by INH and rifampicin for 4 months.

Rifampicin is administered:

– Intravenously (one infusion/day), dose 10 mg/kg/d in adults and 15 mg/kg/d in children between 1 month and 7 months old and 10 mg/kg/d in the newborn.

– Orally (one dose/day), dose 8 to 12 mg/kg/d in adults and 10 to 20 mg/kg/d in children between 1 month and 7 years and 10 mg/kg/d in the newborn.

It is recommended that the capsules and oral suspension be taken fasting at least half an hour before a meal (food reduces the bioavailability of rifampicin).

PHARMACOKINETICS

Bioavailability (oral, fasting)	Almost 100% in adults (capsules), approximately 50% in children between 3 and 36 months old (oral suspension)
Plasma peak	2 to 3 hours (after fasting administration of a single dose of 600 mg)
Time to steady state	From the second week of treatment
Protein binding	80%, mostly through albumin
Metabolism	Mostly converted into desacetyl-rifampicin which is active. Rifampicin is a hepatic enzyme inducer and also induces its own metabolism.
Elimination	80%, in bile
Half-life of	1.5 to 3 hours. Elimination is increased with functional renal insufficiency if the dose exceeds 600 mg/d and in liver dysfunction.

INDICATIONS FOR MEASUREMENT

Measurement is of no use in sensitive tuberculosis in the absence of intercurrent disease. It is however recommended if cultures remain positive after treatment for two months ("non-responder" patients).

Therapeutic monitoring is used to confirm that plasma concentrations are effective in situations where a risk of underdosing exist:

– Severe concomitant disease (particularly cystic fibrosis, diabetes, gastro-intestinal disease).

– Infection with *Mycobacterium* avium or with a multi-resistant organism.

– HIV patients: oral absorption of the anti-tuberculous drugs may be reduced in these people, the incidence of *M. avium* infections is high and plasma rifampicin concentrations may be altered by the antiproteases.

– Tuberculous meningitis.

– Therapeutic interactions associated with imidazole antifungals *(cf. below)* and pyrazinamide.

– Suspected poor adherence to treatment.

Therapeutic monitoring is also used to confirm that the drug has not accumulated, producing a risk of toxicity. This is seen in patients with liver dysfunction or renal insufficiency. Measurements are also recommended if signs of toxicity such as sweating, vomiting, red discolouration of the integument and urine or moderate increase in alkaline phophatase and transaminase develop. Therapeutic monitoring is recommended systematically in children.

INFORMATION

SAMPLE

Preferably serum: heparinised plasma may be used; avoid tubes with separator gel.

Two types of sample may be taken:

– A sample taken immediately before the next dose of the drug: trough concentration (C0).

– A sample taken 3 hours after the oral dose or at the end of an infusion (Cmax).

QUESTIONS FOR THE PATIENT

Are you taking any other medical drugs?

Many therapeutic interactions occur with rifampicin, usually because of its potent enzyme-inducing effect. Patients should be alerted to this risk.

Therapeutic interactions to consider when interpreting plasma concentrations are those involving:

– An imidazol anti-fungal, such as ketoconazole, itraconazole and fluconazole. Reduced plasma rifampicin concentrations due to reduced intestinal absorption.

Pyrazinamide increases rifampicin clearance.

Any request for drug measurement must include the reasons for the request (testing for efficacy or toxicity), the sampling time, treatment start date and/or any change in dosage, dosage information (amount administered, frequency and route of administration) and the age, height and weight of the person whenever possible.

SAMPLE STORAGE AND TRANSPORT

Serum or plasma can be stored at +4°C for a few days beyond which time it should be frozen.

Transport at + 4°C or frozen.

ASSAY METHODS

HPLC method.

NORMAL EXPECTED VALUES

The therapeutic range for rifampicin 3 hours post-dose (Cmax) at steady state is between 3 and 15 mg/l.

After intravenous administration the therapeutic range depends on the dose administered and length of the infusion. For reference, the target Cmax is approximately 10 mg/l for a dose of 600 mg administered as a 1 h 30 min infusion.

FOR FURTHER INFORMATION

■ Dictionnaire Vidal® .

■ Limosin A., Bouquet S., Le Guellec C., Rey E., Venisse N., *Suivi thérapeutique de la rifampicine*. In: Suivi thérapeutique pharmacologique pour l'adaptation de posologie des médicaments. Collection Option/Bio, Ed Elsevier, Paris. 2004: 105-14.

ROTAVIRUS

DEFINITION

The rotaviruses belong to the *Reoviridae* family and *Rotavirus* genus.

They are small double strand RNA viruses (75 nm in diameter), with a genome consisting of 11 segments. They are unenveloped viruses with an icosahedric capsid consisting of 3 circular protein layers, giving them the appearance of a wheel (hence the name rotavirus).

The rotaviruses are very common in animals, particularly in mammals. They are classified into 7 different antigenic groups (from A to G).

Most of the human rotaviruses belong to group A and are responsible for the majority of cases of acute infant gastro-enteritis (between the ages of 6 months and 2 years old).

INTRODUCTION

EPIDEMIOLOGY

The virus is ubiquitous. Infections occur epidemically in winter in temperate regions (from November to March with a peak in December-January). Direct or indirect transmission is via faeco-oral transmission between human beings. Once inside the body the virus multiplies in small bile enterocytes, causing the diarrhoea.

SYMPTOMS

After a short incubation period of 24 to 48 hours, clinical signs of watery diarrhoea without mucus or blood, vomiting and fever develop. Attenuated or even asymptomatic forms of the infection also occur. The main risk however is that of dehydration which occurs in severe disease and often requires hospitalisation.

Occasional cases of adult rotavirus gastroenteritis which is generally pauci-symptomatic are also seen.

These are more common in the elderly and in the immunosuppressed. Reinfections may occur in all cases.

The involvement of rotaviruses in pneumonia and in neurological disease is very contentious.

SEARCH INDICATIONS

– Diagnosis of acute gastro-enteritis in infants and young children, specifically in the context of dehydration.

– In nosocomial studies, rotaviruses are the leading cause of nosocomial infections in paediatric departments.

INFORMATION

SAMPLE

A stool collection is essential and is used for direct detection of the virus. The stool sample is obtained during the acute phase of the disease by placing 2 to 3 g (or ml) in a sterile hermetically sealed container.

QUESTIONS FOR THE PATIENT

Age?

Signs of dehydration?

Clinical features with the diarrhoea?

Treatment?

SAMPLE STORAGE AND TRANSPORT

The stool sample can be transported at room temperature or stored at + 4°C for 72 hours.

It may be frozen at -30°C to be stored for longer periods.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

The specific diagnosis is direct and is performed in the acute phase of the disease because of the large number of viruses excreted in stools.

- **Rapid antigen detection in stool:** This uses antibodies directed against the VP6 protein group of group A rotaviruses allowing these to be detected specifically.

  The methods used are immunoenzymatic or latex particle agglutination.

Immunoenzymatic methods: These are performed on plates or beads that are suitable for large batches. They offer excellent specificity and sensitivity.

Single membrane tests are also available and have the advantage of providing a very rapid answer in 10 to 15 seconds.

Latex particle agglutination methods: These use antibodies against rotaviruses (anti-VP6) bound to latex particles. They are simple to perform single sample tests which produce a very rapid answer. On the other hand their sensitivity and specificity are lower than for the EIA methods.

- **Amplification methods:**

Cell culture is difficult and is not therefore performed.

Molecular biology: RT-PCR methods are used to determine rotavirus genotypes and for environmental testing.

- **Other methods:**

Viral RNA polyacrylamide gel electrophoresis: This is performed in an epidemiological context to differentiate between strains of rotaviruses.

Electron microscopy: This is the reference method which is reserved for laboratories equipped and trained to read the micographs.

Serology (complement fixation reaction) is useful in epidemiological studies.

INTERPRETATION

The laboratory diagnosis of rotavirus infection is based mostly on detecting the virus in stools, which is a simple and fast method.

Interpretation of the result, however, is not always straightforward. Caution is required if a laboratory result is negative despite suggestive symptoms; similarly if a rotavirus is found in a stool sample when clinical features are not gastro-intestinal.

TREATMENT

Treatment is preventative and involves strict hygiene measures. Oral rehydration solutions can be used to correct dehydration. There is no specific antiviral treatment.

FOR FURTHER INFORMATION

■ Garbarg-Chenon A., *Rotavirus,* Encycl. Med. Chir., Elsevier, Paris, 2003.

■ Groupe- Révir, *Rotavirus*. In : Société Française de Microbiologie (ed). LE REVIR, référentiel en virologie médicale, Montmorency; 2M2, 2000: 119-121.

RUBELLA

DEFINITION

Rubella is an eruptive disease of childhood due to a virus belonging to the *Togaviridae* family, *Rubivirus* genus, which develops either sporadically or in epidemics, mostly during the spring. It is usually benign and its importance is due to the risk of congenital malformations in children born to women who are infected during the initial months of pregnancy.

Vaccination is strongly recommended in infants during their second year of life with a booster between 3 and 6 years old, and in women of child-bearing age.

INTRODUCTION

EPIDEMIOLOGY

Approximately 95% of women of child-bearing age in France are seropositive for rubella. Despite effective vaccination the virus continues to circulate, although the incidence of primary rubella infections during pregnancy is low, less than 1 per 100,000 births in France in 2006.

SYMPTOMS

The virus is transmitted via the respiratory tract following direct human-human contact.

Primary rubella infection

Rash accompanied by lymphadenopathy develops after an incubation period of approximately 15 days. Rubella, however, is asymptomatic in approximately 50% of cases. Its evolution has a good clinical outcome but complications (which are rare) are mostly arthralgia and very rarely encephalitis or thrombocytopaenia.

Reinfection

Recovery from rubella usually leaves long-term immunity. Reinfections (asymptomatic) may however occur. The risk of foetal malformations from reinfection during pregnancy is extremely low, although does exist. Around thirty cases of congenital rubella have been published in the literature out of tens of thousands of rubella reinfections.

Congenital rubella

The risk of foetal infection is extremely high at the start of pregnancy (60 to 90%) and then falls during the second trimester (25 to 50%) rising again at the end of pregnancy (100%). The incidence of congenital abnormalities varies by gestational age *(cf. figure 1)*.

Figure 1: Risks of foetal damage

	12th	18th-20th	*Weeks of pregnancy*
Risk: > 80%	Risk: 80 ± 10%	Risk: almost zero	
Possible damage: - neurological - ophthalmological - auditory - cardiac	Possible damage: - auditory		

SEROLOGICAL AND BIOLOGICAL MARKERS

– The virus is present in the pharynx eight days before the rash (contagious period).

– Viraemia is positive for one week before the rash and antibodies become detectable at the time of the rash when the viraemia becomes negative.

– In primary infection, specific IgM develops two weeks after infection and disappears over a variable period (generally 3 to 8 weeks) depending on the person and on assay methods. They are variably present between weeks 8 and 15 *(cf. figure 2)*.

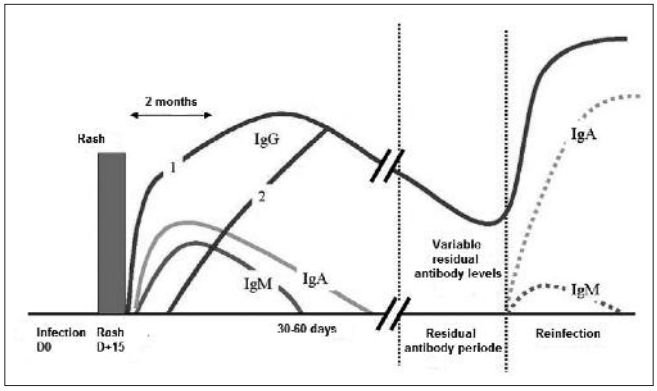

Figure 2 Kinetics of antibodies detected by Elisa during rubella acquired after birth.
Kinetics of IgG varies depending on the person and methods used.

SEARCH INDICATIONS

Determination of immune status– diagnosis of primary postnatal infection: serology

Systematic screening is based only on measurement of specific IgG (or total antibodies). This is mandatory in all pregnant women as early as possible during the pregnancy.

Testing for or confirmation of primary rubella infection is indicated in the following situations during pregnancy:

– contact with an infected person;

– suggestive symptoms or ultrasound abnormalities;

– seroconversion or significant rise in antibody titres (x 2);

A repeated serology test is recommended at 20 weeks of pregnancy (WOP) in non-immunised pregnant women; beyond this period the risks of malformation are almost zero.

When to request specific IgM?

– In seroconversion (seronegative female patient, becoming seropositive 3 weeks later) or significant rise in IgG titre (x 2), to confirm primary infection;

– following contact with an affected person or for a suspicious rash even if IgG titres are stable (**NB:** the IgG plateau may be reached extremely early).

– in diagnosis of antenatal infection: foetal blood, sample taken from 22 WOP under ultrasound guidance;

– in the diagnosis of postnatal infection.

INFORMATION

■ SAMPLES

– Serodiagnosis: Blood collected into a dry tube (an EDTA tube may be used to test for IgG). A fasting sample is not required. If the antibody test is to be performed on foetal blood, ensure that no contamination occurs from maternal blood or amniotic fluid (take a maternal blood sample in parallel).

– Amniotic fluid sample: After 18 WOP and a minimum of 6 weeks after maternal seroconversion to test for viral genome by PCR. The patient's informed consent and certification of the consultation must (in France) accompany the sample.

– Urine samples, pharyngeal secretions, CSF, placenta, for direct specific diagnosis (culture, PCR).

■ QUESTIONS FOR THE PATIENT

– Have you been vaccinated against rubella? If yes, give vaccination date.

– For women: are you pregnant (+ term of pregnancy)?

– Do you have a history of contact with an infected person or of a rubella-like rash?

– Was an initial serological test performed in another laboratory, if so when and what was the result?

■ SAMPLE STORAGE AND TRANSPORT

Store serum at + 4°C for up to 1 week and then freeze at – 20°C (1 year).

Amniotic fluid must be transported in dry ice for transport times exceeding a few hours. Store at – 80°C.

Pharyngeal secretions, urine, CSF, placenta must be transported at + 4°C.

ASSAY METHODS

■ **Serological diagnosis**

Assay of IgG: ELISA methods, haemagglutination inhibition, sensitised latex particle agglutination test (Total Ab).

Assay of IgM: ELISA immunocapture method or indirect method after absorption with rheumatoid factor.

■ **Culture:** on RK13, SIRC or Vero cells: detection of cytopathic activity (direct or indirect) or by immunofluorescence or PCR.

■ **PCR:** testing mostly for congenital infection by amplifying localised sequences in the E1 gene (coding for an envelope glycoprotein).

■ **Complementary method:** useful to help to date the infection in suspected primary infection during pregnancy: measurement of IgG avidity.

INTERPRETATION

■ SYSTEMATIC LABORATORY SCREENING FOR RUBELLA INFECTION

■ **First request for serology:**

– If IgG < threshold: IgG – absent – no immunity present.

In a pregnant woman, a repeat serology test is recommended at 20 WOP. Recommend vaccination after childbirth.

– If IgG > threshold: IgG present, supports immunity.

■ **Second sample, 3 to 4 weeks later:**

– Stable IgG titre: this is probably long-standing immunity although it is not possible to formally exclude recent infection, as the IgG plateau may be reached very early. This result must therefore be interpreted depending on the clinical and laboratory context.

– Significant rise in IgG titre: this serology should be interpreted as a function of the clinical context and by testing for specific IgM on the first sample.

If IgM is negative, primary infection can be excluded: this is serological reactivation. A positive IgM may indicate primary infection, vaccination, reinfection or polyclonal immune system stimulation. IgG avidity is required.

NB: high IgG titres are of no significance because of their large variability.

Interpretation of IgM

– IgM is usually associated with recent primary infection, although it is not however, specific for this.

– IgM is found for a very long time after vaccination, often for more than 6 months and even after 12 months.

– It is occasionally found in reinfections: titres are then generally low and transient.

– IgM is also found in polyclonal immune system stimulation particularly in herpes virus or parvovirus B19 infection.

Interpretation of further investigations performed to help to date the infection: measurement of IgG avidity

A low avidity index (threshold varies depending on the method used) supports recent infection (within 1 to 2 months); a high avidity index excludes recent infection within three months.

■ TESTING FOR RUBELLA INFECTION IN A HISTORY OF RECENT CONTACT WITH AN AFFECTED PERSON

■ **Initial request for serology (less than 10 days after contact): measurement of IgG**

– If IgG < threshold: a second serology sample taken 3 to 4 weeks later can confirm or exclude primary infection.

– If IgG > threshold: patient immunised – immunity present prior to contact.

■ **Second sample (3 to 4 weeks after the first sample): measurement of IgG and/or IgM**

– IgG < threshold: patient not immunised. No infection. Recommend vaccination after childbirth.

– IgG < threshold, IgM positive: sample too early; request a third sample to determine whether IgG develops.

– IgG > threshold, IgM negative: serological reactivation (IgG present at concentration below threshold in the first sample); primary infection can be excluded although this should be confirmed by IgG avidity.

– IgG > threshold, IgM positive: this may be primary rubella infection, reinfection (IgG present at concentrations below the threshold in the first sample) or polyclonal immune system stimulation. Confirm by IgG avidity.

■ LABORATORY TESTING FOR RUBELLA INFECTION IN A LATE SAMPLE AFTER CONTACT (> 15 DAYS) OR IF SYMPTOMS DEVELOP IN A PREGNANT WOMAN

■ **First sample (more than 15 days after contact with the infected person):** measurement of IgG and IgM:

– IgG < threshold, IgM negative: patient not immunised. No infection.

– IgG < threshold, IgM positive: request a second sample to check for development of IgG.

– IgG > threshold, IgM negative: old infection or infection more than 3 to 8 weeks previously.

– IgG > threshold, IgM positive: this may be primary rubella infection, reinfection (IgG present at concentrations below the threshold in the first sample) or polyclonal immune system stimulation. Confirm by IgG avidity.

■ TESTING FOR RUBELLA INFECTION IN THE PRESENCE OF SUGGESTIVE ULTRASOUND SIGNS

■ **First sample: when the abnormalities are detected**

– IgG < threshold, IgM negative: abnormalities not related to rubella infection.

– IgG > threshold, IgM negative: perform a serology control in a sample from the start of pregnancy and if: IgG > threshold, IgM negative on this sample from the start of pregnancy: immunity acquired before pregnancy.

– IgG > threshold, IgM positive: primary infection possible. Confirm by testing IgG avidity.

■ DIAGNOSIS OF ANTENATAL INFECTION

– Testing for specific IgM in foetal blood, taken from 22 WOP under ultrasound guidance.

– Testing for viral genome in amniotic fluid taken by amniocentesis after 18 WOP, a minimum of 6 weeks after seroconversion.

■ DIAGNOSIS OF POSTNATAL INFECTION

This is performed by testing for specific IgM in neonatal blood using an immunocapture method. The virus or its genome can also be tested in urine, pharyngeal secretions or cerebrospinal fluid by viral culture or PCR.

FOR FURTHER INFORMATION

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■ Grangeot-Keros L., *La rubéole et son sérodiagnostic*, Cahier de Formation Bioforma Sérologie, 1992: 7-26.

■ Grangeot-Keros L., *Mesure de l'avidité des IgG*, Encycl Méd Biol (Elsevier, Paris), 2003.

S100B PROTEIN

DEFINITION

S100B protein (name given due to its solubility characteristics) is a dimeric holoprotein with a molecular weight of 21 kDa, composed of two subunits (monodimer with two beta subunits, or heterodimer with one β-subunit and one α-subunit).

S100B is a cytosolic calcium-bonding protein synthesised by glial cells, Schwann cells, melanocytes, chondrocytes and adipocytes. It is present in cerebrospinal fluid and in plasma. Capable of interacting with various membrane proteins, it appears to play a major role in the development and maintenance of cerebral tissue. Its plasma half-life is 2 hours, with elimination taking place through the kidneys.

Synonyms: PS 100, S100 protein.

INDICATIONS FOR MEASUREMENT

– Malignant melanoma: Marker in prognosis and monitoring of treatment effectiveness (S100B protein is not a diagnostic marker).

– Cerebral lesions: Cerebral vascular accidents of an ischaemic nature, cranial trauma, meningeal haemorrhage and viral meningitis-encephalitis.

INFORMATION

■ SAMPLE

Dry or heparinated tube: CSF is collected in a dry tube.

Centrifuge and decant the plasma (or serum) rapidly after collection.

■ ESSENTIAL INFORMATION

Administration of biotin (> 5 mg/day)?

Administration of monoclonal antibodies?

Diagnostic hypothesis?

■ SAMPLE STORAGE AND TRANSPORT

At room temperature for 24 hours. If the analysis is sent to a referral laboratory then freeze within 4 hours of collection.

NB: The measurement can be performed on haemolysed samples, while lactescent samples should generally be avoided.

Send samples frozen at -20°C to the referral laboratory.

ASSAY METHOD

Immunometric (sandwich) method.

NORMAL EXPECTED VALUES

Serum: < 0.15 µg/l

CSF: mean (m) = 0.047 µg/l

PATHOLOGICAL VARIATIONS

An elevation of the S100B protein concentration in serum or plasma has two principal causes: gene over expression, with increased release by astroglial cells and malignant cells, or release following brain cell lysis.

– S100B protein measurement is used as a prognostic factor: Alzheimer's disease, Creuzfeld-Jacob disease, prion disease, and multiple sclerosis (lack of sensitivity and specificity).

– S100B protein is released into the bloodstream in cases of brain tissue lesions in neurotrauma (road accidents). Values observed after 45 minutes: 5.2 µg/l; after 2 hours: 3.2 µg/l. It also rises in cases of cerebral damage as a result of cardiac surgery.

NB: According to certain authors, the absence of raised S100 protein after cranial trauma suggests a favourable prognosis.

– During malignant melanoma (stages 3 and 4), it is a marker for aggressiveness and response to treatment (m > 0.2 µg/l: increase depending on the location of metastases).

FOR FURTHER INFORMATION

■ Beaudeux J.L., *Protéine S-100 bêta*, Encyclopédie médicobiologique, Elsevier, Paris, 2003.

SALMONELLOSES

DEFINITION

Salmonelloses are diseases due to bacteria belonging to the *Enterobacteriaceae* family, *Salmonella* genus. Salmonellae cause 2 main types of infection: gastroenteritis due to food poisoning and typhoid and paratyphoid fevers. They are aerobic-facultative, anaerobic Gram negative bacilli. The *Salmonella* genus currently contains 2 species: *Salmonella enteritica* which is sub-divided into 6 sub-species and *Salmonella bongori*. Serovars are found within each of the sub-species characterised by their somatic (O) antigens, flagellar (H) and in some cases virulence (Vi) antigen. The serovars are commonly known by their antigenic form except for those belonging to the *Salmonella enteritica subsp. enteritica* sub-species which have a specific name.

INTRODUCTION

EPIDEMIOLOGY

The bacterium is transmitted to human beings mostly by drinking contaminated water or eating contaminated food which is either raw or poorly cooked (eggs, meat and particularly poultry, dairy product, raw vegetables, and shellfish), although transmission may occur directly from soiled hands, faeces and urine. The typhoid fevers particularly affect the developing countries with poor hygiene conditions (Africa, Latin America and Asia) and cause approximately 17 million cases annually throughout the world. The typhoid fevers, however, have not been completely eradicated from industrial countries and develop as small epidemics in people who have returned from travel. Community food poisoning infections may cause large outbreaks.

SYMPTOMS

- **Gastroenteritis:** This is caused by ubiquitous Salmonellae found in human beings and animals. The main species responsible are *S. typhimurium, S. enteritidis and S. panama*. It has an incubation period of between 12 and 36 hours depending on the amount ingested, the host's immune status and the strain of bacterium responsible. Symptoms are typically high fever with diarrhoea, abdominal pain and vomiting. The infection usually resolves in 3 to 5 days without treatment in healthy people. On the other hand it is more severe with extra gastro-intestinal forms (septicaemia) which is occasionally fatal in the immunosuppressed, pregnant women and infants.
- **Typhoid and paratyphoid fevers:** These are due to strictly human Salmonellae, *S. typhi, S. paratyphi A* and some strains of *S. paratyphi B. Following* an incubation period of between 1 and 2 weeks, a high fever of 39 - 40°C develops with headache, anorexia, abdominal pain, foetid ochre-coloured diarrhoea and nausea. Patients then develop splenomegaly, a skin rash and "typhus" which are characteristic of the disease and is a state of clouded consciousness or prostration. Latent and attenuated forms of the disease occur commonly.

With treatment the disease resolves after a convalescence period of several weeks. Without treatment, occasionally fatal gastro-intestinal, cardiac, meningeal, respiratory and osteoarticular complications may occur.

SEARCH INDICATIONS

Diagnosis of typhoid and paratyphoid fevers.

Diagnosis of Salmonella gastroenteritis.

Differential diagnosis from other bacterial or viral gastroenteritis.

INFORMATION

SAMPLE

Venous blood for blood cultures.

Stool collected into a sterile container for stool culture.

Other samples: CSF, urine and bile for direct diagnosis.

Serum for antibody testing.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

Have you eaten a potentially contaminated food or have any of your close contacts had food poisoning?

Recent travel to a country with poor hygiene?

Patient's immune status?

Current antibiotic therapy?

SAMPLE STORAGE AND TRANSPORT

Blood cultures must be transported as quickly as possible at room temperature. The bottles should be placed at 37°C in an incubator as soon as possible.

Stool samples, transported as quickly as possible and may be stored for 12 hours at a maximum of + 4°C.

Serum samples to be stored at + 4°C for up to one week and then frozen at – 30°C for one year.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

Isolation and identification of salmonellae.

Isolation of the pathogenic agent from sterile or low level contaminated pathological products is straightforward on ordinary media (Mueller-Hinton). Samples containing multiple microbes (faeces) require selected media for salmonellae and shigellae (SS medium or Hektoen medium).

Biochemical identification is performed by inseminating a standard gallery and enables a species diagnosis to be made.

O, H and Vi antigen identification is used to define the serovar of the Salmonella responsible.

INDIRECT DIAGNOSIS

The classical Widal and Félix serodiagnosis is restricted to typhoid and paratyphoid fevers and involves testing and titration of O and H agglutinin antibodies in the patient's serum. O agglutination occurs slowly (18 hours), is granular and stable at room temperature. The H antigen is derived from

the flagella; H agglutination occurs rapidly (2 hours) in large clouds at 37°C and is easily dissociable. The somatic Vi antigen is found variably and only in *S. typhi* and *S. paratyphi C.*

Classically, anti-O antibodies develop around day 8 of the disease, increase modestly until around week 3 and then disappear over 2 to 3 months. Anti-H antibodies can be detected slightly later at around day 12 and may reach high titres. They then fall very slowly and may persist for years. Vi antibodies are detectable late, limiting their use.

Testing is also possible for so-called minor Salmonella antibodies, particularly *S. enteritidis* and *S. typhimurium*.

INTERPRETATION OF RESULTS

TYPHOID AND PARATYPHOID FEVERS

The blood culture is positive in 90% of cases during the first week of the disease and in 50% of cases at the end of the 3rd week.

Stool cultures are negative at the beginning of the first week and the most reliable diagnosis is obtained between weeks 3 and 5. Five to 10% of patients still excrete bacilli 2 or 3 months after the onset of the disease and 3% after over a year. These are the chronic carriers.

The Widal and Félix serodiagnosis is generally negative during the first week of the disease. It is straightforward to interpret if the anti-O and anti-H titres are high at the same time during a fever: this indicates recent or current infection. Interpretation can occasionally be difficult as the usual kinetics can be altered by various factors:

– early antibiotic treatment can prevent the development of anti-O antibodies;

– TAB vaccination triggers development of anti-O and anti-H with assistance of anti-H alone, at a titre of 100 or 200 for years. AH agglutinins can be unreliable as the vaccine contains less A than T and B;

– anti-O and anti-H antibodies can develop during *Yersinia* and *Brucella*, infections, some rickettsioses, *Candida* infections and acute malaria;

– false positive reactions are seen in some diseases: bowel neoplasia, malignant blood dyscrasias and connective tissue diseases.

MINOR SALMONELLOSES

The diagnosis is made primarily by isolating the bacterium (mostly from stool culture). Testing for antibodies against the most commonly seen minor Salmonellae may be useful retrospectively, although is only a guide.

TREATMENT

CURATIVE

The treatment of typhoid fever is with antibiotics. Because of the increasing resistance of the strains which are isolated it has become essential to perform an antibiotic sensitivity profile as inappropriate treatment can also promote chronic carrier status.

In gastroenteritis due to minor Salmonellae, antibiotic therapy is only prescribed for severe disease in infants, the elderly, pregnant women or the immunosuppressed.

PREVENTIVE

General measures

Hygiene measures: Observe the cold chain, test foods and drinking water and avoid sources of faeco-oral transmission.

National epidemiological surveillance: monitoring of circulating serotypes and antibiotic resistance, testing for chronic carriers (kitchen and food industry staff).

Individual measures

Vaccination against typhoid fever in France is mandatory for occupationally exposed people and the military. The TAB vaccine is poorly tolerated and its effectiveness is very widely debated and has now been replaced by the Typhim Vi© vaccine which is better tolerated and which offers 60% protection in endemic areas (only against *S. typhi*).

POUR EN SAVOIR PLUS

Avril J.L., Dabernat H., Denis F., Monteil H., *Bactériologie clinique : salmonelles,* 3e édition, Paris, Ellipses; 2000 : 189 à 207.

SARS

DEFINITION

The Severe Acute Respiratory Syndrome (SARS) is the first serious transmissible disease of the 21st century. It was initially discovered in south-east Asia in part of China at the end of 2002 and spread rapidly, causing a worldwide epidemic in 2003 with more than 8000 cases and almost 800 deaths. Thanks to unprecedented international action following the WHO worldwide alert of 12 March 2003, the epidemic was contained with isolation and quarantine measures. At the same time, the agent responsible for SARS, a completely unknown Coronavirus (SARS-CoV) was quickly identified. This is an RNA virus which belongs to the Nidoviral order, *Coronaviridae* family and *Coronavirus*. The Coronaviruses infect a large number of animal species and are responsible for pulmonary and intestinal infections. Human beings can also be infected, through faecal-oral or respiratory transmission. The *Coronavirus* genus is divided into 3 groups:

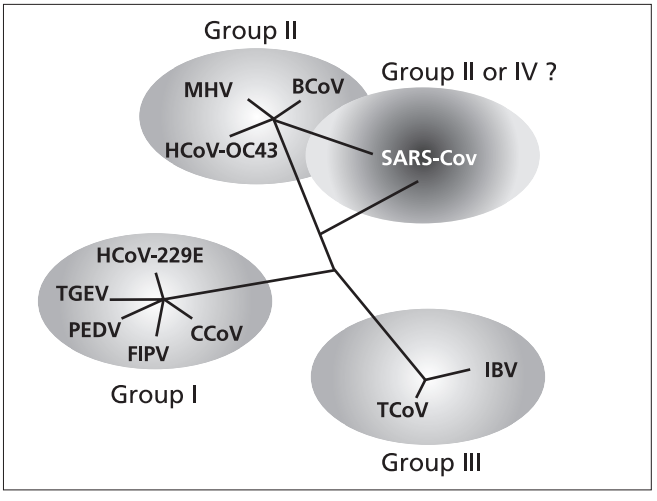
Group I contains different species: *human Coronavirus* 229E (HCoV-229E), *transmissible gastroenteritis virus* (TGEV), *porcine epidemic diarrhoea virus* (PEDV), *canine Coronavirus* (CCoV) and *feline Coronavirus* (FIPV).

Group II contains *human Coronavirus* OC43 (HCoV-OC43), *murine hepatitis virus* (MHV) and *bovine Coronavirus* (BCoV).

Group III contains (TCoV) found in turkeys and (IBV) in aviaries.

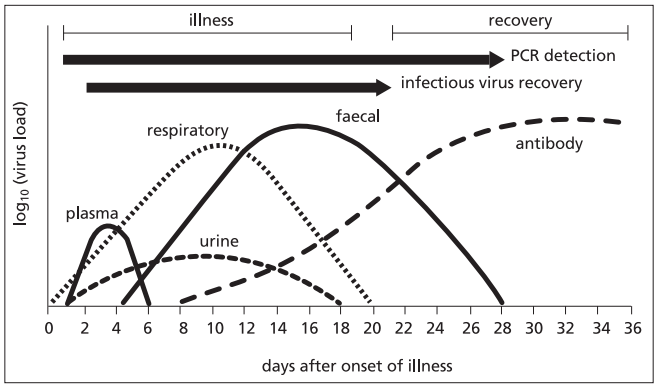
The animal reservoir of the SARS Coronavirus is believed to be the bat. The virus is thought to have spread to human beings via cats and/or civets (wild animals sold in markets and eaten in Southern China).

SARS is an extremely serious threat firstly because no vaccine or treatment exists and secondly because the virus mutates frequently, suggesting that outbreaks will come in the future and making vaccine development difficult.



SYMPTOMS

The symptoms of SARS are relatively non-specific and can also be seen in other respiratory infections. The syndrome begins with sudden-onset fever (over 38°C) generally associated with respiratory signs (dry cough, breathlessness and breathing difficulties). Other symptoms including headache, muscle pain, diarrhoea and general malaise may be present.



EPIDEMIOLOGY

SARS spreads through direct close contact (droplet transmission from sneezing and coughing) with an infected person. Indirect transmission may also occur from soiled objects as the virus is present in faeces and urine.

People suffering from SARS do not appear to be contagious before clinical symptoms develop, which may occur up to 10 days after contact with an affected person.

INDICATIONS FOR BIOLOGICAL DIAGNOSIS

Risk factors for the disease are shown below:

– Close contact with a likely case of SARS within the previous 10 days.

– Travel in an "affected area" declared to the World Health Organisation (WHO) in Asia during the previous 10 days.

– Travel or visit to an establishment in which cases of SARS have been declared (e.g. a hospital) in the previous 10 days.

Definitions of SARS cases have been produced based on WHO recommendations in order to standardise international data. The following are defined:

- **Possible cases:** all people with all of the following signs: fever > 38°C associated with one or more signs of lower respiratory tract disease (cough, dyspnoea, breathing discomfort, auscultatory abnormalities, radiological abnormalities if the chest radiograph has already been performed or loss of digital oxygen saturation if performed) and history within 10 days of the onset of signs of either staying in an area deemed by the WHO to be an area in which local active transmission of SARS occurs (consult http://www.who.int/csr/sarsareas), or close contact with a probable case.
- **Probable cases:** all cases with radiological or pulmonary CT signs of pneumonia in the absence of another diagnosis.

- **Excluded cases:** all possible cases in which symptoms are explained by an alternative diagnosis or who meet the following 4 criteria: good clinical state, no abnormality on chest radiography or CT scan, no drop in lymphocyte count, no contact with a probable case.

  A contact is a person exposed to a possible or probable SARS case who may therefore be at increased risk of contracting SARS. Situations of exposure to a risk include having provided care to a possible or probable SARS case without effective protective measure, or having been in close contact with respiratory secretions or biological fluids from a possible or probable SARS case.

INFORMATION

SAMPLE

Nasopharyngeal samples: for molecular biology testing.

Serum: for serological tests.

DIAGNOSTIC METHODS

The diagnosis is based on epidemiological, clinical, laboratory and radiological criteria.

As soon as patients arrive in hospital the virus must be tested for directly in expectoration by RT- PCR or cell culture (Vero cells), which is reserved for specialist laboratories.

Conventional serological methods (ELISA or Immunofluorescence) can be used to reconsider the diagnosis of unidentified pneumonia.

INTERPRETATION

Only small amounts of the SARS virus are excreted at the start of the disease. Peak excretion occurs approximately 10 days after the onset of clinical signs. The virus (or its genome) is then found in respiratory secretions and faeces. It is only found by RT-PCR in nasopharyngeal secretions, however, in 32% of cases in the initial phases and in 68% of cases, two weeks after the onset of clinical signs. RT-PCR is positive later in faeces, in 98% of patients (after 14 days) and in urine (after 15 days).

Serologically, IgG + IgM are found by ELISA approximately 21 days after the onset of clinical signs. Antibodies (IgG, M or a mixture of both) are found from day 10 after the onset of the disease by IIF on SARS virus-infected cells fixed onto a slide. Dilutions of patient's sera are used to provide a quantitative result. A neutralisation test has been developed although needs to be performed in a BSL level 3 confinement laboratory.

TREATMENT

Apart from isolating patients and protecting care staff, the treatment of SARS is symptomatic. Antibiotics may be required initially if bacterial causes of pneumonia have not been excluded. Oxygen and corticosteroid therapy may be considered depending on the risk of acute respiratory distress syndrome. Ribavirin, which was used in 2003, is now questioned.

FOR FURTHER INFORMATION

- Chan Paul K.S. et *al.*, *Laboratory Diagnosis of SARS 2004*, Emerging Infectious Diseases. 10 : 825-831.
- Institut de Veille Sanitaire : http://www.invs.sante.fr
- Organisation Mondiale de la Santé : http://www.who.int
- Centers for Disease Control : http://www.cdc.gov
- http://www.sarsreference.com

SCC

DEFINITION

SCC *(Squamous Cell Carcinoma)* is a glycoprotein subfraction of tumour antigen TA-4, purified from cervical squamous carcinoma cells and recognised by polyclonal antibodies. SCC is expressed in squamous tissues.

Synonym: TA-4.

INDICATIONS FOR MEASUREMENT

SCC is a laboratory marker of squamous cell carcinomas and the serum marker of choice for cervical pavement epithelium carcinomas. It has prognostic use and is used to monitor treatment in patients suffering from this type of cancer.

INFORMATION

SAMPLE

Serum or plasma collected into EDTA or heparin, not haemolysed – refer to the recommendations for the method used. A fasting sample is not essential although hyperlipaemia can interfere with measurement.

Aspiration fluid (cyst, ascites, etc.).

QUESTIONS FOR THE PATIENT

Disease?

Current treatment (chemotherapy, radiotherapy and surgery), types and date of treatment?

SAMPLE STORAGE AND TRANSPORT

24 hours at + 4°C and at – 20°C beyond this time.

ASSAY METHOD

"Sandwich" immunometric method.

Human anti-mouse antibodies (HAMA), if present, may interfere with some assay methods.

NORMAL EXPECTED VALUES

Usual serum values at < 2 µg/l and may vary depending on the method used.

PATHOLOGICAL VARIATIONS:

RAISED SCC IN MALIGNANT DISEASE

Cervical squamous cancers

At diagnosis:

The sensitivity of SCC in the diagnosis of invasive cervical squamous carcinomas is between 40 and 55%, although only 7 to 18% for *in-situ* carcinomas. Serum SCC concentrations correlate with the initial stage of the disease (histological grade); a high concentration is a poor prognostic indicator.

During treatment monitoring:

After complete surgical excision, SCC returns to normal over 3 to 7 days. It is also used to monitor patients undergoing radiotherapy or chemotherapy.

Measurement is particularly useful for the early detection of recurrence or metastases. Serum concentrations raise an average of 1 to 3 months before clinical and/or radiological symptoms.

Squamous cell bronchial carcinomas

SCC has a sensitivity of 15% for localised disease and 45% for widespread disease in this situation. Because of this measurement is limited to post-treatment monitoring.

Head and neck squamous carcinomas

As above, SCC has low sensitivity in the region of 30 to 40%. Measurement is therefore limited to post-treatment monitoring.

Anal canal squamous carcinomas

SCC has a sensitivity of 45 to 55% at this site with a specificity of approximately 90%. Its main use is in the monitoring of these tumours (early detection of recurrence).

NON-SPECIFIC RISES

Modest rises (generally < 6 ng/ml) in serum SCC concentrations have been described in the following situations:

– Severe respiratory disease, such as sarcoidosis, tuberculosis, chronic bronchitis and emphysema.

– Benign gastro-intestinal diseases and hepatitis.

– Benign gynaecological diseases, such as endometriosis.

– Chronic renal insufficiency.

FOR FURTHER INFORMATION

Micke O., Prott F.J., Schafer U. et *al*., *The impact of squamous cell carcinoma antigen in the follow-up after radiotherapy in patients with cervical cancer*, Anticancer Res 2 000 ; 20 : 5 113-5.

SELENIUM

DEFINITION

Selenium (Se) is an essential trace element which is found widely in nature, generally associated with sulphur-containing minerals in the form of selenide or in volcanic rocks. It is extracted from the by-products obtained from the treatment and refining of some minerals, cements and petroleum and from many conversion industries (electronics, glass and ceramic production, rubber, plastics, pigments, lubricants and cosmetics industry). It is used widely in animal and human nutrition and in pharmacology. The selenium content of a food depends on the type of ground where it was produced and its protein concentration. Fish, shellfish, eggs, cereals, garlic and mushrooms contain more Se than fruits, vegetables and dairy products. Daily requirements in adults and adolescents are estimated to be 50-200 µg/d and range between 30 and 120 µg/d in children over 2 years old and are 1-2 µg/kg body weight/day in infants.

METABOLISM

The main route of entry in an industrial environment is through the lungs, although absorption depends on the form in which the selenium element exists. Similarly, gastro-intestinal absorption varies between compounds. It is greater for organic compounds (selenomethionine and selenocysteine) than for minerals (selenites and selenates). It can be absorbed through the skin.

Selenium is transported bound to red cells and plasma proteins. It is distributed in the liver, kidneys and spleen: 40 to 50% of the total body pool is incorporated into striated muscle in the form of selenomethionine.

The human body contains between 6 and 20 mg of selenium. Its metabolism varies depending on the chemical form; reduction of selenite to selenide by cellular glutathione, incorporation of selenide into selenoproteins via selenocysteine and methylation of selenide into various metabolites which are then removed. The half-lives of elimination vary depending on the chemical form. Elimination is triphasic (1 day, 8-20 days and 68-116 days) after ingestion of a therapeutic dose of selenite, although the route of elimination appears to vary depending on the amount absorbed. Elimination is via urinary, faecal and pulmonary routes, in the free or methylated form.

MECHANISM OF ACTION

Se is an essential constituent of glutathione peroxidise. The active site of this enzyme contains four selenium atoms in the form of selenocysteine. It is an intracellular cytoplasmic enzyme present in red cells and in other tissues involved in oxidative metabolism and in plasma and breast milk. It catalyses the reduction of many organic and inorganic peroxides into alcohol. Glutathione acts as the acceptor and the regeneration of glutathione reduced by the NADP-NADPH system is the determining stage in the reaction.

Glutathione peroxidase protects cell membranes and probably DNA from damage by oxidative metabolism. This anti-oxidant effect is optimised by its association with vitamin E and superoxide dismutase.

Through its anti-oxidant action, Se may play a protective role against the physiological processes of ageing and the development of some degenerative diseases (rheumatoid arthritis, degenerative cardiovascular diseases and tumours, etc.).

SYMPTOMS OF DEFICIENCY

The symptoms of deficiency are only described in extreme severe prolonged deficiency and involve:

– Necrotising focal cardiomyopathy progressing to sudden decompensation and cardiac arrest.

– Peripheral myopathy characterised by increased creatine phosphokinase, reduced muscle tone and conduction disorders.

– Changes in the integument (lightening of the hair, opacification of the nails).

- Macrocytic anaemia and neutropaenia.

Childhood cardiomyopathy known as Keshan's disease in China is attributed to selenium deficiency.

Deficiency may occur in people living in areas where the ground is poor in selenium and whose diet is low in protein, such as vegetarians, the elderly, pregnant or breastfeeding women, infants, patients following medical weight reduction regimes, patients receiving prolonged parenteral nutrition and those with malabsorption syndrome.

SYMPTOMS OF POISONING

No specific chronic disease has been described in human beings due to excess Se. However, workers exposed to Se may develop the following symptoms: apathy, depression, irritability, food intolerance, occasional dermatitis and skin and breath odour reminiscent of garlic. Risks of exposure occur in the glass, paint and pigments, electronics and fungicide industry.

INDICATIONS FOR MEASUREMENT

Measurements of serum or plasma selenium concentration and of glutathione peroxidase activity reflect recent intake. Measurement of these parameters in red blood cells is used to assess nutritional status.

Urinary measurements are useful to monitor exposed workers, urine concentrations increasing during the working week returning to normal 8 days after exposure ceases.

INFORMATION

■ SAMPLE

2 ml of serum or heparinised plasma (do not use a tube with phase separator); 20 ml unacidified urine sample. The sample should preferably be taken at the end of shift at the end of the week.

SAMPLE STORAGE AND TRANSPORT

Blood and urine samples can be stored and transported to the laboratory at room temperature or at between + 2 and + 8°C.

ASSAY METHODS

Electrothermal atomisation atomic absorption spectrophotometry (graphite furnace) with Zeeman correction. Induction coupled plasma-mass spectrometry (ICP-MS).

REFERENCE VALUES

In the general population:

– Serum Se: 60 to 120 µg/l i.e. 0.75 to 1.51 mol/l.

– Urinary Se: 10 to 50 µg/24 h.

FOR FURTHER INFORMATION

■ Lauwerys. R., Toxicologie industrielle et intoxications professionnelles, *sélénium*, 3e édition, Masson.

■ Guide BIOTOX 2002, *Fiche du sélénium plasmatique et urinaire*, INRS.

■ Chappuis P., Les oligoéléments en médecine et biologie, *le sélénium*, Ed. Lavoisier, Tec & Doc, 1991.

SEROTONIN

DEFINITION

Serotonin is a biogenic amine produced from the hydroxylation and decarboxylation of tryptophan, an essential amino acid with an indole structure. It acts as a neurotransmitter in the central nervous system and as a neurohormone secreted into the general circulation. Measurement of serotonin in blood and urine often combined with the measurement of its main metabolite, 5-hydroxyindolylacetic acid or 5-HIAA in urine is used mostly to test for and monitor carcinoid tumours, which are found mostly in the gastro-intestinal tract.

Synonym: 5-hydroxytryptamine, 5-OHT, hydroxytryptamine.

INTRODUCTION

Serotonin is produced from tryptophan by neurones, platelets and enterochromaffin cells (APUD or *Amine Precursor Uptake and Decarboxylation* cells) found mostly in the gastro-intestinal tract (stomach, duodenum, jejunum, rectum and appendix), but also in the pancreas, lungs, bronchi, thyroid and ovaries.

Serotonin is stored in the cells which produce it and is catabolised by oxidation or acetylation and removed in urine mostly as 5-hydroxyindolylacetic acid, and as a small amount in the unchanged form.

Physiologically, serotonin is involved in the vascular system (vasoconstricting effect), platelets (pro-aggregant effect), renin (anti-diuretic action), muscles (tonic effect on smooth muscles, acceleration of gastro-intestinal peristalsis and bowel transit), the nervous system, the hypothalamic-pituitary axis and regulation of the immune response. Pathologically it has been implicated in many disorders including sleep disturbance, migraine, some psychiatric diseases (depression, anxiety and schizophrenia), hypertension and allergy. Abnormal APUD cell proliferation also causes carcinoid tumours which secrete large amounts of serotonin and its metabolites.

INDICATIONS FOR MEASUREMENT

Serotonin measurement is used to investigate and monitor patients for carcinoid tumours. Monitoring the effect of treatment, as a predictive marker for recurrence or metastasis.

Serotonin can be measured in whole blood (the most appropriate sample as it is found both in plasma and particularly in platelets in large amounts), plasma (platelet rich or poor plasma depending on centrifugation speed) or urine and in CSF.

Measurement of serotonin in whole blood as this includes the secretion of serotonin throughout the average lifespan of platelets (9 days) and therefore can be used to assess a recent rise.

INFORMATION

SAMPLE

Whole blood collected into a plastic tube containing EDTA (avoid glass tubes which cause platelet adhesion with release of serotonin which is then destroyed by monoamine oxidase). Samples taken into tubes without anticoagulant (dry tubes) are not recommended as this results in an approximate 30% loss of serotonin.

24 hour urine collection into a plastic container; urine should be acidified in the laboratory by adding hydrochloric acid (HCl) 6N or 12 N to obtain a urine pH of approximately 2 (i.e. 5 to 20 ml of acid depending on the urine volume and pH). Record urine output.

Avoid eating foods rich in tryptophan or serotonin for 48 hours before the sample is collected, such as tomatoes, avocado pears, bananas, dried fruits, citrus fruits, pineapples, kiwis, plumbs, chocolate and molluscs.

QUESTIONS FOR THE PATIENT

Have you followed the diet before the collection was taken?

Type of tumour, types and date of any treatment?

Are you taking any of the following medicinal products: imipramine, clomipramine, desipramine, amitryptyline, maprotiline, mono-amine oxidase inhibitors: moclobemide, toloxatone, or specific serotonin reuptake inhibitors: fluvoxamine, fluoxetine, paroxetine and citalopram. All of these medicinal products influence the metabolism of serotonin.

SAMPLE STORAGE AND TRANSPORT

Samples should be transported promptly to the laboratory.

Assay in whole blood within an hour of sampling, otherwise freeze at – 20°C.

Assay in plasma: centrifuge the sample within an hour of sampling and freeze immediately at – 20°C.

Assay in urine: assay within an hour following the collection or otherwise freeze at – 20°C.

Transport (if > 1 hour): freeze at – 20°C.

ASSAY METHODS

Radio-immunology, immuno-enzymology, high performance liquid chromatography (HPLC, fluorimetric detection).

NORMAL EXPECTED VALUES

For reference (HPLC):

– Whole blood: 50 to 300 µg/, i.e. 0.28 to 1.70 µmol/l.

– Plasma: 10 to 50 µg/l, i.e. 0.06 to 0.28 µmol/l.

– Urine: 50 to 200 µg/24 h.

PATHOLOGICAL VARIATIONS

SEROTONIN AND CARCINOID TUMOURS

Carcinoid tumours are mostly found in the gastro-intestinal tract. They may also affect the ovaries, lungs or pancreas. In the gastro-intestinal tract they represent approximately half

of all appendicitis tumours, 20% of small bowel tumours and 10% of rectal tumours. The tumours are generally asymptomatic but can cause a carcinoid syndrome (in which case the tumour is usually in the small bowel), the major symptoms of which are skin "flushing" in the face, neck and extremities, cardiovascular problems and diarrhoea.

The great majority of carcinoid tumours cause serotonin hypersecretion. Some only secrete serotonin into blood or urine and others secrete 5-HIAA; for this reason all three parameters are usually used for testing. In practice, any rise in the concentration of one of these three markers suggests a carcinoid tumour.

Serotonin concentration in whole blood, plasma or urine is used above all as a diagnostic and monitoring marker when 5-HIAA values are "borderline". Following surgical excision, a rise in the concentration of any of these markers during laboratory follow- up is a sign of recurrence or metastasis.

■ OTHER DISEASES

Raised blood serotonin concentrations have been described in skin cancers as papillomas, some cases of migraine (which nevertheless respond to serotoninergic treatments such as sumatriptan or dihydroergotamine) and hypertension.

Reduced blood serotonin concentrations are reported in metabolic diseases including phenylketonuria or histidinaemia, renal insufficiency, trisomy, some cases of myoclonia and some cases of major depression treated with anti-depressants (imipramine derivatives, MAOI or selective serotonin uptake inhibitors, *cf. above*).

FOR FURTHER INFORMATION

■ Garnier J.P., Benlakehal M., Le Bricon T., *Sérotonine*, Encycl Med Biol, Elsevier, Paris 2003.

■ Garnier J.P., Bousquet B., *Acide 5-hydroxy-indolylacétique (5-HIAA) Sérotonine*. Cahier de formation Biochimie, tome III, Bioforma, Paris, 1996 : 11-19.

SERUM AMYLOID A PROTEIN

DEFINITION

Serum amyloid A protein (SAA) is an acute phase inflammatory protein. It is produced by hepatocytes under the influence of cytokines, particularly interleukin 1 and has a plasma half-life of approximately 10 hours. Like C-reactive protein (CRP), it is present in very small amounts in healthy people; serum concentrations may rise very quickly (in 6 to 12 hours) and to very high levels (up to 1,000 times the baseline value) in response to an inflammatory stimulus and then return to normal values over a few days. It is not widely used, however, as an inflammatory marker as it appears to be too sensitive, less specific and more difficult to measure than CRP. In practice it is mostly used as a marker of secondary amyloidosis.

Synonyms: SAA, serum amyloid A protein.

INTRODUCTION

SAA is an apolipoprotein present in large amounts in place of ApoA1, in "inflammatory" HDL3. Macrophages accumulate cholesterol from cell membrane debris at the site of inflammation. SAA may take part in the removal and catabolism of cholesterol contained in these macrophages, leading to the formation of so-called "inflammatory" HDL3 from circulating LDL. This is then bound in the liver.

INDICATIONS FOR MEASUREMENT

As a marker of inflammation or infection with rapid kinetics. Its indications for use are potentially the same as those of CRP, although it is far less widely used than CRP.

INFORMATION

SAMPLE

Serum (dry tube); a fasting sample is necessary; centrifuge clouded samples (turbidity interferes with the analysis).

QUESTIONS FOR THE PATIENT

Are you taking oestrogens or leflunomide? Oral oestrogens (oral contraception and hormone replacement therapy) increase serum SAA concentrations. They reduce concentrations when administered transdermally. Leflunomide reduces hepatocyte SAA synthesis.

SAMPLE STORAGE AND TRANSPORT

Serum can be stored for one hour at room temperature and for several months at -20°C. After the sample is taken, the serum must be separated and frozen promptly as SAA adsorbs onto plastic when the serum is stored at +4°C. Transport frozen at -20°C.

ASSAY METHODS

Liquid immunoprecipitation methods, turbidimetry or nephelometry.

NORMAL EXPECTED VALUES

Serum SAA values in healthy people are usually < 15 mg/l.

PATHOLOGICAL VARIATIONS

Serum SAA concentrations increase from 6 hours after the onset of an inflammatory process and may reach very high values (x 1000). Levels depend on the patient's clinical features, the activity of the disease and the treatment administered. Values return to around normal in 3 to 4 days if the inflammatory reaction resolves without complications.

INTERPRETATION IN DISEASE

- **Diagnosis of infectious bacterial and fungal diseases and inflammatory diseases**

  Serum SAA reaches values of between 100 and 1000 mg/l in bacterial and fungal diseases, cancers, tissue injuries, myocardial infarction and auto-immune diseases such as vasculitis or rheumatoid arthritis. Moderate rises (10 to 100 mg/l) are often seen in viral infections, systemic lupus erythematosus and local inflammatory reactions with tissue damage (cystitis, cerebral infarction and appendicitis).

- **As an aid to the diagnosis of appendicitis in children**

  A study conducted in 60 children hospitalised for abdominal pain with suspected appendicitis has shown that the leukocyte count, serum CRP and serum SAA were higher during the initial assessment in children who underwent surgery for acute appendicitis (42/60), than in those whose condition improved spontaneously during the 24 hours after admission (control group: n = 18). The serum SAA was not raised in any cases in the control group, whereas only 21.4% of these children had a normal serum CRP. The sensitivity and specificity of each of these tests were 76% and 75% for leukocytes > 10 x 109/l, 62% and 94% for CRP > 10 mg/l and 86% and 83% for SAA > 45 mg/l. SAA had a better discriminatory value than CRP or white cells in the diagnosis of acute appendicitis in this study.

- **Aid to differential diagnosis of infection/rejection after organ transplantation**

  When measured jointly with CRP, the literature reports SAA to have discriminatory value between infection and acute rejection in patients who have had a renal (or kidney + pancreas), or bone marrow transplant being treated with cyclosporin A and corticosteroids. Schematically, CRP appears to be reduced by immunosuppressants in acute rejection but not in infection, whereas serum SAA remains raised in both situations.

- **In secondary amyloidosis**

  Serum SAA concentrations are raised in all types of amyloidosis. It is higher, however, in secondary (due to infection, tumour or chronic inflammatory disease, etc.) than in primary amyloidosis. The rise in serum SAA concentration correlates with clinical disease in patients with confirmed amyloidosis.

FOR FURTHER INFORMATION

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SEX HORMONE BINDING GLOBULIN

DEFINITION – INTRODUCTION

Sex hormone binding globulin (SHBG) is a glycoprotein dimer with a molecular mass of 90 kDa, made up of 373 amino acids. It is produced in the liver.

The main property of SHBG is its high affinity for 17β-hydroxysteroids *(cf. table)*; DHT or 5α-dihydrotestosterone has the greatest affinity, the affinity of testosterone and estradiol being only a third and a fifth of this respectively.

Steroid and abbreviation	SHBG binding
Dihydrotestosterone (DHT)	300
Androstanediol (3α or 3β)	160
Testosterone (T)	100
Estradiol (E2)	60
Androstenediol	50

Obviously, neither the 17-ketosteroids nor epitestosterone bind to SHBG.

Although SHBG has high affinity for these steroids, it has limited capacity and most testosterone (49.9%) and estradiol (78%) is bound to albumin.

Only a very small fraction circulates free, not bound to proteins.

Binding of steroids to SHBG makes them inaccessible to target cells and metabolism. Until recently, only the non-protein bound steroid fraction was thought to be biologically active. It has emerged, however, that the steroid-albumin complex easily dissociates and the albumin-bound fraction may also therefore be available to target cells *(cf. testosterone)*.

High affinity SHBG receptors are also found in membranes of target cells for oestrogens and androgens. SHBG binds to these receptors forming an [SHBG. R_{SHGB}] complex.

SHBG therefore plays a role in regulating the ratio of unbound testosterone and estradiol fractions. Both of these steroids bind to the same site although testosterone has greater affinity than estradiol. On the other hand, estradiol has greater affinity for albumin than testosterone and as a result unbound testosterone (T) and estradiol (E2) concentrations depend on SHBG and albumin.

Increased SHBG causes greater binding of T than E2 and unbound E2 therefore rises producing a pro-oestrogenic effect. Conversely when SHBG falls the unbound T fraction increases causing an androgenic effect.

SHBG is produced in the liver and circulating concentrations are altered by many substances. SHBG increases in response to both natural and synthetic oestrogens, thyroid hormones, progesterone, anti-epileptic drugs and rifampicin. Oestrogens have greater effect after oral than percutaneous administration and anti-oestrogens such as clomifene and tamoxifen also cause a rise in SHBG because of their weak pro-oestrogenic activity.

Conversely, SHBG is reduced by natural and synthetic androgens, glucocorticoids, progestogens (except for progesterone) and insulin. This fall is believed to be secondary to reduced synthesis. Some substances such as danazol and norgestrel displace bound testosterone from SHBG causing a rise in the free testosterone fraction, which produces a fall in SHBG synthesis.

Synonyms: SHBG = *Sex Steroid Binding Protein* = *Sex Binding Protein* = SBP = TeBG = *Testosterone-estradiol Binding Globulin*.

INDICATIONS FOR MEASUREMENT

SHBG measurement is recommended in order to refine the interpretation of testosterone and estradiol concentrations, by calculating the free testosterone index, which is the ratio of total testosterone concentration to SHBG multiplied by 100. SHBG measurement allows the effect of hormones which influence hepatic SHBG synthesis (oestrogens, androgens, progestogens and thyroid hormones), insulin-resistance and cardiovascular risk to be assessed.

INFORMATION

■ SAMPLE

SHBG can be measured both in serum and in heparinised plasma. EDTA and citrate, however, are not recommended as anticoagulants. Blood may be taken at any time during the day, although as SHBG measurement is generally combined with that of androgens, the sample should preferably be taken in the morning.

■ QUESTIONS FOR THE PATIENT

All medicinal products which influence circulating SHBG concentrations should be reported, such as oestrogens, androgens, progestogens, thyroid hormones, anti-oestrogens (clomifene and tamoxifen), insulin, danazol, anti-epileptic drugs and rifampicin.

■ SAMPLE STORAGE AND TRANSPORT

Serum or plasma samples can be stored for 3 days at + 4°C for 1 month at – 20°C. Transport at + 4 °C if less than < 3 days.

ASSAY METHOD

SHBG is measured immunologically with an isotopic (RIA or IRMA) or non-isotopic label. It can also be measured on some auto-analysers, particularly using electrochemoluminescence.

REFERENCE VALUES

SHBG concentrations range between 18 and 114 nmol/l in women with menstrual cycles regardless of the phase of the cycle. SHBG falls at the menopause to circulating concentrations ranging between 18 and 70 nmol/l. Concentrations in adult men less than 50 years old are between 13 and 71 nmol/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

In the newborn SHBG concentrations are similar in both sexes and of the same order of magnitude as are found in adults. Concentrations then rise to approximately 3 months of age and fall thereafter. During the pre-pubertal period concentrations are higher than in adults. In the peri-pubertal phase a fall occurs, attributed to the rise in circulating androgens associated with adrenal maturation (adrenarche). SHBG concentrations subsequently fall more in boys than in girls, reaching adult concentrations. SHBG does not vary during the 24 hour cycle or during the menstrual cycle.

SHBG increases greatly in pregnancy, reaching concentrations of 5 to 8 times higher in the third trimester than in non-pregnant women. A very large fall follows after childbirth, although concentrations only return to similar values to non-pregnant women after 6 to 12 weeks.

Concentrations in men remain constant until the 4^{th} – 5^{th} decade and then gradually rise. This rise is not due to reduced testicular function as SHBG concentrations are not affected by orchidectomy and do not correlate with the fall in T. On the other hand, insulin GH and IGF-1 which also fall with age may explain these changes in SHBG.

PATHOLOGICAL VARIATIONS

Raised SHBG

SHBG rises in both primary and secondary hypogonadism in men. Note that both SHBG and testosterone are raised in the androgen insensitivity syndrome.

Hyperthyroidism, cirrhosis, anorexia nervosa and malnutrition are generally associated with a rise in SHBG.

Reduced SHBG

SHBG falls in androgen over-production syndromes in women, producing a rise in the unbound testosterone fraction.

SHBG is also reduced in hypothyroidism, hyperprolactinaemia, acromegaly and insulin-resistance states with hyperinsulinism as seen in the polycystic ovarian syndrome and obesity. Reduced SHBG is a risk factor for type 2 diabetes and cardiovascular diseases.

SHBG can be seen as marker of hyperinsulinism and insulin-resistance states.

FOR FURTHER INFORMATION

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- Fortunati N., *Sex hormone-binding globulin: not only a transport protein,* What news around the corner, J Endocrinol Invest 1999; 22: 223-34.
- Pugeat M., Crave J.C., Tourniaire J., Forest M.G., *Clinical utility of sex-hormone-binding globulin measurement*, Horm Res, 1996; 45: 148-55.

SHIGELLOSIS

DEFINITION

Shigellosis is an acute infectious proctocolitis caused by a bacterium belonging to the *Enterobacteriaceae* family, *Shigella* genus. It is not the most common of the diarrhoeal diseases but it is undoubtedly the most serious in its characteristic dysentery form, killing between 600,000 and 1 million people throughout the world each year (mostly young children). The species of bacteria responsible are: *Shigella dysenteriae, Shigella flexneri, Shigella boydii* and *Shigella sonnei*. Each of these 4 species is divided into serotypes according to their somatic (O) antigens. The Shigellae are short, Gram negative, aerobic-facultative anaerobic bacilli.

Synonyms: shigellosis, bacillary dysentery.

INTRODUCTION

EPIDEMIOLOGY

Human beings are the only reservoir for Shigella. Shigellosis is cosmopolitan and particularly occurs in sub-tropical countries (South-East Asia, Equatorial Africa, Central America, etc.). Transmission is faeco-oral from food or water contaminated by the faeces of patients or healthy carriers, although direct human-human transmission also occurs between the patient and his/her close contacts. It particularly affects children in developing countries where hygiene conditions are precarious or inadequate. The commonest and most pathogenic species of bacteria are *Shigella dysenteriae* serotype 1 which is responsible for epidemics and *Shigella flexneri* which causes an endemic form of the disease. *Shigella sonnei* is the main agent responsible for bacilliary dysentery in France and the United States.

PATHOPHYSIOLOGY

Shigellae have an invasive destructive potential on the colonic mucosa. After entering through the mouth they invade the intestinal epithelial cells and the tissue of the rectal and colonic mucosa where they multiply, leading to the formation of mucosal ulcers, causing severe inflammatory enterocolitis. The pathogenic potential of the bacterium is also related to the presence of toxins, such as the shiga toxin which has enterotoxic and neurotoxic activity and is produced in large amounts by *Shigella dysenteriae serotype 1*. *S. sonnei* and *S. flexneri* produce endotoxins with cytotoxic and enterotoxic activity.

SYMPTOMS

The severe form is the typical acute dysentery in adults, although attenuated or even asymptomatic forms and prolonged forms of the disease are also seen.

- **Acute dysenteric form** - After a short incubation period a fever of 39-40°C develops associated with deterioration in general health, abdominal pain sometimes accompanied by vomiting, straining and, tenesmus and abundant continuous watery diarrhoea which becomes bloody with mucus and pus. It resolves rapidly without treatment. Without effective treatment the convalescent becomes a healthy carrier for several weeks or months. The severity of the disease is due to the occasionally fatal complications which may occur in dysentery, such as neurological damage, bacteraemia and septicaemia originating from the intestine, dehydration which is particularly worrying in infants and is due to the fever and permanent stool production, intestinal complications including obstruction or colonic perforation, the haemolytic uremic syndrome, acute renal failure or late complications such as Reiter's syndrome which is a combination of arthritis, urethritis and conjunctivitis in people with HLA-B27.
- **Other forms** - Attenuated forms are seen commonly and involve simple diarrhoea associated with abdominal pain and a relatively mild fever.

SEARCH INDICATIONS

Diagnosis of shigellosis in a patient with an acute dysenteric form returning from a stay in a tropical area.

Differential diagnosis with other infectious parasitic (acute intestinal amoebiasis and bilharziosis), bacterial (salmonellosis, etc.) or viral (enterovirus, etc.) diarrhoea or dysentery.

INFORMATION

SAMPLE

Stool sample collected into a sterile container for stool culture.

Rectal swab may be useful in young children and infants.

Blood cultures are not performed commonly unless complications develop.

Serum for serological diagnosis.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

Return from a stay in a tropical area?

History of shigellosis in the patient's close contacts?

Current antibiotic therapy?

SAMPLE STORAGE AND TRANSPORT

Stools should be sent as soon as possible at room temperature, failing which in a suitable transport medium, stored for a maximum of 12 hours at + 4°C.

Serum can be stored for 1 week at + 4°C and then frozen at – 20°C for 1 year.

DIAGNOSTIC METHODS

DIRECT BACTERIOLOGICAL DIAGNOSIS

- **Macroscopic examination of stool** - This provides information about the presence of blood, mucus or pus in the stool.
- **Microscopic examination** - This reveals the presence of large amounts of polynuclear cells associated with imbalance of the bacterial flora with a large number of Gram negative bacilli.
- **Stool culture** - For the isolation of Shigella from the sample on selective media.

- **Biochemical and antigenic identification** - This is used to confirm the result of stool culture and identify a specific serotype.
- **Antibiotic sensitivity profile** - Is performed because of the increased acquired resistance of Shigella to antibiotics.

SEROLOGICAL DIAGNOSIS

Antibodies develop 8 to 10 days after the onset of the disease. Testing is performed by an agglutination technique with possible titration of O agglutinins or by the complement fixation reaction. Cross-reactions are found with other Enterobacteriaciae, particularly *Escherichia coli*. Antibody testing is mostly of epidemiological use.

TREATMENT

CURATIVE

Appropriate antibiotic therapy combined with rehydration. The antibiotics currently used are a quinolone for 2 or 5 days or a 3rd generation cephalosporin.

PREVENTIVE

This relies on improving hygiene conditions and combating faecal contamination. A vaccine is under investigation.

FOR FURTHER INFORMATION

Germani Y., Sansonetti P., *Shigellose et infections à Escherichia coli entéro-invasifs*, Encycl Med Chir. Elsevier, Paris, 8-026-A-10 ; 1999 : 9 p.

SILVER

DEFINITION AND SYNONYMS

Silver (Ag) is a light-coloured metal used in industry in the form of various alloys with copper, aluminium, or cadmium, as solder wire and as a catalyst in the chemical industry in the manufacture of silver nitrate, in photography and in the manufacture of various silver articles (silverware and jewellery).

Therapeutically, it has been known as an anti-bacterial agent since early times and is still used today in the forms of collyria and creams, particularly in burned patients. Silver is also a constituent of some dental amalgams from which it may leach in minimal amounts.

INTRODUCTION

Silver is absorbed by the gastro-intestinal (18% of the amount ingested are absorbed) and pulmonary (almost 90% of the amount inhaled is absorbed) tract and very slightly through the skin. It is stored in the reticulo-endothelial system and is excreted almost entirely in the faeces (half life 1 to 50 days) and to a very small extent, in the urine and sweat (< 8%).

Acute poisoning is rare and may be characterised by haemolytic anaemia, haemorrhagic gastroenteritis, liver failure, impaired renal tubule function and acute pulmonary oedema. Growth retardation may also occur in young children.

Excessive chronic silver absorption is responsible for argyria or argyrosis which is characterised by local or systemic tissue impregnation with silver precipitated as silver sulphide. Blue-grey pigmentation is seen in the skin or conjunctiva of the eye at contact points.

Facial pigmentation extending to the rest of the body, pigmentation of the conjunctiva, cornea and lens without deterioration in vision and pigmentation of the respiratory mucosa without clinical features are seen in systemic poisoning.

INDICATIONS FOR MEASUREMENT

The main indication for measuring silver is in iatrogenic poisoning due in particular to the use of antibacterial creams.

Measurement of blood and urine silver may be useful for the biological monitoring of occupational exposure although these are rarely used. Blood and urine concentrations in exposed subjects are higher than in unexposed subjects but no correlation with extent of exposure has been demonstrated. The information currently available does not allow any limit values to be proposed in exposed workers.

INFORMATION

SAMPLE

Dry tubes are used for serum silver measurement. Tubes containing heparin are used for measurement in whole blood. It is recommended that tubes specially designed for trace element analysis are used.

There are no specific sampling precautions to be observed for silver measurement. The time of sampling during the day or in the week is unimportant for monitoring occupational exposure, either for measurement in whole blood, serum or urine.

QUESTIONS FOR THE PATIENT

Has the patient recently received a medicine containing silver?

– Collyrium: Silver nitrate, silver proteinate?

– Cream: Silver sulfadiazine?

– Oral solution or tablets containing silver salts?

Is the patient occupationally exposed to silver?

SAMPLE STORAGE AND TRANSPORT

If analysis is to be performed later, the whole blood can be stored at +4°C, whereas serum must be separated from erythrocytes and stored at +4°C for up to a week or frozen at –20°C for longer a period of storage.

Urine samples are stored at +4°C for up to a week or frozen at –20°C for longer periods.

ASSAY METHODS

The assay method of choice for silver is induction coupled plasma mass spectrometry (ICP-MS) which provides a level of sensitivity consistent with measuring physiological concentrations. Electrothermal atomic absorption spectrometry can also be used, although is far less sensitive.

NORMAL EXPECTED VALUES

Reference values in the general population:

Serum: < 0.5 µg/l

Whole blood: < 0.8 µg/l

Urine: < 2 µg/l

There are no guideline values in France, Germany or United States for occupationally exposed subjects.

PATHOPHYSIOLOGICAL VARIATIONS

Silver contained in therapeutic creams is not absorbed by healthy skin but it is absorbed by burned skin. Blood silver concentrations in these patients are proportional to the burned skin surface area although not to the depth of the burn. Serum concentrations may rise considerably from the sixth hour after the first application and reach several tens of µg/l. Urine concentrations also increase significantly, rising to values as high as 500 µg/l.

Argyrosis occurs in serum concentrations of > 2 µg/l.

FOR FURTHER INFORMATION

- Fiche Biotox Argent. www.inrs.fr

SIROLIMUS

DEFINITION

Sirolimus belongs to the macrolide family which has immunosuppressant activity. It is indicated for use in the prevention of organ rejection in adult patients, at mild to moderate immunological risk, who have received a renal transplant. Sirolimus is started in association with cyclosporin microemulsion and corticosteroids for 2 to 3 months. It is then continued as maintenance treatment with corticosteroids only, and only if, the cyclosporin can be stopped gradually. If not, a change in immunosuppressant therapy must be considered.

Synonyms: Rapamycin (initial name of sirolimus).

MECHANISM OF ACTION

Sirolimus inhibits T lymphocyte activation by blocking intracellular signal transduction. After binding to a specific cytosol protein FKPB12, the sirolimus – FKPB12 complex inhibits activation of the target mTOR *(mammalian Target Of Rapamycin)* protein which is a kinase essential for progression of the cell cycle. mTOR inhibition blocks several specific signal transduction pathways resulting in inhibition of lymphocyte activation, causing immunosuppression.

METHODS OF ADMINISTRATION

Sirolimus is marketed as 1 mg coated tablets and a 1 mg/ml oral solution.

Initiation treatment (for 2 to 3 months after transplantation associated with cyclosporin): loading dose of 6 mg *per os*, then 2 mg once daily.

Maintenance treatment: after gradually stopping the cyclosporin over a period of 4 to 8 weeks, adjust the sirolimus dose to obtain trough sirolimus concentrations within the therapeutic range *(cf. below)*.

METABOLISM

Bioavailability (oral form)	14% (concomitant administration of cyclosporin)
Concentration peak	1 to 3 hours
Half-life of elimination	In stable renal transplant patients: 62 hours The useful half-life, however, is reduced: steady state concentration is obtained after an average of 5 to 7 days.
Metabolism	By cytochrome P450 3A4 (CYP 3A4) and glycoprotein P. Seven main metabolites including hydroxylated, demethylated and hydroxydemethylated derivatives are found in blood. Sirolimus remains the main compound found in blood and contributes 90% of the immunosuppressant activity.
Elimination	Approximately 98% in faeces; 2% in urine.

INDICATIONS FOR MEASUREMENT

The need for blood sirolimus measurements is stated in the MA for dose adjustment. These measurements are justified for the following reasons:

– Intra and inter-concentration variability.

– Sirolimus is a potent and potentially toxic immunosuppressant. The main adverse effects are general problems such as peripheral oedema, fungal, viral or bacterial infections, cardiac problems (tachycardia), haematological abnormalities (anaemia and thrombocytopaenia) and metabolic abnormalities (hypercholesterolaemia, hypertriglyceridaemia, hypokalaemia and increased transaminases), arthralgia, respiratory (pneumonia), renal and urinary problems (urinary infections and pyelonephritis). Overdose predisposes to and maintains infections and increases the risk of malignant tumours, particularly skin tumours and lymphomas.

– Numerous therapeutic interactions alter the metabolism of sirolimus (risk of lack of effect or overdose).

In order to reduce fluctuations in blood concentrations sirolimus must always be taken 4 hours after the cyclosporin and always either with or without food (large increase in Cmax, Tmax and AUC if a fat rich meal is taken at the same time).

Monitoring of plasma sirolimus concentrations is recommended in all patients. Monitoring must be particularly strict in patients with hepatic renal insufficiency, when a potent CYP3A4 inhibitor or inducer is being taken concomitantly or if the dose of cyclosporin is greatly increased or reduced. Cyclosporin inhibits the metabolism of sirolimus and plasma sirolimus concentrations fall when the cyclosporin is stopped. In order to remain effective, the dose of sirolimus must be increased by a factor of approximately 4 to take account of the disappearance of the therapeutic interaction and increased immunosuppressant requirement.

In practice, sirolimus should be measured when any concomitant drug treatment liable to change serum sirolimus concentrations, 5 to 7 days after starting treatment or changing dose.

When moving from the solution form to the tablet form it is recommended that trough sirolimus concentrations be measured after 1 to 2 weeks to confirm that they are within the therapeutic range.

INFORMATION

SAMPLE

Whole blood collected into EDTA.

Samples for measurement of trough sirolimus concentration (C0) are taken immediately before the next dose. In general, dosage adjustments should be based on several trough concentration results obtained more than 5 days after starting treatment or a previous change in dose.

QUESTIONS FOR THE PATIENT

Any request for drug measurement must include the reasons for requesting (testing for efficacy or toxicity), the sampling time, treatment start date and/or any change in dose, dosage information (amount administered, frequency and route of administration) and the age, height, and weight of the person wherever possible.

State the time since transplant and the date when treatment was started.

Concomitant treatments at risk of producing therapeutic interactions with sirolimus:

A) Drugs (or substances) which increase blood sirolimus concentrations

– Cyclosporin,

– Macrolides and related compounds, such as erythromycin, clarithromycin and telithromycin,

– Azole anti-fungals, such as ketoconazole, itraconazole and fluconazole,

– Calcium channel blockers, such as nicardipine, nifedipine, diltiazem and verapamil,

– HIV anti-proteases, such as ritonavir, nelfinavir, indinavir, saquinavir, amprenavir and lopinavir,

– Others: danazol, cisapride, metoclopramide, bromocriptine and cimetidine,

– Grapefruit juice alters cytochrome P450 3A4 metabolism and must be avoided.

B) Drugs which reduce blood sirolimus concentrations

– Anti-epileptics such as carbamazepine, phenobarbital, phenytoin and primidone,

– Rifampicin,

– St. John's Wort.

■ SAMPLE STORAGE AND TRANSPORT

Store whole blood for up to 1 week at + 4°C, and at – 20°C beyond one week.

Transport at + 4°C.

ASSAY METHODS

– High performance liquid chromatography linked to mass spectrometry.

– Immunoassays.

NB: Chromatographic methods give results which are on average approximately 20% lower than those obtained by immunoassay.

NORMAL EXPECTED VALUES

During initiation treatment: trough (C0) sirolimus concentration (associated with cyclosporin):

– 4 to 12 ng/ml by chromatography,

– 4.50 to 14 ng/ml by immunoassay.

During maintenance treatment (after cyclosporin is stopped).

– 12 to 20 ng/ml by chromatography,

– 15 to 25 ng/ml by immunoassay.

FOR FURTHER INFORMATION

■ Dictionnaire Vidal®.

■ Royer B., Davani S., Bérard M., Kantelip J.-P., Muret P., *Suivi thérapeutique pharmacologique de l'acide mycophénolique, du sirolimus et de la ciclosporine au moyen du « C2 »*, Ann Biol Clin 2003 ; 61 : 251-8.

SOLUBLE COMPLEXES

DEFINITION

Soluble complexes are formed from combination of fibrin monomers with either fibrinogen, fibrin degradation products or fibrinogen degradation products (FDP).

INTRODUCTION

Thrombin, the key coagulation enzyme, cleaves soluble fibrinogen detaching fibrinopeptides A and B from the fibrinogen molecule. The fibrin monomers produced polymerise, forming fibrin gel, which is stabilised by factor XIII.

Physiological fibrinolysis degrades the fibrin clot and maintains the circulation of blood in the vascular lumen. Plasmin, the key enzyme of fibrinolysis degrades the fibrin clot and the fibrinogen molecule into FDP. In disseminated intravascular coagulation (DIC), thrombin, which is produced to excess beyond the body's ability to inhibit it, forms fibrin monomers from the fibrinogen molecule. These fibrin monomers can bind to fibrinogen molecules and/or FDP and form reversible complexes, the soluble complexes.

The presence of fibrin monomers and therefore of soluble complexes indicates disseminated intravascular coagulation.

INDICATIONS FOR MEASUREMENT

In clinical situations in which DIC is suspected, soluble complexes provide evidence in favour of a diagnosis. They contribute to the differential diagnosis between acute primary fibrinolysis (negative soluble complexes) and fibrinolysis secondary to DIC (positive soluble complexes). Soluble complexes are also useful to monitor DIC.

INFORMATION

SAMPLE

Samples should be collected into citrate, concentration 3.2% (0.109 M), 1/10 (0.5 ml per 4.5 ml of blood). 3.8% (0.129 M) are acceptable. CTAD tubes (citrate, theophylline, adenine, dipyridamole) are recommended in patients receiving heparin. No other anticoagulant may be used.

The sample should preferably be taken in the morning between 0700 and 1100 hours with the patient resting, seated, for at least 5 minutes. A light low fat snack is permitted but caffeine, tobacco and physical activity must be avoided during the hour before the sample is taken. Tubes must be centrifuged promptly after the sample is taken. Check that no micro-clots are present and discard haemolysed or lipaemic samples.

For more information refer to the "General pre-analytical conditions in haemostasis" section.

ESSENTIAL INFORMATION

Clinical and laboratory context.

SAMPLE STORAGE AND TRANSPORT

The samples obtained are stable for a few hours at + 20°C. They can be frozen and stored for up to 3 months at – 20°C or for 6 months at – 70°C. Plasma samples must be transported frozen at – 20°C within 4 hours of sampling.

ASSAY METHODS

The haemolysis tube ethanol test is based on the principle of dissociating soluble complexes with ethanol at + 4°C. A protamine sulphate test is also available. These tests may produce false negative results particularly when fibrinogen concentrations are low.

A haemaglutination technique is available in which reagent red blood cells are sensitised with fibrin monomers. This test is not suitable for newborn babies. The haemaglutination test appears to be more specific and more sensitive than the ethanol and protamine sulphate tests.

NORMAL EXPECTED VALUES

Results are qualitative: presence or absence of soluble complexes.

Physiologically, when the coagulation and fibrinolysis processes are in steady state, no soluble complexes are formed.

PATHOPHYSIOLOGICAL VARIATIONS

Soluble complexes develop whenever large amounts of thrombin are generated exceeding the body's ability to inhibit them. They are seen in DIC (the major causes of which are obstetric, multiple injury and surgery), acute intravascular haemolysis, disseminated malignancy and acute promyelocytic leukaemia, severe infection and poisonous snake-bites.

As soluble complexes consist of fibrin molecules and fibrinogen molecules or FDP they cannot be present if the fibrinogen concentration is very low (and therefore a negative test does not exclude DIC).

The diagnosis of DIC is now based mostly on the use of scores, one of which was proposed in 2002 by the *International Society for Thrombosis and Haemostasis*, and has been validated in several studies and correlates well with mortality rate and organ failure.

Diagnostic algorithm for "decompensated" DIC according to the *International Society for Thrombosis and Haemostasis*

Risk assessment: is the patient suffering from a disease known to be associated with DIC? *If yes: perform the test; if not, do not use this algorithm.*

– Perform the global coagulation assessment test (platelet count, prothrombin time, fibrinogen, fibrin degradation markers, fibrin degradation products, d-dimers and soluble fibrin monomers)

– Assess the results of the tests

Platelets (> 100 = 0; < 100 = 1; < 50 = 2)

Markers of fibrin degradation (no increase: 0; moderate increase: 2, large increase: 3)

Prolongation of Quick time (< 3 seconds = 0; > 3 seconds but < 6 seconds = 1; > 6 seconds = 2)

Fibrinogen concentration (> 1 g/l = 0; < 1g/l = 1)

– Calculate score:

If score ≥5: compatible with "decompensated" DIC: repeat score daily;

If score < 5: suggests but does not confirm "compensated" DIC: repeat in 24-48 hours.

FOR FURTHER INFORMATION

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SOMATOSTATIN

DEFINITION

Somatostatin is a cyclical peptide described in 1973 as a hypothalamic hormone inhibiting growth hormone release. It exists in two forms, a 14 amino acid peptide and a 28 amino acid peptide and notably is also produced outside of the hypothalamus. It has been found in other neuronal structures including the cortex, thalamus, spinal cord and cerebellum and in the gastro-intestinal tract, where it is mostly found in the stomach, duodenum, ileum and the pancreas.

High somatostatin 14 and somatostatin 28 concentrations are found in the cerebral cortex and duodenum. Somatostatin 14 is the predominant form in all tissues compared to somatostatin 28, except for the ileum and duodenum.

Synonym: SRFI (Somatotropin Release Inhibiting Factor).

INTRODUCTION

Somatostatin is believed to have a paracrine action in the nervous system, whereas it has both paracrine and endocrine actions in the hypothalamus. In other tissues it is recognised to have both paracrine and endocrine actions together with an autocrine action in the stomach.

These actions occur through receptors, 5 different sub-types of which (sst1 to sst5) have been described to date. The genes for these receptors are each located on a different chromosome. The sst2 receptor gene can produce two different proteins by alternative splicing. The sst2 and sst5 receptors are expressed in somatotrope cells, whereas the sst4 receptor is predominant in thyrotrope cells. All 5 types of receptor are expressed in human pituitary tumours and their density, measured by scintigraphy, is an important prognostic indicator for the anti-secretory, anti-proliferative effect of somatostatin analogues.

By binding to its receptors, somatostatin inhibits adenylate cyclase, activating Gi protein. It inhibits release of GH, but not its synthesis of GH which may explain the GH rebound after the effect of somatostatin is removed. The sst1 receptor also activates the phosphoinositide pathway. In addition, somatostatin inhibits TSH secretion and secretion of the pancreatic peptides, insulin and glucagon, the metabolic effects of which are involved in regulating growth hormone secretion.

Together with somatoliberin *(Growth Hormone Releasing Hormone or GHRH)*, hypothalamic somatostatin plays an important role in determining the pulsatility of growth hormone as both peptides have antagonistic effects on growth hormone secretion.

The somatostatin-somatostatin receptor system appears to be an important relay for the modulating actions of extra-neuronal substances such as glucocorticoids, glucagons, sex hormones, amino acids and glucose.

The suppressing effect of somatostatin on growth hormone secretion is also used in the treatment of acromegaly with potent synthetic agonists. These agonists, octreotide, lanreotide and vapreotide, are also used in gastro-intestinal disease to treat some carcinoid tumours and in the treatment of gastro-intestinal bleeding from oesophageal varices.

INDICATIONS FOR MEASUREMENT

Somatostatin measurement is indicated when a bioactive peptide-secreting duodenal gastric or pancreatic gastro-intestinal tumour is suspected particularly in the investigation of type I multiple endocrine neoplasia (MEN I).

INFORMATION

■ SAMPLE

The assay is performed on plasma (blood sampled into EDTA + aprotinine).

The sample may be taken fasting or post-prandially (4 hours after the start of a meal).

■ SAMPLE STORAGE AND TRANSPORT

The sample must be centrifuged promptly and the separated plasma frozen at – 20°C within an hour of sampling until assay. Transport samples frozen.

ASSAY METHODS

Somatostatin is measured in blood by a direct, isotopic or non-isotopic immunoassay.

EXPECTED VALUES

In normal subjects:

– Fasting: < 26 ng/l;

– Post-prandial (4 h): < 80 ng/l.

PATHOPHYSIOLOGICAL VARIATIONS

■ IATROGENIC VARIATIONS

Antidopaminergic agents such as domperidone and the antidepressants can increase plasma somatostatin concentrations, causing incorrect interpretation of a result.

■ PATHOLOGICAL VARIATIONS

Only somatostatin releasing gastro-intestinal tumours or somatostatinomas cause high levels in peripheral blood of over twice normal.

These tumours are found in various sites in the gastro-intestinal tract, particularly in the pancreas.

Somatostatin secretion may be associated with abnormal secretion of gastrin and/or VIP *(Vasoactive Intestinal Peptide)*.

FOR FURTHER INFORMATION

■ Olias G., Viollet C., Kusserov H., Epelbaum J., Meyerhof W., *Regulation and function of somatostatin receptors*, J Neurochem 2004, 89 : 1 057-91.

SPECIFIC IgE

DEFINITION

Whilst as early as 1921, passive transfer experiments demonstrated that the allergic response involved a specific so-called "reagin" antibody, it was not until 1966 that a fifth class of immunoglobulins, IgE, were shown to be the support for "reagin activity". IgE is a 190 kDa monomeric immunoglobulin, consisting of 2 identical light chains (κ and λ) and 2 identical heavy chains (ε) which are extensively glycosylated. Its plasma half-life is short (2 days), although increases (3 to 4 days) when IgE reaches the increased levels characteristic of atopy. These immunoglobulins are heat labile and lose their homocytotropic activity on heating.

IgE is known to play a role in the development of immediate hypersensitivity reactions but also contributes considerably to anti-parasitic immunity.

The biological effects of IgE act through receptors which differ in their affinity, protein family (immunoglobulin and lectin superfamily) and cell expression. High affinity receptors (FcεRI) exist in two isoforms, depending on the cell line; the αβγ2 tetrameric form is expressed constitutionally and at high densities on cell membranes, tissue mastocytes and circulating basophils; and the αγ2 trimer which can be expressed (at low densities) on a small contingent of monocytes, macrophages, dendritic cells and platelets, even in normal people and on some blood or tissue eosinophils in atopy. The FcεRII or CD23 receptors are lectins with low affinity for IgE and are present on some dendritic cells and on a minor contingent of monocytes, macrophages, eosinophils and platelets. Whether free or after binding IgE, the receptors are mobile and are distributed uniformally over the cell surface.

Bridging between IgE by a multivalent allergen causes them to aggregate resulting in the signal transduction cascade, leading to the release of mediators of anaphylaxis (histamine, leukotrienes, prostaglandins, cytokines and tryptase). Clinically these mediators result in the well-known symptoms of rhino-conjunctivitis, asthma, urticaria, angio-oedema, gastro-intestinal problems and even anaphylactic shock.

Allergic diseases are ranked the 4th most common worldwide by the WHO and have major economic and social public health consequences. There is understood to be a minimum of 4.3 million people in France currently diagnosed as having an allergy, which equates to 7% of the population. According to some authors, however, almost 25% of the population of France is believed to be affected by the disorder, three quarters of people with an allergy, not being aware of their condition. Epidemiological studies have shown that the prevalence of allergic disorders has increased markedly over the last 30 years and that it appears to have doubled over 20 years.

IgE-mediated allergy in the pathological sense of the term, occurs as a result of a combination of genetic, immune and inflammatory system-related and various environmental factors.

Allergens are mostly proteins and can be classified depending on the route through which they enter the body. Aeroallergens mostly enter through the respiratory but also epicutaneous routes and make up the majority of sensitisations; trophallergens (food allergens) enter mostly through the gastro-intestinal tract; and finally, allergens can also enter through various other routes such as hymenopter venoms, drugs and various chemicals.

These disorders, which combine a wide range of clinical symptoms with multifactorial causes, can therefore make the diagnosis occasionally extremely difficult. Diagnosis is based on the clinical history, skin tests (prick-tests or even the intra-dermal reaction), the choice of which is guided by the clinical history, and possibly from the results of measuring circulating specific IgE. Provocation tests, which are not without risk, are only indicated when clinical features, skin tests and laboratory findings are inconsistent, for a limited number of allergens.

INDICATIONS FOR MEASUREMENT

- **Specific IgE screening tests for several allergens contained in a mixture (food allergens, respiratory allergens or mixed allergens)**

  For clinicians not specialised in allergy medicine, these provide an overall response as to whether or not specific IgE for allergens contained in the mixture are present. The composition of the mixtures is chosen depending on allergen prevalence.

In respiratory allergy, the screening tests contain the main environmental, respiratory airborne allergens (mites, animal hair, pollens and moulds). These tests correlate well with a clinical diagnosis of allergy and further patient investigations are required if the result is negative in the face of suggestive clinical features. Negative reactions have different possible causes, such as the allergen concerned not being present in the mixture, single allergen sensitisation to one allergen in the mixture, absence of circulating IgE, etc. It should also be noted that in young children, initial sensitisations to food allergens occur before sensitisation to respiratory allergens.

The screening tests perform variably in food allergy and depend on the patient's age. Allergen prevalence is extremely different between adults and children. Animal allergens predominate in children (milk and egg white), whereas plant allergens predominate in adults (rosaceous plants, latex-fruit group and umbelliferous plants). The tests used are targeted from the patient's clinical history.

- **Identification tests – Single specific IgE (RAST)**

  A good correlation is seen between the immediately read skin tests and concentrations of the corresponding specific IgE in most patients with immediate allergy to common allergens. The main indications for measuring specific IgE are:

As an aid to diagnosis in the following situations:

– Inability to perform skin tests (widespread atopic dermatitis, patients receiving anti-histaminergic agents or skin test allergen unavailable).

– Inability to interpret skin tests (poor skin reactivity or dermographism).

– Inconsistency between skin tests and the patient's clinical history.

For monitoring treatment and as an aid to the decision to reintroduce:

– Before starting treatment (desensitisation, etc.).

– Before reintroducing the food. Measurement of specific "unitary" IgE levels have become particularly useful since the work by Sampson et al. (*JACI* 1997; 100: 444-51), who showed that threshold values could be defined giving a 95% probability of finding a positive oral provocation test result. Since then, other authors, (Hill et al., *Clin Exp Allergy* 2001; 31: 1031-1035 and Rance et al., *J Allergy Clin Immunol* 2002; 109: 1027-33) have found often very different threshold values. This is not surprising as the positive (PPV) and negative (NPV) predictive values depend closely on many factors:

– Patient characteristics, such as age, causal diseases and environment;

– The prevalence of allergy in the cohort studied;

– Assay methods for the specific IgE;

– Cross-reactions, which occur more often *in vitro* than in skin tests, and may produce positive laboratory results, particularly in patients allergic to pollen.

Each centre that performs stimulation tests must therefore establish its own threshold values, to reduce the number of stimulation tests performed, as these are onerous, dangerous and expensive. These threshold values can never be used directly to confirm or refute a diagnosis of food allergy.

INFORMATION

■ SAMPLE

Serum (dry tube) or possibly heparinised plasma.

A fasting sample is not essential. Measurements are not influenced by drugs (antihistaminergic agents, corticosteroids, antidepressants, etc.).

■ SAMPLE STORAGE AND TRANSPORT

Store at between + 2 and + 8°C for up to one week or frozen at – 20°C for several years. Repeated freeze-thaw cycles should be avoided.

ASSAY METHODS

Regardless of the method used, specific IgE are sandwiched between an allergen fixed to a support and labelled human anti-IgE. The labelling signal rises with increasing IgE concentrations. There is no international standard for specific IgE and results are generally expressed against the international standard for total IgE (kU/l). Measurement ranges are normally between 0.35 and 100 kU/l.

EXPECTED VALUES

The finding of high specific IgE concentrations for one or more allergen, in a patient's serum, reflects the presence of sensitisation but does not imply that the sensitisation is pathogenic. A cause-effect relationship must be shown between the development of clinical signs and the exposure to the allergen in order to prove allergy. If this relationship is not found, it may be necessary to use respiratory, nasal, bronchial or oral stimulation tests to establish that the sensitisation is pathogenic.

PATHOLOGICAL VARIATIONS

Depend on the extent and frequency of exposure and treatment (avoidance, desensitisation, etc.).

FOR FURTHER INFORMATION

■ Vervloet D., Magnan A., *Traité d'allergologie,* Flammarion Médecine-Sciences, 2003.

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STEATORRHOEA

DEFINITION

24-hour faecal fat excretion (often incorrectly called 24 hour steatorrhoea) is requested to investigate and quantify intestinal absorption and digestion effects in order to characterise malabsorption or maldigestion syndromes. Steatorrhoea is defined as an increase in fat excretion above normal limits.

Measurement of faecal fat alone does not differentiate maldigestion (intra-luminal enzymatic origin) from malabsorption (parietal and vascular origin). If however, measurement is combined with faecal microscopy the clinician may be guided towards maldigestion or malabsorption.

INTRODUCTION

Unlike proteins, fats in faeces come exclusively from the diet. Measurement of 24-hour fat excretion (or steatorrhoea) is therefore used either diagnostically or to monitor treatment in order to characterise and quantify maldigestion syndromes associated with endoluminal dysfunction (exocrine pancreatic, biliary and gastric insufficiency), malabsorption syndromes associated with small bowel wall abnormalities (villous atrophy, infiltration or inflammation of the *amina propria*) or due to chronic small bowel bacterial colonisation.

The digestion of dietary lipids is complex: long chain triglycerides (LCT) begin to be digested in the stomach under the action of gastric lipase produced by the fundal principal cells. In the duodenum, LCT are solubilised in the presence of bile acids and are then hydrolysed by pancreatic lipase in the presence of colipase to form monoglycerides and fatty acids. The monoglycerides formed are then hydrolysed into glycerol and fatty acid, which can be absorbed by the enterocyte.

Phospholipids, principally phosphatidylcholine, are hydrolysed by the action of phopholipase A2, forming fatty acids and lysolecithine.

Finally, cholesterol esters are hydrolysed by pancreatic cholesterase.

An increase in fat excretion may therefore occur as a result of disturbance of the different more or less intricated mechanisms involved in their digestion and absorption: biliary abnormalities (emulsification), pancreatic (digestion), enterocyte (absorption and resynthesis) and lymphatic (drainage) and motor abnormalities.

INDICATIONS FOR MEASUREMENT

Faecal fat measurement is a first line stool investigation.

It is used in chronic diarrhoea to identify organic disease involving maldigestion or malabsorption.

Measurement of fats and faecal chymotrypsin activity is used in exocrine pancreatic disease to assess adherence to treatments and their effect.

The test is also requested in the presence of any deficiency associated with abnormalities of the fat soluble vitamins (ADEK) and to identify inadequate dietary intake or even malnutrition, particularly in young children and in the elderly.

INFORMATION

SAMPLE

Faecal fat excretion results can only be interpreted if all of the faeces produced over a given period have been collected into specific pots, taking care not to mix faeces and urine. For ease and hygiene, the whole 24 hour faecal collection should be collected into one or more opaque pots of sufficient volume (the usual pot volume used is 1 litre) stored in a refrigerator. The date should be recorded on each pot allowing a daily faecal weight to be measured and the average daily weight to be calculated over the collection period.

It is standard practice to collect faeces over a period of three whole consecutive days, also taking care to collect faeces past overnight. A single collection day is probably insufficient, although can be considered in people with small spontaneous variations in bowel transit and in young children. In the newborn, the collection must be obtained by positioning a "urinocol" bag over the anus in order to only collect faeces and avoid them being dehydrated by absorption into the nappy.

Fat quantification can be underestimated if the patient reduces the amount he/she eats and when the investigation is performed standardised conditions are desirable. To do this, the diet should be supplemented with 50 g of additional fat daily (i.e. 5 x 10 g butter portions) in order to obtain a daily intake of at least 100 g of fat. Supplementation should begin 3 days before the collection and continued during the 3 days of the collection. The request should state whether the patient is or is not receiving supplementation and this fact should be taken into account when the results are analysed.

QUESTIONS FOR THE PATIENT

Radiological investigations involving opacification of the bowel and colonoscopy preparations which alter the bowel ecosystem must be avoided for the 8 days before and during the collection. Care must be taken during the collection period to avoid interferences which may change the analysis of the results such as laxatives, paraffin oil or local bowel medications.

Oil-bearing fruits/nuts (peanuts, walnuts, hazelnuts and avocados) must be avoided for 3 days before and during the 3 days of the collection: these are very rich in intracellular fat and interfere with fat measurement.

SAMPLE STORAGE AND TRANSPORT

All of the faeces (24, 48 or 72 hours) or an aliquot obtained after homogenising the 24 hour sample with a record of the daily faecal production should be transported as soon as possible at room temperature to the laboratory. The samples should be stored at + 4°C if the measurements are performed immediately or frozen at – 20°C if they are to be performed later.

ASSAY METHODS

Faecal fat is measured using a titrimetric (van de Kamer) or gravimetric (Jeejeebhoy) method after extracting the fats. The main batch method used is the Van de Kamer method.

NORMAL EXPECTED VALUES

If the factors required to correctly interpret the test (fat supplementation, 2 to 3 day faecal collection) are observed, adult faecal fat excretion should normally be between 2 and 6 g/24 hours. A fat excretion of more than 7 g per day is acknowledged to be pathological and the term steatorrhoea is used for values over 7 g.

The normal fat absorption coefficient is ≥ 95%.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Physiological variations in bowel transit explain the preference for a collection of all faeces passed over 3 consecutive days. The 3 day faecal collection should be carefully homogenised and measurements performed on an aliquot of the homogenates. Results are reported as an average value per 24 hours.

Increased bowel transit time in healthy volunteers caused by laxatives may cause steatorrhoea although this is never more than 14 g per day. Similarly, moderate steatorrhoea may be found in some idiopathic motor or secretory diarrhoeas.

■ PATHOLOGICAL VARIATIONS

Faecal fat excretion must be interpreted according to each individual patient's context: age, bowel and non-bowel symptoms, and past surgical history.

A faecal fat excretion of more than 14 g per day in a patient without a past surgical history is a significant indicator of fat maldigestion or malabsorption. Concomitant macro and microscopic examinations may help to understand the mechanism of the chronic diarrhoea.

The presence of long chain triglycerides which stain with Sudan III and Bailanger reagent, together with poorly digested meat fibres suggests fat maldigestion, which may have many causes.

– ***Gastrectomy:*** absence of secretin or cholecystokinin secretion abolishes the synchronicity between arrival of the food bolus in the duodenum and release of pancreatic enzymes.

– ***Gastric insufficiency or hypergastrinaemia*** disturbs the pH of chyme by reducing or increasing pH respectively which in particular no longer enables pancreatic lipase to act under optimal conditions (pH 6.8); producing steatorrhoea; for example with hypergastrinaemia in pancreatic gastrinoma (Zollinger-Ellison syndrome), the abnormal increase in gastric acidity inhibits enzymatic activity in the duodenum, precipitating glycocol-conjugated bile salts and reducing the esterification of fatty acids, which prevents chylomicron formation.

– ***Exocrine pancreatic insufficiency*** insufficiency causes a fall in lipase secretion, which is essential to digest long chain triglycerides.

- In exocrine pancreatic insufficiency (chronic pancreatitis or cystic fibrosis), steatorrhoea develops when less than 10% of the pancreatic enzyme secretory capacity remains. It may be helpful to combine measurement with faecal elastase for further investigation of the exocrine pancreas.

- In children with cystic fibrosis, a mucus plug can obstruct the pancreatic ducts and reduce mixing of pancreatic enzymes with the food bolus.

– ***Biliary insufficiency*** causes a fall in intra-luminal concentration of bile salts which are essential to emulsify fats:

- In acute or chronic liver disease or biliary tract atresia.

- With bacterial proliferation, bile acids are unconjugated and reabsorbed more quickly, reducing their concentration in the faecal bolus.

- With drugs which interfere with the entero-hepatic bile salt cycle, such as cholestyramine.

- In extensive small bowel resection (short small bowel syndrome).

The presence of large amounts of fatty acids and soaps, absence of long chain triglycerides which stain with Sudan III and well digested muscle fibres suggest fat malabsorption, which has many causes:

– ***Villous atrophy*** *causing reduced absorption surface area:* coeliac disease, tropical sprue, extensive small bowel inflammatory disease, scleroderma, amyloidosis, etc.

– ***Reduced absorption surface area*** *due to extensive small bowel resection (short small bowel syndrome).*

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■ Fine K.D., Schiller L.R., *AGA technical review on the evaluation and management of chronic diarrhea*, Gastroenterology 1999 ; 116 : 1464-86.

■ Modigliani R., Bonnet J., *Syndrome de malabsorption de l'adulte*. In : Rambaud J.C., ed. Traité de gastro-entérologie, Paris : Flammarion Médecine-Sciences, 2000 : 157-69.

STOOL EXAMINATION

DEFINITION

There is a variety of examinations that can be performed on the faeces:

– Physical examination of the stools can involve the determination of mean weight and/or dry weight; macroscopic examination; microscopic examination which can be direct or after staining, the latter facilitating the identification of blood cells, various sorts of fat and components that stain with iodine. Microscopy can also be employed to judge the effectiveness of digestion of muscle fibres and starches from food.

– Measurement of total lipids.

– Determination of faecal nitrogen.

– Chemical examination: pH, organic acids, ammonia, detection of biliary pigments (stercobilin and/or bilirubin) and human haemoglobin.

– Faecal electrolytes and calculation of the osmotic gap.

The combined results of a stool examination must carry a report pointing towards a diagnostic or therapeutic outcome. The qualitative and quantitative data should be taken as a whole and interpreted in the clinical context. This should help define fatty diarrhoea due to malabsorption or maldigestion; secretory diarrhoea; osmotic diarrhoea; motility disorders causing frequent stools; inflammatory diarrhoea; organic or functional constipation; and levels of fat and nitrogen excretion which reflect a poor dietary intake.

INTRODUCTION

Although it is usually easy to recognise the existence of chronic diarrhoea, the diagnosis of aetiology is sometimes difficult, as there are so many possible causes. In this context, the stool examination is designed to assess digestive metabolism in a non-invasive way.

The measurement of the mean daily weight of fresh and of dry stools gives an objective assessment of diarrhoea. The measurements of daily fat excretion looking for steatorrhoea and of 24 hour nitrogen excretion in the search for protein losing enteropathy or for creatorrhoea, are performed for the diagnosis of digestive disorders or for their monitoring during therapy. We thus seek to characterise and to quantify disorders of digestion due to endoluminal abnormalities, such as exocrine pancreatic insufficiency, biliary insufficiency and gastric dysfunction; and disturbances of absorption related to lesions of the small intestinal wall (villous atrophy, infiltration or inflammation of the lamina propria) or to luminal abnormalities (chronic bacterial colonisation of the small intestine etc.). The measurement of faecal electrolytes makes it possible to assess loss with a view to differentiating secretory and osmotic diarrhoeas. Finally, chemical examination of the stools is performed with a view to assessing colonic function.

As for all functional studies of bowel excretion, the results are interpreted in the context of the individual patient: on age, gender and the suspicion that a specific illness is present and needs to be confirmed. Does he/she have a diagnosed and/or treated gastro-enterological condition and, if so, has the effectiveness of treatment or even of compliance with treatment been evaluated? Has surgery previously been performed on the digestive tract?

SEARCH INDICATIONS

Stool examination is the more complete functional analysis of the stool. It is prescribed for the investigation of chronic diarrhoea in order to confirm the nature of the symptoms and to assess whether these are organic or functional, and then to explore the mechanism and even the cause. Such an examination can identify iatrogenic diarrhoea and/or symptoms in the elderly, who are unable to separate urinary and faecal excretion or have alternating diarrhoea and constipation. This examination also has importance in the assessment of nutritional status, because it can define whether food intake is inadequate, particularly in young children and the elderly. In surgical patients these tests can contribute to the evaluation of the absorptive capacity and adaptability of the digestive tract.

INFORMATION

SAMPLE

The results of faecal studies can only be properly interpreted if all the stools produced over a given period have been collected in the appropriate containers, with care taken not to mix stools and urine. With convenience and hygienic considerations in mind, the whole of the 24 hour stool output is collected in one or more opaque containers of sufficient volume (1 litre being the usual size) stored in the refrigerator in plastic bags. Each pot is labelled with the date of collection so that each day's output can be weighed and a mean daily weight for the period of collection can be calculated. It is usual to collect faeces over three consecutive full days, making sure that collection continues through the night. Collection over a single day is probably insufficient, but can be considered in those who display only slight variations in rate of transit and in young children. In the new-born baby, collection must be performed using an "urinocol" type pouch placed over the anus so as to collect stool only and to avoid drying of the stool on the nappy.

As the amount of faecal fat excretion can be under-estimated if the subject reduces oral intake, it is desirable to perform this test under standard conditions. The fatty load can be increased by adding 50 g per day to the usual diet (for example, 5 x 10 g packets of butter), so as to ensure that the daily intake is equal to at least 100 g of fat. This supplementation should commence 3 days before the collection and continue during the 3 days of the collection. Whether supplementation has been employed or not must be specified on the request form and an allowance made for it when analysing the results.

In the 8 days before the collection and during the collection period itself, it is essential to avoid X-ray examinations employing opacification of the digestive tract and preparative

procedures for colonoscopy, which alter the behaviour of the bowel. Over the collection period, care must be taken to avoid administration of agents which might disturb the analysis, such as laxatives including paraffin; agents used for mucosal cytoprotection; or drugs which slow intestinal transit. The consumption of oil containing foods such as peanuts, walnuts, hazelnuts and avocados, is not permitted for 3 days before the collection of stools and during the collection period itself. As they have high intracellular fat content, they interfere with lipid analysis.

■ SAMPLE STORAGE AND TRANSPORT

The whole stool collection (24, 48 or 72 hours) or an aliquot obtained after homogenisation of a 24 hour sample is sent as rapidly as possible to the laboratory together with information on faecal excretion day by day. Samples are stored at + 4°C if the assay is to be performed within 48 hours or at – 20°C if measurements are to be made later.

ASSAY METHODS

– The mean weight of fresh faeces in g/24 hours is determined by weighing the 24 hour stool specimen and the mean is calculated over 2 to 3 days according to the period of collection.

– The amount of dry matter (dry weight) in g per 100 g faeces (%) is measured by weighing an aliquot before and after drying by incubation overnight at 70°C.

– Macroscopic stool examination involves a description of consistency, colour, presence or absence of mucus, of vegetable matter, of undigested food, of frank blood and of parasites.

– An aliquot of faeces is diluted 1/3 in physiological fluid and is examined microscopically under a variety of conditions. Examination is performed directly, both with and without polarised light. Examination of stained material is also carried out using Sudan III dye, Bailenger reagent and Lugol's iodine. Staining is performed with equal volumes of stool and of the reagent. This examination can be used to assess digestive function and to identify abnormal constituents (qualitatively or quantitatively) or iatrogenic material. The majority of these examinations are performed at a magnification of x 10.

– Faecal fats can be assayed either by titration (Van de Kamer's method) or by a gravimetric technique (Jeejeebhoy's method) after fat extraction in organic solvent. The principal technique employed in published series is that of Van de Kamer.

– After mineralisation of the specimen, faecal nitrogen is measured using Kjeldhal's method or by a technique for the detection of mineralised nitrogen by catharometry or by chemoluminescence.

– Human haemoglobin can be detected immunochemically.

– Faecal pigments (bilirubin and stercobilin) are measured colorimetrically.

– For determination of faecal pH an electrometric technique is applied to an aliquot of stool which is diluted 1/10 in demineralised water.

Organic acids produced by bacterial metabolism of carbohydrate and ammonia are assayed by titration.

Sodium, potassium and chloride ions are measured using atomic emission spectrophotometry on a 1/10 dilution of an aliquot of stool in demineralised water.

– Faecal osmolarity is theoretically equal to 290 mosm/l. The faecal osmolar gap is calculated according to the following formula: 290 – 2 x [Na^+ (mmol/l) + K^+ (mmol/l)].

NORMAL EXPECTED VALUES

If the tests have been carried out in conformity with the conditions above (see *SAMPLE STORAGE AND TRANSPORT*), the normal values in the adult are as follows:

– Mean fresh weight: 80 to 200 g/24 hours.

– Dry weight: 18 to 23%.

– Macroscopic appearance: formed brown stools.

– Microscopic appearance: any residual muscle fibres are well digested and any lipid present is in a saponified form. Carbohydrate is present in small amounts in vegetable cells, more or less filled with starch.

– Faecal fat excretion: 2 to 6 g/24 hours.

– Nitrogen excretion: 0.8 to 2 g/24 hours.

– Absence of human haemoglobin.

– Presence of stercobilin.

– pH between 6 and 7.

– Organic acids: 14 to 16 mmol/100 g.

– Ammonia: 2 to 4 mmol/100 g.

– Sodium 1 to 10 mmol/24 hours – potassium: 8 to 20 mmol/24 hours.

– Chloride: 1 to 10 mmol/24 hours.

– Faecal osmolarity: 290 mosm/l.

– Osmolar gap: > 50 and < 125 mosm/l.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Because of physiological variations in bowel transit, it is advisable to collect stools over 3 consecutive days. The 3 day specimen is carefully homogenised and assays are performed on an aliquot of the homogenate. The results are quoted in mean value per 24 hours.

In the healthy subject the administration of laxatives produces acceleration of intestinal transit, which can cause steatorrhoea. This never exceeds 14 g per day. Similarly, in certain diarrhoeas due to idiopathic motility disorders or secretory problems, a moderate increase in fat and protein excretion may be seen.

■ PATHOLOGICAL VARIATIONS

The different elements of stool analysis are dealt with separately (methods, validation and interpretation) but it is vital that the interpretation of any one element takes account of all the available results from the examination of the faeces, especially the mean fresh weight, the dry weight and the microscopic analysis. Interpretation also depends on the knowledge of the medical, surgical and therapeutic context for the patient. This section does not deal with surgical conditions.

– Diarrhoea can be confirmed by determining the mean fresh weight and dry weight of stools: mean fresh weight greater than 300 g/24 hours and/or dry weight less than or equal to 12%. These values can suggest certain mechanisms of organic chronic diarrhoea, because faecal excretion in functional diarrhoea rarely exceeds 500 g per day. An excretion rate greater than 1,000 g per day is suggestive of secretory diarrhoea.

Low stool weight may reflect poor intake or constipation.

– On macroscopic examination of the stool one can define its consistency and demonstrate the presence of abnormal elements such as superficial blood (piles), melena, mucus, parasites, undigested food, vegetable matter or even iatrogenic material (tablets, suppositories, barium, etc.). These findings are taken into consideration in the final report.

– Microscopic examination is useful in differentiating diarrhoea due to malabsorption (normal constituents present but in large amounts) from diarrhoea due to inadequate digestion. In the latter situation, one can identify residual poorly digested muscle fibres and undigested lipids present, in the form of long chain triglycerides, which have the appearance of optically empty lipid droplets. These stain orange with Sudan III reagent and pink/violet with Bailenger reagent. Bailenger's reagent can be used to differentiate between triglycerides of alimentary origin, which stain rose/violet, and laxative mineral oils such as paraffin oil, which do not stain.

Microscopic examination without staining can also be performed to identify signs of colonic problems (streaks of mucus) and the stigmata of inflammatory infiltration of the mucosa: white cells with greater or lesser degrees of damage and/or red cells and/or Charcot-Leyden crystals. Such examination can also detect parasites and drugs which might interfere in assays. The presence of ammonium and magnesium phosphate crystals reveals that the specimen is contaminated with urine. This can lead to a false diagnosis of diarrhoea. There is also an increase in the pH and ammonia.

Staining with Lugol's iodine completes this aspect of the examination. This can reveal starch, which is normally absent or present in only small amounts, but it can also identify bacteria from the caecum or right colon that are not normally seen in the stool. When these elements are seen as well as digestible vegetable cells in large amounts, it is probable that one is dealing with a motility diarrhoea due to accelerated colonic transit.

– Fat excretion greater than 7 g per 24 hours suggests an abnormality of intestinal function. When it exceeds 14 g per day, then a defect in digestive function or malabsorption must be considered likely. There are numerous causes. By considering, in addition, the stool weight, protein excretion and the microscopic examination, it may be possible to differentiate steatorrhoea due to motility problems, which are due to poor digestive function or due to malabsorption. Digestive malfunction is secondary to "endoluminal" problems, such as pancreatic exocrine insufficiency, biliary insufficiency or impaired gastric function. Malabsorption can be due to abnormalities of the small intestinal wall such as villous atrophy, infiltration of the bowel wall or local inflammation. It can also result from abnormalities within the lumen of the small bowel such as bacterial overgrowth.

– Reduction in mean stool weight and fat and protein excretion reveals a deficiency in nutritional intake.

– A reduction in pH points towards colonic fermentation of carbohydrates which have not been absorbed in the small bowel. If this is associated with an increase in organic acids derived from bacterial induced metabolism of carbohydrates, then an isolated defect in carbohydrate absorption is strongly suggested, provided that the sample has been correctly stored, so as to prevent bacterial fermentation *in-vitro*.

– An isolated increase in pH and ammonia suggests urinary contamination of the stool or an entero-vesical fistula.

– Abnormalities in electrolyte values point to alteration of electrolyte secretion in the colon or small intestine. This may be a reflection of a disease process or of previous surgery.

– An osmolar gap less than 50 mosm/l is characteristic of secretory diarrhoeas such as microscopic colitis, endocrine diarrhoea, villous tumour and that due to use of non-osmotic laxatives.

– An osmolar gap greater than 125 mosm/l characterises osmotic diarrhoea. This can be due to malabsorption of a carbohydrate or may be iatrogenic secondary to the use of osmotic laxatives such as lactulose, lactitol, sorbitol, mineral salts or macrogols (PEG).

– An abnormality restricted to the qualitative elements of the examination may suggest a functional intestinal problem (with normal excretion of nutriments) which can be accompanied by signs of increased motility (bilirubin and the presence in stool of material derived from carbohydrate).

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STRONGYLOIDIASIS

DEFINITION

Strongyloidiasis is a human intestinal parasitic infection which affects 30-60 million people in the world. It is caused by *Strongyloides stercoralis* which belongs to the class of Nematoda and the super-family Rhabditoidea.

Adults: 2-3 mm in length.

– The parthogenetic female is strongyloid (with a single oesophageal bulge), measures about 2.5 mm, and lives in the intestinal mucosa.

– The non-parthogenetic female is rhabditoid (with two oesophageal bulges), measures 1 mm, and lives free in the external environment.

The ***egg*** has a fine, transparent shell and measures 50 x 30 µm. It resembles the eggs of Ankylostoma.

Larvae:

– The *rabditiform L1 larva* measures about 250 µm in length and has a double oesophageal bulge.

– The *filariform L2 larva* has only a single oesophageal bulge and measures 500 µm.

– The *infective filariform L3 larva* measures 600 µm, and has a single oesophageal bulge and a double-branched posterior.

INTRODUCTION

EPIDEMIOLOGY

The disease is transmitted via human faeces and humans are the main reservoir for the parasite.

– *Strongyloides stercoralis* is endemic in the tropics and sub-tropical regions (the Caribbean and Central Equatorial Africa) but it is also found in the temperate zone (southern Europe, southern USA).

– The usual route of infection involves a larva penetrating the skin when the victim comes into contact with contaminated soil or water (swimming, walking through mud, etc.).

LIFE CYCLE

Man is the definitive host.

Adult parthogenetic females reside in the intestine where they lay unembryonated eggs which transform into rabditiform larvae (L1) which are excreted in the faeces.

The subsequent events depend on external conditions. In conducive conditions, a long sexual cycle commences; and, if conditions are adverse, an asexual cycle begins. There is also a third endogenous alternative which leads to autoinfection.

Long sexual cycle

If the external temperature is 20°C and the environment is damp, the larvae (L1) directly transform into male or female rabditiform adults. The females lay eggs which give rise to second-generation rabditiform larvae and then infective filariform larvae (L3). After penetration of the skin, the L3 larvae enter the host's circulation and are carried to the lungs. They pass into the airways and up to the pharynx from where they reach the small intestine to become parthenogenetic females.

Short asexual cycle

If external conditions are unconducive, the L1 larvae transform directly into infective L3 filariform larvae.

Endogenous autoinfection cycle

This occurs inside the intestine.

L1 larvae directly transform into infective filariform larvae (L3) inside the intestinal lumen or at the anus (without any external maturation step). This results in the phenomenon of autoinfection which underlies longstanding infection with the parasite *(see the Summary Table below)*.

SYMPTOMS

Sub-clinical, asymptomatic forms are common.

Clinical forms progress through three distinct phases:

Invasion phase

The filariform larvae penetrate the skin causing itching with a variable degree of papular erythema lasting 2-3 days. The infection event often passes without being noticed.

Pulmonary phase

The larvae migrate to the lungs and cause coughing or asthma-like shortness of breath, even sometimes Loffler's syndrome.

Chronic phase

The worms install in the duodenum 20-30 days after initial infection.

– Systemic signs: Lassitude, loss of appetite, weight loss or growth retardation in children.

– Gastrointestinal symptoms: Duodenitis, stomach ache, diarrhoea alternating with constipation, nausea and vomiting.

– Dermatological symptoms: Urticaria, perianal pruritus, *larva currens* or transient waves of skin rash on the buttocks, thighs or abdomen. These are seen in endogenous autoinfection.

– Pulmonary symptoms: Coughing and asthma attacks.

Disseminated strongyloidiasis

In subjects who are immunodepressed (due to AIDS, leukaemia or immunosuppressive drugs, e.g. following transplantation,) the parasite load may become very heavy with dissemination throughout the body. This is due to exuberant expansion of the endogenous autoinfection cycle and causes a severe clinical picture with massive diarrhoea, impaired lung function, and septicaemia or meningitis due to Gram-negative bacilli being carried by the worms. More than 80% of victims die which is why it is so important to prevent this complication.

SEARCH INDICATIONS

– In a subject who has visited an endemic zone with suggestive symptoms and/or hypereosinophilia.

– As part of a systematic work-up to investigate unexplained eosinophilia.

– Before the prescription of an immunosuppressive drug (notably for organ transplantation) or a long-term course of a corticosteroid, or in an HTLV-positive subject from Africa or the Caribbean.

INFORMATION

SAMPLE

Whole blood: Draw into EDTA for a full blood count.

Faeces: Collected into a clean, dry recipient, to detect larvae.

Serum: For serology and a total IgE assay.

Other samples: If disseminated strongyloidiasis is suspected then sputum, duodenal aspiration biopsy, bronchial lavage, BAL, CSF and urine samples may be taken.

QUESTIONS FOR THE PATIENT

Symptoms and date of onset?

Have you visited an endemic region?

Any reason for immunosuppression?

Are you taking any drugs for roundworm infection?

SAMPLE STORAGE AND TRANSPORT

Serum: At + 4°C or frozen at –30°C for up to one year.

Faeces: Preferably passed at the laboratory; otherwise, store at room temperature.

DIAGNOSTIC METHODS

NON-SPECIFIC DIAGNOSIS

Hyperleukocytosis with hypereosinophilia, with potentially very high counts (10-30.10^9/l) during the invasion phase. The counts follow a saw-tooth pattern with peaks corresponding to waves of larval migration.

Total IgE: Elevated.

DIRECT DIAGNOSIS

By analysing faecal material for *Strongyloides stercoralis* larvae, this is only possible during the chronic phase.

Direct examination

Larvae will only be observed in a lump of faecal material squashed between microscope slide and cover slip if the patient is very heavily infected because female worms lay a small number of eggs and only do so irregularly. Eggs may be detected in such an examination if intestinal transit is particularly fast.

Current concentration methods

Ritchie, Bailanger, Junod, etc. are relatively ineffective when it comes to detecting larvae.

Baermann method

An enrichment/extraction method based on the larva's tropism for warm water. It should be performed using freshly passed, soft stools. This is the method of choice for the detection of *S. stercoralis* larvae.

Coproculture: On agar or carbon and is only relevant if the cycle is sexual.

INDIRECT DIAGNOSIS

The immunoenzymatic, indirect immunofluorescence and agglutination techniques available are based on antigens from larvae of *S. stercoralis or S. ratti*. There is cross-reactivity with other nematodes (filaria, ascaris, anisakis, etc.).

INTERPRETATION OF RESULTS

Strongyloidiasis is often diagnosed in a patient who presents hypereosinophilia after visiting in an endemic region.

Serological results lack specificity and can only be used to establish a presumptive diagnosis.

A parasitological examination makes it possible to establish a definitive diagnosis, as long as only freshly passed faeces are examined and a series of several successive examinations are carried out two or three days apart (if the results are all negative).

TREATMENT

ANTIHELMINTH DRUGS

A number of drugs are effective:

– Ivermectin (the drug of choice)

– Albendazole

– Thiabendazole

The efficacy of treatment should be checked one, six and twelve months after the beginning of treatment, by confirming the normality of the eosinophil count and the absence of larvae (by parasitological examination).

PREVENTION

– General: Management of the risk of faecal contamination through hygiene measures.

– Individual: Avoid direct contact with water and soil by wearing footwear.

FOR FURTHER INFORMATION

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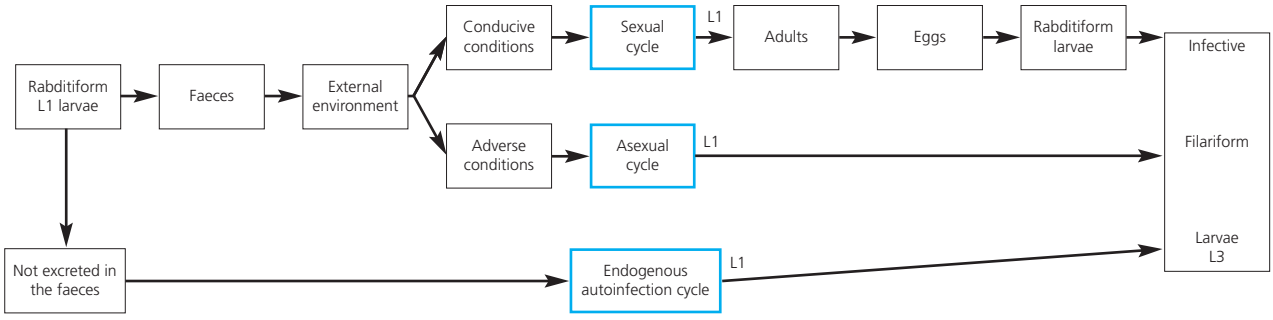
Gentilini M., Duflo B., *Anguillulose*. Dans : Médecine tropicale, Paris: Flammarion, 1999 :188-191.

The three Strongyloides stercoralis life cycles



STYRENE

DEFINITION AND SYNONYM

Styrene is a monocyclic aromatic hydrocarbon and a higher homologue of benzene. It is used as a solvent or synthetic intermediary in plastic manufacture.

Synonyms: vinylbenzene, ethenylbenzene, phenylethylene.

INTRODUCTION

The main route of industrial styrene absorption is through the lungs (60% of inhaled styrene is absorbed), although it is also very well absorbed through the skin in liquid form. It is mostly metabolised in the liver by cytochrome P450 mono-oxygenases into an intermediary metabolite, styrene – 7,8-oxide. The intermediate metabolite is then detoxified through two pathways, a predominant pathway leading to production of the styrene glycol which is metabolised into mandelic acid, phenylglyoxylic acid, benzoic acid and hippuric acid; the second very minor pathway (< 1% of absorbed styrene) involves glutathione conjugation producing mercaptupuric acids. More than 90% of the styrene is excreted as metabolites in the urine, only 3% being excreted through the lungs and a very small amount in the unchanged form in urine or sweat. Mandelic acid is excreted in a biphasic manner with half-lives of 4 hours and 18 hours; the half-life of phenylglyoxylic acid is longer (7 hours). Almost all is removed over four days, although minimal accumulation may occur during the working week in people exposed through their work.

The features of acute toxicity are mostly due to the irritant and central nervous system depressant nature of styrene (concentration difficulties, muscle weakness, nausea, irritation of the eyes, nose and throat): inhalation pneumonia may also occur following inhalation. Chronic toxicity from repeated exposure is characterised by organic mental disorders, respiratory tract irritation, sensory peripheral neuropathy, irritation dermatitis, haematological problems developing, after several months to years of exposure (thrombocytopaenia, leukopaenia, then anaemia due to bone marrow aplasia), gastro-intestinal and renal problems, mutagenicity, carcinogenicity, and reproductive disorders.

INDICATIONS FOR MEASUREMENT

Styrene, and particularly its urinary metabolites (urinary mandelic and phenylglyoxylic acids) are measured for the preventive purposes and to monitor occupationally exposed workers. Guideline values are available: mandelic and phenylglyoxylic acid concentrations in an end of shift urine sample reflect exposure during the same and two previous days. In view of the large inter-individual variations, results must be interpreted from a group of workers. Combined measurement of mandelic acid and phenylglyoxylic acid is recommended as these concentrations appear to correlate well with health (particularly in neurological damage). It should be noted that following extensive exposure (more than 150 ppm, or three times the maximum guideline exposure value), styrene metabolism becomes saturated, reducing the utility of mandelic and phenylglyoxylic acid measurement.

Measurement of styrene in blood immediately at the end of the working shift reflects exposure on the same day, whereas if the sample is taken before the working shift, concentrations reflect exposure on the previous day. Styrene levels in blood correlate well with the extent but not duration of exposure. Styrene measurement in an end of shift urine sample has been proposed as an indicator of exposure and is believed to be the parameter which correlates best with air styrene concentrations to be specific and not influenced by exposure to other solvents. This marker however requires further investigation before being validated. Styrene measurement in expired air immediately at the end of the working shift appears to reflect very recent exposure, although this marker is not widely used in practice.

INFORMATION

SAMPLE

Blood measurements are performed on whole blood collected into EDTA.

Urine samples (10 ml) for styrene measurement are collected into a glass bottle with a PTFE closure. Urine samples for assay of metabolites (15 ml) are collected into an unused polyethylene or polystyrene bottle at the end of shift at the end of the week. Patients should be informed to avoid drinking alcohol on the day of the sample as alcohol slows and reduces excretion of the two urinary styrene metabolites.

QUESTIONS FOR THE PATIENT

Mandelic acid and phenylglyoxylic acid must be interpreted taking account of possible interferences: be aware of alcohol ingestion which reduces their excretion, cutaneous absorption, medicines, co-exposure to solvents metabolised into mandelic acid (ethylbenzene, phenylglycol, etc.) and exposure to other solvents (acetone, toluene, benzene, xylene, etc.) because of competitive inhibition.

SAMPLE STORAGE AND TRANSPORT

Samples can be stored and transported for a few days at + 4°C.

ASSAY METHODS

The assay methods available for styrene are mostly head space gas chromatography or gas chromatography linked to mass spectrometry.

Assay methods for the urinary metabolites are chromatographic (HPLC).

NORMAL EXPECTED VALUES

The French guideline value for styrene (limit value) in blood is 550 µg/l at the end of shift (20 µg/l before the start of the shift).

End of shift urinary styrene values are in the region of 85 µg/ g of creatinine. No guideline value for styrene in urine has been established.

The French guideline value for mandelic acid is 800 mg/g of creatinine at the end of shift (300 mg/g of creatinine before the start of the shift).

The French guideline value for phenylglyoxylic acid is 240 mg/g of creatinine at the end of shift (100 mg/g of creatinine before the start of the shift).

FOR FURTHER INFORMATION

- Fiche Biotox : *Styrène*. www.inrs.fr

SYPHILIS

DEFINITION

Syphilis or the pox, is a highly contagious infectious disease due to a spirochete, *Treponema pallidum subsp pallidum* (pale treponema), which belongs to the Spirochete order and *Treponemaceae* family. Spirochetes are mobile, spiral helicoidal bacteria (Length: 8 to 14 µm and width: 0.15 to 0.20 µm).

INTRODUCTION

Syphilis is generally transmitted by sexual intercourse and more rarely across the placenta (congenital syphilis). Transfusion risks are avoided by systematic donor screening. After the epidemics reported in the 1990s in the USA and in eastern countries, the worrying rise in cases calls for greater awareness of the infection.

SYMPTOMS

Untreated syphilis progresses classically in several stages:

- **Silent incubation period** (approximately 3 weeks). This is the phase in which the treponema develop in cutaneous or mucosal epithelium at the primary infection site. The serological diagnosis is still negative at this stage.
- **Primary syphilis** (1 to 2 months): development of the painless chancre (highly contagious ulceration) and satellite lymphadenopathy. Serological tests become positive (IgM followed by IgG).
- **Secondary syphilis** (6 months to 1 year): the dissemination phase to all tissues with successive flares of highly contagious mucosal erosions and maculo-papular skin rashes (lymphadenopathy, fever, etc.) All serological tests are strongly positive.
- **Tertiary syphilis** or late symptomatic stage (several years after infection): a third of people develop skin or visceral cardiovascular or neurological lesions.

  Congenital syphilis is secondary to foetal infection across the placenta. It is increasingly severe with more recent maternal syphilis and the closer it is to childbirth. The following types are distinguished:

  – Early congenital syphilis occurs from birth up to the age of 2 years old (80% of diagnoses are made in the 1st year of life).

  – Late congenital syphilis develops after 2 years, usually between 8 and 10 years old.

INDICATIONS FOR MEASUREMENT

Signs of primary (chancre), secondary (maculo-papular skin rashes) or tertiary syphilis.

Serology in pregnant women.

INFORMATION

SAMPLES AND TRANSPORT CONDITIONS

Smear of serous fluid from a genital ulcer. In a healed ulcer: make scars across the ulcer with a vaccine needle.

Serum (5 ml of whole blood collected into a dry tube): transport at + 4°C.

CSF (for meningitis or encephalitis): transport at + 4°C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

Direct testing is only performed in primary and secondary lesions. The test must be performed before any antibody treatment is given.

- **Dark background microscopy.** This is a test on fresh samples between slide and cover slip. The treponema are mobile with regular refringent spira, contrasting sharply against the dark background. This method lacks specificity particularly from the oral, anal or rectal mucosa, where non-pathogenic Spirochetes may be found. Its major use is in providing a rapid diagnosis.
- **Direct immunofluorescence:** Uses a monoclonal antibody reacting with the *T. pallidum* 47 kDa antigen. The use of this method is that it can be applied to fixed smears, which do not require dark background microscopy.
- **Silver impregnation staining (Fontana-Tribondeau).** This is decreasingly used.

SEROLOGICAL DIAGNOSIS

As *T. pallidum* cannot be cultured *in-vitro*, the laboratory diagnosis of treponematoses is mostly serological.

Diagnostic tests use a combination of methods with non-treponema (cardiolipin) antigens which detect anti-phospholipid antibodies, combined with treponema antigens which detect antibodies specific to pathogenic treponema. The combination of TPHA and VDRL (or RPR) is used to diagnose primary or secondary syphilis with a sensitivity of 84%. Indirect immunofluorescence or FTA-abs generally provides early confirmation of a positive TPHA.

The serological diagnosis of syphilis is well characterised in France: two tests are mandatory, with at least one from each group (group 1: VDRL latex, VDRL stain, VDRL charcoal; group 2: TPHA, FTA abs, EIA). If the reactions are positive or inconsistent, each group must be titred combined with testing for specific IgM.

- **Group 1:** test using non-treponema antigens (cardiolipin).

VDRL *(Venereal Disease Research Laboratory)* uses an antigen produced from a suspension of cholesterol microcrystals onto which cardiolipin molecules are adsorbed. Antibodies develop over 7 to 10 days after formation of the chancre. The quantitative VDRL is inexpensive and fast and is the usual test to evaluate response to treatment. It becomes negative approximately 1 year after treatment of primary syphilis and 2 years after treatment of secondary syphilis. False positive reactions may occur in patients with anti-phospholipid antibodies or in different inflammatory syndromes.

■ **Group 2** tests using treponema antigens

TPHA *(Treponema Pallidum Hemagglutination Assay)*. This is a passive haemagglutination reaction with an antigen obtained from a lysate of *T. pallidum* adsorbed onto red blood cells. Results are expressed qualitatively as (+), or quantitatively as the reciprocal of the dilution. The test becomes positive slightly later than the VDRL and FTA, 10 to 15 days after development of the chancre. TPHA antibody titres are not significantly influenced by antibiotic therapy.

FTA-abs *(Fluorescent Treponemal Antibody test-Absorbed)*. The antigen is obtained from a preparation of *T. pallidum* fixed onto a slide. The titre is expressed as the reciprocal of the last dilution which produces a positive result. This is the earliest and longest lasting reaction in untreated or late treated syphilis and allows an IgG or IgM response to be identified using human anti-IgG or IgM labelled with fluorescein. The utility of IgM is that it identifies active infection, congenital syphilis and neuro-syphilis. The presence of IgM in CSF confirms the diagnosis of neurosyphilis.

ELISA (immunoenzymatic methods)

These use native or recombinant treponema antigens fixed onto the solid phase of a micro-titre plate. Different studies have shown these to offer excellent sensitivity and specificity compared to the TPHA at all stages of infection. The automatable tests are particularly suitable for qualitative screening of large numbers of samples. Their use is limited by the high reagent cost compared to its test tariff in France.

■ **IgM antibodies**

Two methods are used to detect anti-treponema IgM:

– FTA-abs IgM on whole serum or 19S (IgM) FTA-abs on serum fractionated by ultracentrifugation or gel filtration,

– ELISA immunoenzymatic IgM methods after IgM immunocapture.

A finding of positive IgM indicates active infection. IgM is the earliest serological marker and is found variably in the tertiary stage of the disease. IgM in a patient with untreated latent syphilis suggests infection which is still active. The IgM disappears with effective treatment. The finding of an anti-treponema IgM in a newborn indicates congenital syphilis, although a negative result does not exclude the diagnosis. IgM found in CSF confirms the diagnosis of syphilis and requires treatment in a patient suspected of neuro-syphilis whose meningeal barrier is intact (normal CSF/serum albumin ratio).

■ **Nelson test**

This involves placing a suspension of living *T. pallidum*, complement-stripped patient serum and guinea pig complement in contact. By binding complement, serum IgG causes immobilisation and lysis of the treponema. The Nelson test only becomes positive in the secondary phase of syphilis and shows high antibody titres (1,000 to 2,000) which fall very slowly during latent and late treated or untreated phases. It is used to confirm advanced syphilis but offers limited information about whether the infection is active or not. It is a difficult test to perform (requiring an animal house, maintenance of the strain and the method lacks reproducibility). It is now only very rarely performed.

■ **Immunoblot or Western Blot**

Highly specific serological confirmation can be obtained by characterising the immunodominant antigens of a lysate of *T. pallidum*, separated by electrophoresis using Western Blot or immunoblot (recombinant proteins fixed to a test strip). The most specific proteins are the 15 kDa, 17 kDa, 47 kDa and transmembrane TmpA proteins. Studies have shown Western Blot to offer excellent sensitivity compared to TPHA at all stages of infection, particularly primary infection. It also appears to offer very good specificity vis-à-vis serum containing antibodies liable to cause cross-reactions (cardiolipins, rheumatoid factor and nuclear antibodies, only VDRL positive etc.). Note however that the Western Blot does not distinguish endemic treponematoses from syphilis any more than the other methods. Western Blot can be now proposed as the specific confirmatory test when "classical reactions" are equivocal or inconsistent (for example in autoimmune diseases). The major use of the Western Blot is to identify characteristic profiles of stages of the infection, which are difficult to distinguish using current techniques (latent syphilis, serological recovery).

INTERPRETATION

The different situations, which the laboratory specialist may encounter in performing diagnostic treponema tests are shown in table 1. Whilst the diagnosis of active secondary infection is generally straightforward because of the very high antibody titres, interpretative difficulties arise when serologies are moderately positive when, in the absence of clinical information, it is often difficult to differentiate between latent syphilis and serological recovery of previously treated syphilis. It would appear wise to confirm a positive TPHA with an FTA abs or, depending on the context, using Western Blot. It is then important to exclude active infection by confirming that specific IgM is not present. The decision to treat will depend on a combination of many factors including the patient history (history of previously treated or untreated syphilis), symptoms, age and sex (young women of child-bearing age, etc.), antibody titre, VDRL positivity etc. A diagnostic algorithm is offered in figure 1.

In conclusion, syphilis and the endemic treponematoses remain a worldwide public health problem despite differences in prevalence between countries and disparities in the quality of medical care. Lack of knowledge about the diagnostic and monitoring methods risks serious forms of the disease emerging, such as congenital syphilis and neurosyphilis. *T. pallidum* is still sensitive to penicillin and it should therefore be possible to envisage eradicating the treponematoses, the only known reservoir of which is human beings.

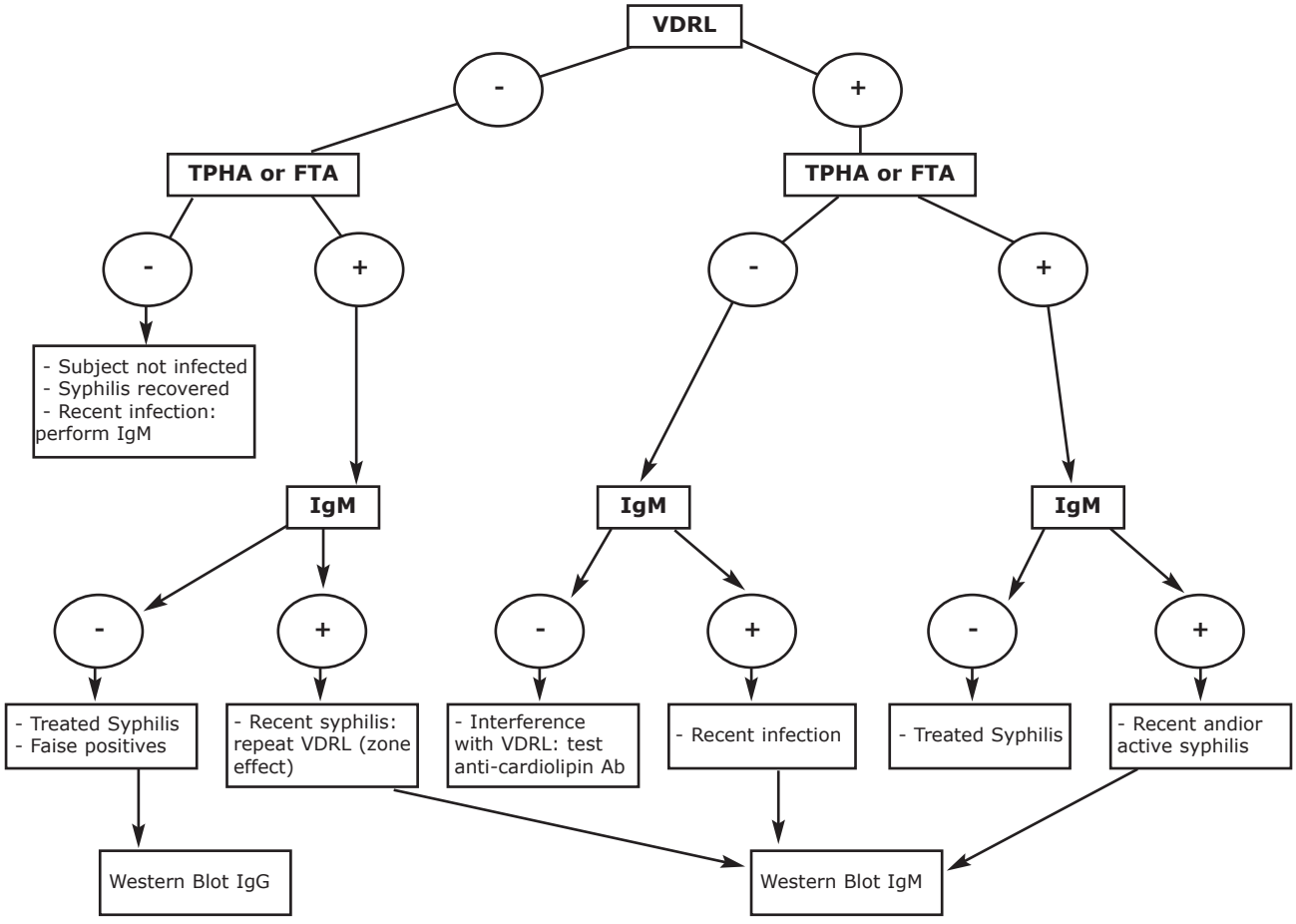
Table 1: Change in antibodies in the different stages of syphilis.

Stage of infection	IgM (FTA)	FTA-ABs	VDRL	TPHA
Early primary	10 to 80	200 to 400	Negative	Negative
Advanced primary	80 to 320	800 to 3200	2 to 8	80 to 320
Secondary	320 to 640	12800 to 81900	16 to 4096	10240 to > 1 million
Untreated latent	0 to 80	3200 to 12800	8 to 12	1280 to 10240
Tertiary	0 to 0	3200 to 51200	16 to 28	10240 to 655360
Recovery	0	400 to 3200	0 to 4	160 to 1280

From AP HAMELIN, Abrégés, Maladies sexuellement transmissibles, 1991:13-49.

Figure 1

Laboratory algorithm for the serological diagnosis of syphilis



FOR FURTHER INFORMATION

■ *Le point sur la syphilis*, numéro spécial, BEH n° 35-36 (28 août 2001). http://www.invs.sante.fr/beh/2001/35-36/index.htm

TACROLIMUS

DEFINITION

Tacrolimus is an immunosuppressant which inhibits calcineurin and is used in the treatment of refractory corticosteroid-resistant rejection of any organ and in the preventative and curative treatment of renal and hepatic transplant rejection.

Synonym: FK 506.

PHARMACOKINETICS

Bioavailability (oral form)	10 to 20%: reflux intestinal glycoprotein P and pre-systemic metabolism by cytochrome P450 3A4 (CYP 3A4).
Concentration peak	1 to 3 hours.
Half-life	In healthy people: 43 hours In liver transplant patient: approximately 12 hours In renal transplant patient: approximately 15 hours
Protein/red cell binding	Binds to albumin and alpha-1 glycoprotein; extensively incorporated into red blood cells.
Metabolism	Extensive and varies according to intestinal and hepatic CYP 3A4. Eight identified metabolites, the predominant one being a 13-O-demethylated metabolite which however has minimal pharmacological activity (6.4% of that of tacrolimus).
Elimination	Bile (enterohepatic cycle).

METHODS OF ADMINISTRATION

Average oral dosage: 0.1 to 0.3 mg/kg/day in 2 doses, strictly fasting, 1 hour before or 2 hours after meals.

Injectable: administered by continuous intravenous infusion of 0.01 to 0.03 mg/kg/day.

INDICATIONS FOR MEASUREMENT

The need for regular blood tacrolimus measurements during treatment is stated in the summaries of product characteristics (SPC) of the marketing authorisation (MA) for the following reasons:

– Intra and inter-individual variability of blood concentrations (low bioavailability and extensive variable metabolism by CYP 450 3A4).

– Potent potentially toxic immunosuppressant (narrow therapeutic margin): a correlation has been shown between blood concentrations and therapeutic effect and between blood concentrations and toxicity. Tacrolimus is nephrotoxic, neurotoxic and diabetogenic. In addition, excessive immunosuppression may predispose and maintain infections and proliferative disease.

– Numerous therapeutic interactions alter the metabolism of tacrolimus (risk of lack of effect or overdose). In general, these interactions are superimposable on those of cyclosporin. However, the signs of toxicity are approximately 3 times more severe with tacrolimus than cyclosporin. The earliest sign of a therapeutic interaction with tacrolimus is an increase in plasma concentrations of the drug (before the rise in serum creatinine).

INFORMATION

SAMPLE

Whole blood sampled into an EDTA tube with separator gel. Heparinised tubes must not be used.

– Sample taken for measurement of trough tacrolimus concentrations (C0), immediately before the next dose.

– Possibly, a 2 hour sample (± 10 minutes) after the dose (C2), or possibly pharmacokinetic analysis (combined 0-4 h or 0-12 h samples to estimate the AUC).

Trough tacrolimus concentrations must be monitored early when starting immediate post-transplantation treatment and then throughout treatment.

QUESTIONS FOR THE PATIENT

Any request for drug measurement must include the reasons for the request (testing for efficacy or toxicity), the sampling time, treatment start date and any change in dosage, dosage information (amount administered, frequency and route of administration), together with the age, height and weight of the person, wherever possible.

State the type of transplant and time from the transplant, together with the date when treatment was started?

Renal function (serum creatinine)?

Current treatments at risk of causing therapeutic interactions with tacrolimus?

A) Medicinal products (or substances) which increase blood tacrolimus concentrations

Macrolides and related substances, such as erythromycin and clarithromycin.

Azole antifungals, such as ketoconazole, itraconazole and fluconazole.

Calcium channel blockers, such as nicardipine, nifedipine, diltiazem and verapamil.

HIV anti-proteases, such as ritonavir, nelfinavir, indinavir, saquinavir, amprenavir and lopinavir.

Voriconazole and danazol.

Grapefruit juice (through inhibition of Cytochrome P450 3A4).

B) Medicinal products which reduce blood tacrolimus concentrations

Antiepileptics, such as carbamazepine, phenobarbital, phenytoin and primidone.

Rifampicin and rifabutin.

Non-nucleoside anti-retroviral agents, such as efavirenz and nevirapine.

St. John's Wort.

SAMPLE STORAGE AND TRANSPORT

Transport and store for up to 7 days at room temperature or at + 4°C, and at – 20°C beyond this time.

ASSAY METHODS

Immunoassay.

NORMAL EXPECTED VALUES

The C0 therapeutic ranges vary depending on the type of transplant, post-transplantation time and clinical and pathological state of the person.

The result must be interpreted taking account of clinical situations and concomitant medications altering blood tacrolimus concentrations.

The values below are given for indicative purposes:

Minimum effective trough concentration: 5 ng/ml or 10 ng/ml immediate post-transplantation (higher risk of rejection).

Toxicity threshold > 20 ng/ml.

Therapeutic margin: tacrolimus associated with corticosteroids and azathioprine or mycophenolate mofetil

– Adults: 10-15 ng/ml from D0 to D42, then 5 to 10 ng/ml

– In children: 10 -20 ng/ml early post-transplant then 5 to 15 ng/ml.

FOR FURTHER INFORMATION

■ Dictionnaire Vidal®.

■ Billaud E., Garaffo R., Royer-Morrot M.J., *Suivi thérapeutique du tacrolimus*. In : Suivi thérapeutique pharmacologique pour l'adaptation de posologie des médicaments, Collection Option/Bio, Ed Elsevier, Paris, 2004 : 295-303.

TAENIASIS

DEFINITION

The taeniases are strictly human helminthiases in which human beings are the final host. Taenia are cestodes or flatworms belonging to the *Plathelminthes* branch of the *Cyclophyllidae* family, *Taeniidae* sub-family.

– The intermediary host for *Taenia saginata* is the cow.

– The intermediary host for *Taenia solium* is the pig. Taenia are better known by the name of "solitary worms", as a single worm is found in human beings in most cases. They are large cestodes with segmented bodies made up of a chain of 500 to 2000 rings extending a scolex, which carries 4 suction pads. The terminal rings have a branched uterus with no laying orifice. *T. saginata* measures 4 to 10 metres long. *T. solium* is shorter and its head has 2 crowns of hooks (armed taenia).

This section only examines *T. saginata*. *T. solium* is considered in cysticercosis.

INTRODUCTION

EPIDEMIOLOGY

Taenia saginata is cosmopolitan and infection occurs from eating beef. It is particularly common in tropical Africa and Asia. This form of taeniasis is relatively common in France from eating raw or inadequately cooked beef

LIFE CYCLE

Taenia saginata is a small intestine parasite in human beings. Once they mature, the final rings detach from the chain and pass through the anal sphincter reaching the external environment where they release eggs or embryophores containing a hexacanth (6 hooked) embryon. Once released into nature the embryophores are eaten by the intermediary host (cow), in which they cross the bowel wall, entering the circulation and reaching the muscles where they change into cysticerci or vesicular larval forms filled with fluid and containing the scolex of the future adult taenia. Human beings become infested by eating raw or poorly cooked beef or ingesting a cysticerci larva, which changes into an adult taenia in 2 to 3 months. The rings of the adult then detach at maturity.

SYMPTOMS

The symptoms of *T. saginata* are predominantly gastro-intestinal, and are often relatively well tolerated. The symptoms include epigastric cramps, nausea, abdominal pain, bulimia or anorexia and diarrhoea. These may be associated with systemic problems such as palpitations, change in character and sleep disturbance, although in most cases the diagnosis is made from the appearance of rings in faeces or the patient's bed linen as the clinical features are often asymptomatic.

SEARCH INDICATIONS

– Diagnosis of *T. saginata* taeniasis in a person with suggestive clinical signs, particularly when rings are discovered in the patient's stool or bed linen.

– Differential diagnosis from *T. solium* taeniasis.

INFORMATION

SAMPLES

– Anal sample taken with adhesive cellophane (Scotch tape-test): performed at the end of the afternoon.

– Serum samples.

USEFUL INFORMATION

– Clinical symptoms? Have rings been seen in bed linen or faeces?

– Dietary habit (beef)?

LABORATORY DIAGNOSIS

– ***Non-specific diagnosis:*** eosinophilia is common and relatively mild with occasionally high levels initially.

– ***Parasitological diagnosis:*** this is based on examining rings found in the patient's bed linen or faeces which are examined for their transparency on microscopy. The first rings appear in faeces approximately 3 months after the infecting meal. The diagnosis can also be made from an anal sample using the "scotch test" method revealing embryophores remaining attached to the anal margin when the rings force their way through the anal sphincter. The species identification is obtained using China ink staining which identifies the many uterine branches in *Taenia saginata*. It is possible to detect eggs or antigens by molecular biology methods although this is not routinely performed.

– ***Indirect diagnosis:*** serological diagnosis is available but is not used diagnostically.

TREATMENT

Treatment is with niclosamide 2 g as a single dose (adults and children over 6 years old; 1 g between 2 and 6 years old), or praziquantel, 10 mg/kg as a single dose.

The most effective prophylaxis is avoiding eating raw or inadequately cooked meat.

FOR FURTHER INFORMATION

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■ Gentilini M., *Cestodoses*, Médecine-Sciences Flammarion ; p 243 à 245.

TATI

DEFINITION – INTRODUCTION

TATI (Tumour-associated trypsin inhibitor) is a low molecular weight polypeptide (6 kDa), initially isolated from the urine of women suffering from ovarian cancer. Because of its structural similarity with pancreatic secretory trypsin inhibitor (PSTI), it is able to inhibit trypsin, hence its name.

In serum, it has a short half-life in the region of 6 minutes and is removed by the kidneys. TATI is normally measured in blood and occasionally in some aspiration fluids, notably from ovarian cysts (TATI concentrations in these fluids do not however always correlate with serum concentrations).

A rise in serum concentrations has been described in several types of cancer, particularly urological (bladder or kidney), gynaecological cancers in women (notably ovarian, cervical and endometrial cancers) and in gastro-intestinal cancers (pancreas, stomach, liver, biliary tract and colon).

INDICATIONS FOR MEASUREMENT

To determine prognosis and monitor treatment in patients suffering from mucinous ovarian cancers, bladder or renal and some gastro-intestinal cancers.

Because of its low molecular weight and renal excretion, it has been proposed as a marker of renal function.

INFORMATION

SAMPLE

Blood sample taken into a dry tube (serum) or EDTA or heparinised tube. A fasting sample is not necessary.

QUESTIONS FOR THE PATIENT

In the context of cancer, type of tumour and current treatment, such as chemotherapy, radiotherapy and surgery (types and dates of treatment).

SAMPLE STORAGE AND TRANSPORT

Store and transport at + 4°C for up to 48 hours. Freeze beyond this time.

ASSAY METHODS

Radioimmunoassay.

NORMAL EXPECTED VALUES

For reference: Serum TATI: < 15 µg/l.

PATHOLOGICAL VARIATIONS

Increased serum and/or urinary TATI concentrations in oncology

– TATI is occasionally proposed as a complementary marker to CA 125 in monitoring mucinous ovarian cancers. It has a sensitivity in this disease of around 45% at stage I and between 95 and 100% at stage IV.

– It has high sensitivity (75 – 95%), but low specificity in pancreatic cancer.

– It is raised in approximately 2/3 of patients with renal carcinoma and serum concentrations correlate with the stage of the disease.

– It is of prognostic value in gastric cancers (a high pre-operative serum concentration is a poor prognostic indicator) and in bladder cancers. A recent study in 157 patients followed up for 10 to 12 years has shown that patients whose serum TATI was initially raised (> 21 µg/l in this study) had a significantly shorter survival than patients with normal serum TATI concentrations ($p < 0.001$).

Increases in other diseases

– It may be increased in pancreatitis and also in cirrhoses or biliary obstruction (cross-reaction with PSTI, released into the circulation in these situations). Unlike PSTI, TATI however is normal in pancreatectomised patients.

– In renal insufficiency *(cf. below)*.

– Raised serum TATI concentrations have also been reported in multiple myeloma and other malignant blood dyscrasias.

TATI as a marker of renal function

Tramonti G *et al.* compared TATI to β2 microglobulin and creatinine and demonstrated its utility as a marker of glomerular filtration rate (GFR). This study, which was conducted on 198 patients, reported a significant rise in serum TATI concentrations in patients with renal dysfunction. Serum TATI concentrations in patients whose GFR was < 20 ml/min were 13 times higher than in patients whose GFR was > 100 ml/min (β2 microglobulin was increased by a factor of 8 and creatinine by a factor of 5). The authors concluded that serum TATI concentrations rose earlier and more than β2 microglobulin or creatinine when glomerular filtration was reduced and proposed TATI as a marker to assess renal function.

FOR FURTHER INFORMATION

- Stenman U.H., *Tumor-associated trypsin inhibitor*, Clin Chem, 2002 ; 48 : 1 206-9.
- Tramonti G., Ferdeghini M., Annichiarico C., Donadio C., Norporth M., Mantuano E., Bianchi C., *Assessment of tumor-associated trypsin inhibitor (TATI) as a marker of renal function*, J Nephrol, 2003 ; 16 : 663-72.
- Kelloniemi E., Rintala E., Finne P., Stenman U.H., *Tumor-associated trypsin inhibitor as a prognostic factor during follow-up of bladder cancer*, Urology 2003 ; 62 : 249-53.

TEICOPLANIN

DEFINITION

Teicoplanin is an antibiotic belonging to the glycopeptide family reserved for hospital use (in France). Its antibacterial spectrum is limited to methicillin sensitive or resistant Gram positive, aerobic or anaerobic bacteria. It is a time-dependent bactericidal antibiotic administered intravenously (injected over 1 minute or as a 30 minute infusion) or intramuscularly. It is used:

– for prophylaxis against infectious endocarditis in adults who are allergic to beta-lactams in dental surgery and in urogenital and gastro-intestinal procedures at an intravenous dose of 400 mg at induction of anaesthesia;

– in the treatment of multi-resistant Gram positive bacterial infections in adults and children. the usual dose (moderately severe infections) is 400 mg (6 mg/kg) IV as a single injection (on the first day) as loading treatment, followed by 200 mg/day (3 mg/kg/d) by IV or IM injection as maintenance treatment. The loading dose in severe infections is 400 mg every 12 hours IV for 1 to 4 days with a maintenance dose of 400 mg/d as one IV or IM injection. The loading dose is switched to maintenance therapy as soon as an effective trough concentration is obtained. Doses need to be adjusted in infants, children, and the elderly over 65 years old and in patients with renal insufficiency.

PHARMACOKINETICS

Teicoplanin is not absorbed orally.

Bioavailability (intra-muscular route)	94%
Distribution	Biphasic following IV administration: Rapid first phase: 1/2 life: 20 to 30 min Slower second phase: 1/2 life: 1.6 to 4 h
Time to steady state	48 hours
Protein binding	90 to 95%, to albumin
Metabolism	Very limited (approximately 3%)
Elimination	More than 80% in urine in the unchanged form
Half-life of terminal elimination	70 to 100 hours; increased in patients with renal insufficiency

INDICATIONS FOR MEASUREMENT

Large inter-individual variability of teicoplanin kinetics is seen, explaining the lack of correlation between the dose administered and plasma concentrations. The main risk is of under dosing, resulting in treatment failure. The efficacy of the antibiotic is assessed by measuring trough concentrations. Whilst teicoplanin is less nephrotoxic than vancomycin (4.8% *vs* 10.7%), it is not however devoid of nephrotoxicity and monitoring of plasma concentrations is used to confirm that the dose is effective but not in the toxic range. Therapeutic monitoring is essential in children.

Measurement of trough concentrations is recommended, combined with serum creatinine measurement, 48 hours after starting treatment or after each change in dose. Measurement of peak concentrations (an estimate of toxicity) does not appear to be essential in view of the wide therapeutic margin of teicoplanin.

INFORMATION

SAMPLE

Serum or heparinised plasma.

Avoid tubes with separator gel.

Take the sample once steady state has been reached 48 hours after starting treatment or 48 hours after changing dose, immediately before the next dose for the trough concentration. A measurement 8 days after starting treatment is also recommended. For peak concentrations, the sample should be taken 30 minutes after an IV injection or 2 to 3 hours after an IM injection.

QUESTIONS FOR THE PATIENT

Teicoplanin is an antibiotic reserved for hospital use (in France). All requests for drug measurements must include the reason for requesting (investigation for efficacy or toxicity), the sampling time, treatment start date and/or any change in dosage, dosage information (amount administered, frequency and administration route) and the age, height and weight of the person wherever possible.

SAMPLE STORAGE AND TRANSPORT

Separate serum or plasma promptly. Serum or plasma can be stored for a few days at + 4°C.

It is recommended that samples be transported frozen at – 20°C.

ASSAY METHODS

Immunological method (FPIA).

High performance liquid chromatography.

NORMAL EXPECTED VALUES

The "effective" trough concentration must be > 10 mg/l. In severe infection, however, particularly in *Staphylococcus aureus* endocarditis the preferred effective range is around or even over 30 mg/l. Doses can be switched to maintenance treatment as soon as the effective trough concentration is achieved.

The "expected" peak concentration is between 40 and 60 mg/l. In practice, this is of limited use in monitoring teicoplanin, which is a time-dependent antibiotic.

Doses should be separated if concentrations are excessive or in patients with renal insufficiency.

FOR FURTHER INFORMATION

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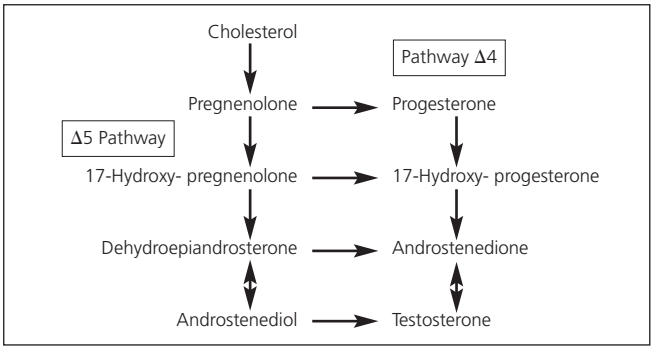
TESTOSTERONE

DEFINITION

Testosterone (T), the most important of the human androgens, is synthesised in the gonads and the adrenal cortex from cholesterol, which is first converted into pregnenolone. The latter is then converted to testosterone via one of the two pathways shown in the diagram below. The delta-4 pathway (or Δ4) comprises the sequence: progesterone, 17-hydroxyprogesterone and androstenedione, while the delta-5 pathway (or Δ5) is: 17-hydroxypregnenolone, dehydroepiandrosterone and androstenediol. The transfer from one pathway to the other can occur at any point under the action of 3 beta-hydroxysteroid dehydrogenase.



Synthesis in the testes takes place in the Leydig cells via both pathways, although delta-5 seems to be preponderant. Testosterone can be formed in the ovaries in the three compartments, the follicle, the corpus luteum and the stroma, along both biosynthetic pathways.

Other than in the gonads and adrenal cortex, testosterone can be formed from its precursors, androstenedione and dehydroepiandrosterone, in the liver and in peripheral tissues, notably including adipose tissue and muscles.

Blood testosterone can thus be of several origins, depending on sex and age.

In adult men, it comes mainly from testicular secretion, whereas in boys before puberty the principal source is the peripheral conversion of androstenedione.

Testosterone in women chiefly originates from the peripheral conversion of androstenedione, both during the child-bearing years and after the menopause *(see Table)*.

After the menopause, the contribution by ovarian secretion becomes virtually non-existent, while adrenal cortex secretion and peripheral conversion from precursors remain at similar levels to those observed in the course of the child-bearing years.

Origin of blood testosterone (%)

Men		
Testicular secretion	Adrenal cortex secretion	Peripheral conversion
95	–	5 (Δ4A)*
Women of child-bearing age		
Ovarian secretion	Adrenal cortex secretion	Peripheral conversion
25	20	50-60 (Δ4A)*

** precursor*

Whatever its origin, testosterone circulates in the blood, mostly bound to carrier proteins but there is a very small proportion of free testosterone. It binds to three proteins: SHBG (or "SBP") (sex hormone-binding globulin) or TeBG (testosterone-estradiol-binding globulin), CBG (corticosteroid-binding globulin, also known as transcortin) and to serum albumin. The bond to SHBG is specific and the binding site is the same as that for estradiol. The synthesis of SHBG is controlled by androgens and oestrogens *(see SBP)*. Any elevation in testosterone production results in a reduction of SBP and a consequent rise in metabolic clearance. This means that the hyperproduction of testosterone may either not alter the plasma concentration of this steroid, or raise it to a much less significant degree.

The levels of fractions bound to the various carrier proteins vary according to sex *(see Table)*. In women, the percentages of bound and free fractions does not vary significantly during the menstrual cycle. In contrast, since the protein levels rise during pregnancy, the bound fraction becomes much larger.

Status in plasma

Free fraction (%)	Bound fraction (%)		
	SBP	CBG	Albumin
Adult men			
2.23	44.30	3.56	49.90
Women of child-bearing age			
1.36	66.00	2.26	30.40
Women during the third trimester of pregnancy			
0.23	50.70	< 0.20	46.30

INTRODUCTION

From a physiological standpoint, until recent years, only the fraction not bound to proteins was considered to be biologically active. It has recently been shown, however, that the fraction bound to serum albumin was also available to the target cells. The total of free fractions and the fraction bound to albumin is therefore referred to as bioavailable testosterone.

The main effects of testosterone are the following:

– regulation of the secretion of LH

– initiation and maintenance of spermatogenesis

– formation of the male phenotype during sexual differentiation

– sexual maturation at puberty.

Testosterone crosses the cell membrane by diffusion. In the cytoplasm, it either binds to a receptor or is first reduced to dihydrotestosterone (DHT) by the action of 5α-reductase, the DHT then binding to the receptor. The complex thus formed migrates to the nucleus and binds to the nuclear action site. It is important to note that DHT and testosterone share the same cytosolic receptor: preferential binding of DHT is determined by the availability of 5α-reductase in the tissue. Muscle tissue, for example, has no 5α-reductase activity and testosterone is the sole stimulant of muscle development in puberty.

The testosterone-receptor complex is responsible for sexual differentiation in the Wolffian ducts, which subsequently become the epididymis, the vas deferens and seminal vesicles, as well as for sexual dimorphism of muscle development. The DHT-receptor complex is responsible for the virilisation of external genital organs during embryogenesis, and the formation of the prostate and other secondary sexual characteristics at puberty.

Testosterone also exercises negative feedback on the production of gonadotropins after *in situ* aromatisation to estradiol.

METABOLISM

Testosterone is metabolised in two pathways: the 17β-hydroxy pathway leading to androstanediol via DHT, and the 17-oxo pathway leading to the 17-oxosteroids or 17-ketosteroids, androsterone and etiocholanolone. These metabolites are combined and then eliminated in urine in the form glucuronides or sulphates.

Finally, a very small fraction of testosterone, from the bloodstream or formed *in situ* in the liver from precursors, is conjugated with glucuronic acid. Testosterone glucuronide is eliminated in the urine. Representing only a tiny fraction (approximately 1%) of total testosterone production, it does not accurately reflect its production in the body.

INDICATIONS FOR MEASUREMENT

Measurement of testosterone is recommended when investigating:

– virilism and hirsutism in girls and women

– sexual ambiguity

– problems with puberty: either precocious or retarded in both girls and boys

– sexual disorders in men, regardless of age: impotence, spermatogenesis problems or gynaecomastia

– testicular or ovarian tumours

– male or female infertility.

INFORMATION

Total testosterone and the bioavailable and free fractions can be measured in plasma or serum, although certain methods for measuring the bioavailable and free fractions require the use of serum. Samples must be collected before 10 a.m. to take account of circadian rhythms. Fasting is also required if bioavailable testosterone is to be measured. Stress and physical activity should be avoided.

■ NECESSARY INFORMATION

It is advisable to report the administration of compounds which modify testosterone concentrations through direct or indirect action on the gonads or adrenal cortex (gonadotropins, ACTH, clomiphene citrate, tamoxifen, glucocorticoids, sex hormones or danazol), or by affecting concentrations of SHBG or its binding to testosterone (clomiphene citrate, tamoxifen, danazol, sex hormones or spironolactone).

■ SAMPLE STORAGE AND TRANSPORT

Decanted plasma and serum are stored at + 4°C and delivered to the laboratory in that condition (stable for several days) for a measurement of total testosterone. For bioavailable or free testosterone, they must be frozen to – 20°C in the hours following collection, if measurement is deferred or the duration of transport is greater than 48 hours. Samples can be stored at – 20°C for 6 months to 1 year.

ASSAY METHODS

Total testosterone can be measured by immunological methods, using an isotopic tracer or not. Currently-available antisera are sufficiently specific, particularly with regard to dihydrotestosterone (DHT), for direct measurement in the serum or plasma fraction: this is valid for men, women and children.

Measurements of bioavailable testosterone or the fraction not bound to SHBG make use of the ability of the latter to be precipitated by ammonium sulphate at semi-saturation. In a first phase, the SHBG in the sample, together with the testosterone bound to it, is precipitated by an equal volume of a saturated solution of ammonium sulphate. After centrifugation, testosterone is measured in the supernatant fluid by the same immunological technique used for measuring total testosterone.

Measuring free testosterone is not easy; the reference method uses steady dialysis through a semi-permeable membrane. A kit is commercially available for direct measurement of the fraction not bound to plasma proteins.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Total testosterone concentrations in boys at birth are higher in peripheral blood than in umbilical cord blood, with the opposite being observed in girls. Concentrations then fall in both sexes. From day 15 in boys, they rise to a peak during the 2nd or 3rd month in parallel with LH.

Variations in total testosterone in plasma or serum as a function of age and the stage of puberty in boys

Age	*2 - 11 d*	*12 - 120 d*	*4 - 5 m*	*6 - 23 m*
Testosterone (ng/ml)	0.1 - 0.9	0.5 - 4.8	< 0.05 - 1.5	< 0.05 - 0.3
Age	*< 11 years*	*11 - 13*	*11 - 15*	*15 - 8*
Stage of puberty	1 a	1 b	2	3 - 4
Testosterone (ng/ml)	< 0.05 - 0.3	< 0.05 - 1.2	0.2 - 3.0	1.2 - 7.0

The results may be expressed in ng/ml or nmol/l. To convert ng/ml to nmol/l, multiply by 3.47.

In both sexes, testosterone then remains at low concentrations until the onset of puberty. Adult concentrations are not reached in boys until the age of 20 years. Variations in bioavailable testosterone, and those in testosterone not bound to proteins, are closely parallel to variations in total testosterone. In adult men, concentrations of total testosterone do not change significantly between 20 and 50 years of age.

For a correct interpretation of results, account must be taken of circadian and circannual variations and of the pulsatile character of testicular secretion.

In elderly men, total testosterone remains at concentrations comparable to those in young adults until an age which varies greatly in different studies. Bioavailable and free testosterone, on the other hand, start to decline from the 5th decade.

Variations in bioavailable testosterone as a function of age in men*

Age (years)	Bioavailable testosterone* (ng/ml)
20 - 35	1.0 - 3.7
36 - 50	0.8 - 3.2
51 - 70	0.3 - 2.9

** According to Nahoul and Roger (1990)*

Total testosterone concentrations in girls remain very low until the onset of puberty, then rise steadily to reach adult levels at the end of puberty.

Variations in total plasma or serum testosterone depending on age and the stage of puberty in girls

Age (years)	< 9	9 - 12	9 - 13	10 - 15	12 - 17
Stage in puberty	1 a	1 b	2	3 - 4	menstruating
Testosterone (ng/ml)	< 0.05 - 0.2	< 0.05 - 0.3	< 0.05 - 035	0.1 - 0.75	0.1 - 0.75

During ovarian activity in women, concentrations of total testosterone do not vary significantly over the menstrual cycle. Testosterone diminishes after the menopause but concentrations remain constant thereafter.

Variations in total plasma or serum testosterone in post-menopausal women

Age (years)	< 60	60 - 70	> 70
Testosterone (ng/ml)	0.15 - 0.50	0.10 - 0.50	< 0.05 - 0.50

The free and bioavailable fractions vary in parallel with total testosterone and remain below 2.3 pg/ml and 0.15 ng/ml respectively.

PATHOLOGICAL VARIATIONS

In men

Low testosterone concentrations allow a diagnosis of:

– simple delayed puberty in boys, or

– hypogonadism.

The primary origin of the hypogonadism is established in the presence of elevated LH concentrations, while a collapse in LH is characteristic of secondary hypogonadism. In the latter case, the reduction in LH may be caused by a disorder of the hypothalamus or pituitary.

Among the hypogonadotropic hypogonadisms, a distinction has to be made between the congenital and the acquired. The congenital conditions include isolated gonadotropic insufficiency, Kallmann's syndrome, Prader-Willi syndrome, Laurence-Mood syndrome and panhypopituitarism. The second category includes suprasellar tumours, hyperprolactinaemia caused by adenoma and post-traumatic or post-radiotherapy hypogonadism.

Hypogonadotropic hypogonadisms include gonadal dysgenesis, Klinefelter's syndrome and disorders of testosterone biogenesis caused by deficiencies in the following enzymes:

– 17α-hydroxylase

– 17-20 desmolase

– 3β-hydroxysteroid dehydrogenase, and

– 17β-hydroxysteroid dehydrogenase.

Testosterone levels are also reduced in cases of malnutrition, renal failure or atrophic myotonia, and in chronic illnesses caused by certain toxins, such as lead and alcohol.

In adult men, for a diagnosis of age-related androgen deficiency characterised by reduced libido, erectile dysfunction and a general feeling of being unwell, it is now established that the diagnosis must be based on concentrations of bioavailable testosterone rather than those of total testosterone. Furthermore, authors generally refer to the norm for men of less than 50 and not to that of men of corresponding age.

Elevated testosterone concentrations may also be observed in sexually-precocious boys, this condition being of central origin or secondary to a Leydig cell tumour.

In women

Testosterone levels may be elevated in the following conditions:

– congenital adrenal hyperplasia caused by a deficiency in 21-hydroxylase, 11β-hydroxylase or 3β-hydroxysteroid dehydrogenase,

– cushing's disease or Cushing's syndromes secondary to an ectopic secretion of ACTH or an adrenal gland tumour,

– ovarian and adrenal tumours,

– ovarian dystrophy.

FOR FURTHER INFORMATION

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TETRACHLOROETHYLENE

DEFINITION

Tetrachloroethylene or perchloroethylene ($Cl_2C = CCl_2$) is a colourless, volatile liquid with a characteristic odour. It is insoluble in water but miscible with most organic solvents, greases, oils and resins. It is used for dry cleaning of clothing, degreasing and cleaning metal parts, finishing textiles, extracting oils and greases and diluting paints and varnishes. The commercial product is stabilised by an anti-oxidant to prevent its decomposition on contact with air and moisture into trichloroacetic acid and hydrochloric acid, which can cause corrosion of metal surfaces. The limiting value of exposure from the air in business premises in France is fixed at 50 ppm i.e. 335 mg/m^3 (average exposure value).

METABOLISM

Tetrachloroethylene is mainly absorbed via the respiratory tract, but also through dermal exposure. It partly accumulates in adipose tissue. Most of the product absorbed (80%) is eliminated in an unchanged form in exhaled air, while less than 20% is metabolised to trichloroacetic acid (TCA, 3% the absorbed dose) by a saturation process in the liver, and eliminated in urine. The half-life for elimination of tetrachloroethylene from the blood varies from 7 hours to 8 days; the corresponding value for urinary TCA is 80 hours, resulting in an accumulation over a week.

MECHANISM OF ACTION

The toxic effects of tetrachloroethylene are linked to its lipophilic nature; in particular, damage to cerebral phospholipids and amino acids may be responsible for its neurotoxic effects.

SYMPTOMS OF POISONING

■ ACUTE POISONING

The inhalation of tetrachloroethylene causes:

– anaesthetic depression of the central nervous system (CNS): signs of inebriation followed by confusion. At very high concentrations, coma with respiratory difficulties and cardiac dysrhythmia.

The ingestion of tetrachloroethylene causes:

– Digestive problems, such as abdominal pain, nausea, vomiting and diarrhoea.

– CNS depression with deglutition pneumopathy, cough and bronchopulmonary superinfection.

– Effects on the liver (cytolysis) and kidneys (proteinuria and haematuria).

Cutaneous contact results in phlyctenules and serious ocular lesions.

■ CHRONIC POISONING

Chronic intoxication results in:

– Dermatitis and ocular irritation.

– Enzymatic induction with elevation of GGT and hepatotoxicity.

– Respiratory and digestive disorders.

– Neurological problems with headache, difficulties with balance and somnolence, psychic problems of memory, concentration and mood.

A carcinogenic effect is probable (CIRC, 1995).

INDICATION FOR MEASUREMENT

In exposed workers, a measurement of blood tetrachloroethylene is a good reflection of exposure in the previous week and correlates well with atmospheric concentrations (samples being collected approximately 16 hours after exposure ceases). This is a specific indicator but it varies with workload and the fat weight of the subject. Avoid contamination by collecting samples in a clean atmosphere in the morning, before work starts.

A measurement of urinary trichloroacetic acid (TCA) at the end of the working week reflects exposure during that week. However, this metabolite represents only a small fraction of the quantity absorbed, and also is not specific to exposure to tetrachloroethylene (interference from trichloroethylene, trichloromethane and tetrachloroethane). This biological marker can thus not be used at an individual level for low exposure levels.

A measurement of tetrachloroethylene in the urine is also suggested, since there is a good correlation with atmospheric concentrations.

INFORMATION

■ SAMPLE

5 ml of whole blood, heparinised or EDTA: tubes containing Merthiolate should not be used. Samples should preferably be collected at the beginning of a shift and the end of the week (e.g. Friday morning).

20 ml from one urine sample, non-acidified: samples should preferably be collected at the end of a shift and the end of the week (TCA or Tetrachloroethylene).

Urinary tetrachloroethylene should preferably be collected in a glass flask with a Teflon stopper.

■ SAMPLE STORAGE AND TRANSPORT

Samples of whole blood or urine can be stored and transported to the laboratory at ambient temperature or between 2 and 8°C.

ASSAY METHODS

Gas chromatography with detection by flame ionisation.

REFERENCE VALUES

INRS 2004:

– Blood tetrachloroethylene: guide value in France → 1 mg/l before the last shift of the working week.

– Urinary tetrachloroethylene: < 75 µg/g of creatinine.

– Urinary TCA: reference value < 1 mg/g of creatinine
recommended value if exposed to tetrachloroethylene < 5 mg/g de creatinine.

FOR FURTHER INFORMATION

■ Toxicologie industrielle et intoxications professionnelles, *Tétrachloroéthylène*, Lauwerys. R., 3ème édition, Masson.

■ Fiche toxicologique tétrachloroéthylène, édition 2004, INRS.

THEOPHYLLINE

DEFINITION

Theophylline, a bronchodilator, belongs to the xanthine family (as caffeine), and is indicated in the treatment of asthma (children and adults), spastic forms of chronic obstructive bronchopneumopathies and newborn baby apnoea. It is also useful for patients being taken off ventilation. It is marketed in oral and intravenous forms.

PHARMACOKINETICS

Bioavailability (oral administration)	100 %
Plasma peak	1 to 2 hours for rapid forms
Steady state	2 to 5 days (1 to 2 days in newborn babies; 6 days in the premature)
Protein binding	60% in adults 36 % in newborn babies or cases of cirrhosis
Metabolism	Hepatic
Clearance	Urinary (10 to 13 % in unchanged form)
Clearance half-life*	Premature babies = 30 hours Newborn babies = 24 hours 30 months to 9 years = 3 to 4 hours Adult non-smokers = 5 to 9 hours

* In addition to age-related variations, the clearance half-life is greater in cases of severe liver failure (20 to 30 hours in subjects with cirrhosis), acute heart failure, respiratory insufficiency, obesity, acute infections of the upper respiratory tract or severe bronchial obstruction, fever, diets rich in fats and the consumption of alcohol. The half-life diminishes in smokers (4 hours) and when the diet is rich in proteins.

INDICATIONS FOR MEASUREMENT

The numerous factors in individual clinical variations (liver or respiratory failure, etc.) and therapeutic considerations (drug interactions), combined with the low therapeutic index of the molecule, justify the monitoring of plasma concentrations in order to optimise dosage.

Measurement is indicated in the following cases:

1. Therapeutic inefficacy, in spite of treatment at the usual dose.
2. If signs of overdose appear: in adults, these may be nausea, vomiting, epigastric pain, agitation, insomnia, tachycardia, headache, delirium, convulsions, hyperthermia or cardiac arrest. Signs of overdose in children are agitation, logorrhoea, mental confusion, repeated vomiting, hyperthermia, tachycardia, ventricular fibrillation, convulsions, hypotension, respiratory problems, and hyperventilation followed by respiratory depression.
3. Patient-related changes in the metabolisation of theophylline (respiratory failure, renal failure, *cf. supra*) or when certain drugs are taken in combination:

   – reduced plasma concentrations of theophylline when the following are combined: carbamazepine, pheonobarbital, phenytoin, primidone, rifampicin and rifabutin.

   – increased plasma concentrations of theophylline (risk of overdose due to reduced catabolisation of theophylline) when the following are combined: enoxacin, viloxazine, cimetidine, erythromycin, clarithromycin, josamycin, roxithromycin, fluconazole, ciprofloxacin, pefloxacin, fluvoxamine, ticlopidine, ritonavir, allopurinol and mexilitine.
4. Particular indication in neonatology (utilisation of theophylline in primitive apnoea and withdrawal of ventilation assistance): the existence of a particular form of metabolisation in premature and newborn babies leading to the formation of caffeine makes it essential to monitor the plasma concentrations of theophylline and caffeine.

INFORMATION

SAMPLE

Preferably serum. EDTA or heparinised plasma possible.

Avoid calcium and magnesium chelators, avoid tubes with phase separation gel.

For therapeutic monitoring, a fasting sample is necessary, at steady state, after 3 to 5 times the half-life i.e. 2 to 5 days for treatment with theophylline.

Theophylline administered orally

– Sampling for measurement of the trough theophylline concentration, immediately before the next dose.

– Sampling at peak (Cmax), 2 hours after administration for a rapid form or 4 hours after administration of a slow-release form.

Theophylline administered intravenously

– Sampling for measurement of trough concentration, just before the start of perfusion.

– Sampling at peak (Cmax), 30 minutes after the end of perfusion.

If intoxication is suspected, sampling and measurement must be carried out urgently.

ESSENTIAL INFORMATION

The following information is essential in any request for drug measurement: the reasons for prescribing (investigation of efficacy or toxicity), the time of sample collection, the date of commencement of treatment and/or any change in dosage, and pharmacological data (quantity administered, frequency, administration route), together with the age, height and weight of the subject when possible.

– Current treatments which might interfere with the metabolisation of the theophylline *(see list below).*

SAMPLE STORAGE AND TRANSPORT

Store for 8 days at + 4°C; 3 months at – 20°C. Transport at + 4°C (if < 8 days).

ASSAY METHODS

The most commonly-used methods are immunological by competition.

NORMAL EXPECTED VALUES

The therapeutic range (effective trough concentration) is as follows:

– in adults: 8 to 20 mg/l.

– in children aged less than 10 years: 6 to 11 mg/l.

– in children aged 10 to 16: 8 to 14 mg/l.

Beyond a plasma concentration of 20 mg/l, the first signs appear of overdose (nausea, vomiting, etc. *see above*) requiring treatment to be stopped; plasma concentrations in excess of 35 mg/l are life-threatening (convulsions, hyperthermia, cardiac arrhythmia and cardio-respiratory arrest).

Conversion factor: 1 mg/l = 5.50 µmol/l.

FOR FURTHER INFORMATION

■ Dictionnaire Vidal®.

■ Manchon M., *Dosage des médicaments*, tome II, Cahier BIO-FORMA N° 9, Paris, décembre 1997, chapitre théophylline : 79-88.

THIOCYANATES

DEFINITION

The thiocyanate anion $(SCN)^-$ is a metabolite of hydrocyanic acid and cyanides. The ions result from the conversion of cyanide ions $(CN)^-$ by enzymes:

- thiosulphate sulphur transferase (Lang's rhodanese) (liver, kidneys, spleen, adrenal glands and thyroid)

- β mercaptopyruvate transsulphurase (Sorbo) (digestive tract, liver and kidneys)

Thiocyanates are of low toxicity and are eliminated unchanged, essentially by the kidneys. They have a half-life of approximately 10 days.

Synonyms: SCN-, cyanide ions.

INTRODUCTION

Thiocyanates are produced by the enzymatic detoxification of cyanides (80%). They are active as competitive inhibitors of iodide capture by the thyroid and may result in hypothyroidism with goitre. They are found in urine, blood, saliva and sweat. High concentrations are observed in smokers and certain workers exposed to cyanides. Acute intoxication is accompanied by metabolic acidosis, weakness, hypotension, confusion, psychotic behaviour, muscle spasms and convulsions.

INDICATIONS FOR MEASUREMENT

– Indicator of exposure to cyanides and hydrocyanic acid (occupational medicine).

– Monitoring of treatments using sodium nitroprusside (antihypertensive).

INFORMATION

■ SAMPLE

– Preferably serum; EDTA or heparinised plasma is sometimes possible depending on the recommendations from the laboratory.

Urine (in occupational medicine): a samples should be collected in plastic bottles immediately after the end of a working shift (end of day, after exposure).

– The subject should be fasting since the previous day.

■ QUESTIONS FOR THE PATIENT

Pathologies suspected, clinical and environmental data?

Monitoring of treatments using sodium nitroprusside?

■ SAMPLE STORAGE AND TRANSPORT

– Serum: after centrifuging and decanting, the sample is stable for several days at +4°C. Certain spectrophotometric methods require prior deproteinisation of the serum or plasma.

– Urine: without additives. After centrifuging, store at +4°C for a maximum of 24 hours. For longer periods, freeze at -20°C. Measurement is combined with that of a urine creatinine measurement after 24 hours.

ASSAY METHODS

Measurements are performed either directly on the thiocyanate ion, or on the cyanide ion obtained after oxidation. Measurement can be direct or indirect (deproteinisation, ion exchange, etc.).

Spectrophotometric methods (UV, visible, IR).

Electrometric methods (voltammetry).

Chromatographic methods.

Selective electrodes.

NORMAL EXPECTED VALUES

Reference values in the general population:

– Non-smokers:

Serum: < 0.6 mg/100 ml

Urine: < 2.5 mg/g of creatinine.

– Smokers:

Serum or plasma: ≤ 1.5 mg/100 ml.

Urine ≤ 5 mg/g of creatinine.

INTERPRETATION

Certain foods (cabbage, maize, swede, turnip, cassava, beans, etc.) are sources of thiocyanate ions in the body. In addition, vitamin C diminishes the detoxification of cyanides to thiocyanates. The consumption of tobacco increases the thiocyanates concentration in serum and urine (see *"normal values"*).

Due to the essentially urinary clearance of thiocyanates, it is important to verify the integrity of renal function in patients.

– Exposed subjects:

Urine: non-smokers: < 4.1 mg/g of creatinine.
smokers: < 10 mg/g of creatinine.

– Treatment with sodium nitroprusside: daily measurement of blood thiocyanate levels is essential for long-term treatments or treatments for more than 48 hours, and in patients with hepatic or renal insufficiency. The plasma concentration should not exceed 5 mg/100 ml. Signs linked to thiocyanate toxicity have been observed with values of 8 to 12 mg/100 ml.

FOR FURTHER INFORMATION

■ *Annales de toxicologie analytique*, Société française de toxicologie analytique, 2000; 12, 2, 131-136.
■ Dictionnaire Vidal®.
■ Fiche Biotox – INRS 2002.

THROMBIN TIME

DEFINITION

The thrombin time is the time taken from citrated plasma to coagulate in the presence of thrombin. It investigates the initial stages of fibrin formation (proteolytic effect of thrombin and polymerisation), i.e. factor I or fibrinogen but not clot stabilisation by factor XIII (fibrin stabilisation factor). It is sensitive to heparin, hirudin and all thrombin inhibitors (bivalirudin, argatroban and dabigatran, etc.).

INDICATIONS FOR MEASUREMENT

Diagnosis of a bleeding disorder or aetiological work-up of the cause of venous thrombosis, to test for afibrinogenaemia or hypo or dysfibrinogenaemia.

With an unexplained lengthening of the PT or ACT.

INFORMATION

■ SAMPLE

The sample should be taken into 3.2% citrate (0.109 M), volume 1/10 (0.5 ml per 4.5 ml of blood). Tubes containing 3.8% citrate (0.129 M) are acceptable. Blood can also be collected into CTAD tubes (citrate, theophylline, adenine, dipyridamole). No other anticoagulant may be used.

A fasting sample is preferable and coffee, smoking and alcohol must be avoided for an hour before the sample is taken. A light low fat snack is permitted.

The tubes must be centrifuged promptly after the sample is taken and the test must be performed within 4 hours. Confirm no microclots are present and discard haemolysed or lipaemic specimens.

For further information refer to the *"General preanalytical conditions in haemostasis"* section.

■ QUESTIONS FOR THE PATIENT

Current treatments, and particularly medical drugs, which prolong the thrombin time:

– Unfractionated heparin or UFH,

– Low molecular weight heparins or LMWH: no increase or very slight increase,

– Hirudin and derivatives,

– New thrombin inhibitors: dabigatran, argatroban, etc.

– Thrombolytic treatments: streptokinase, urokinase, rt-PA and tenecteplase.

■ SAMPLE STORAGE AND TRANSPORT

For 4 hours at laboratory temperature, 1 week at -20°C and – 80°C beyond this time. It is recommended that samples be thawed quickly in a water bath at 37°C immediately prior to analysis.

ASSAY METHODS

By measurement of the coagulation time at 37°C of a platelet-poor plasma in the presence of a defined amount of thrombin (human or animal).

NORMAL EXPECTED VALUES

Patient time ≤ control time + 3 seconds.

State the type of thrombin in the reagent used, whether human or animal.

IATROGENIC AND PATHOLOGICAL VARIATIONS

■ TREATMENT WITH HEPARIN OR HIRUDIN AND ITS DERIVATIVES

– The thrombin time is very greatly increased beyond 60 seconds on UFH treatment. In practice, it is not suitable to monitor these treatments, as it is too sensitive.

– The LMWH have little effect on the thrombin time at prophylactic doses, although at least some of them such as tinzaparin, which has greater anti-thrombin activity than the other LMWH can prolong it at curative doses.

– The "new" anti-thrombotics, which target thrombin (hirudin and derivatives, dabigatran), can prolong the thrombin time and a modified thrombin time (Hemoclot®) is used to monitor dabigatran treatment.

– Thrombolytics prolong the thrombin time.

■ CONSTITUTIONAL FIBRINOGEN DEFICIENCIES

– Afibrinogenaemia, hypofibrinogenaemia (quantitative deficiency): risk of bleeding.

– Dysfibrinogenaemia (qualitative defect: normal amounts of non-functional fibrinogen). The constitutional dysfibrinogenaemias are usually asymptomatic and occasionally present with moderate bleeding and in approximately 10% of cases are associated with venous or arterial thromboses.

■ ACQUIRED ABNORMALITIES

– Disseminated intravascular coagulation, primary or secondary fibrinolysis,

– High levels of FDP or D-dimers,

– Hepatocellular insufficiency,

– The presence of a specific thrombin inhibitor (anti-thrombin) in plasma: circulating anticoagulant, dysglobulinaemia, bovine anti-thrombin antibodies develop, after treatment with some biological adhesives containing bovine thrombin (distinguished by normal thrombin time when human thrombin is used),

– Inflammatory states with pronounced hyperfibrinogenaemia,

– Acquired dysfibrinogenaemias.

■ ACTION FOR PROLONGED THROMBIN TIME

– Repeat the test on an equal part mixture of patient plasma and control plasma. Correction of the time suggests fibrinogen deficiency (plasma concentration < 1 g/l); failure to correct suggests an inhibitor with anti-thrombin activity is present (heparin, hirudin or its derivatives, dabigatran, circulating anticoagulant, high concentrations of D-dimers or FDP or monoclonal immunoglobulin in myeloma or Waldenström's disease).

– To determine whether the abnormality is due to fibrinogen, the thrombin time can be measured replacing the patient's plasma in the correction test with serum (ensuring that the serum contains no fibrinogen): dysfibrinogenaemia is suspected if the thrombin time is prolonged and corrects in the presence of serum and confirmed by a discrepancy between measurements of fibrinogen using chronometric and immunological methods, or even by weighing.

– The presence of heparin in the sample can be confirmed from a normal reptilase time.

FOR FURTHER INFORMATION

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■ Samama M.M., Elalamy I., Conard J., Achkar A., Horellou M.H., *Hémorragies et thromboses : du diagnostic au traitement*, Collection « Les abrégés », Édition Masson, Paris, 2004.

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THYMIDINE KINASE

DEFINITION

Thymidine kinase (TK) is an enzyme catalysing the phosphorylation of deoxythimidine during DNA synthesis. It is directly associated with cell proliferation. Several studies have shown that serum TK activity reflects tumoral proliferation activity and correlates with the prognosis for various cancers, in particular malignant haemopathies (Hodgkin's and non-Hodgkin's lymphoma, leukaemia and multiple myeloma), and cancer of the breast, ovary or bladder. Three isoforms of TK have been identified: TK1 (foetal and cytosolic), TK2 (mitochondrial), and TK3, described only in embryonic fibroblasts and certain cell lines. 95% of TK activity in serum is due to TK1. Serum TK increases during cell division phases. TK1 has been described as a marker for proliferation in primitive bladder cancers, making it possible to assess prognosis and monitor post-operative patients. TK2 is the only physiologically-active thymidine kinase in rest cells. It plays an important part in the maintenance of a sufficient pool of deoxynucleotide triphosphates (dNTP's) in the mitochondria. Mutations in the gene coding for TK2 have been associated with a loss of mitochondrial DNA, mainly affecting the skeletal muscles. It is also implicated in the mitochondrial toxicity which is associated with prolonged treatment by nucleoside analogues (such as azithromycin).

INDICATIONS FOR MEASUREMENT

Determination of prognosis and post-therapeutic monitoring of patients with cancer, particularly cancer of the bladder and multiple myeloma. This marker is little-used, however.

INFORMATION

SAMPLE

In dry tubes (serum). A fasting sample is not necessary.

NB: large, random circadian variations (sometimes > 100%) in serum TK have been shown. Certain authors have in fact suggested taking repeated samples over a 48-hour period in order to monitor patients with this marker.

QUESTIONS FOR THE PATIENT

In a context of cancer, type of tumour and treatment in progress: chemotherapy, radiotherapy and surgery (modalities and date of treatment)?

SAMPLE STORAGE AND TRANSPORT

Centrifuge, decant and store at +4°C.

Transport at +4°C.

ASSAY METHODS

Radioimmunological assay.

NORMAL EXPECTED VALUES

Serum TK activity < 6.1 U/l (RIA).

PATHOLOGICAL VARIATIONS

Serum TK activity increases in various cancers:

– In cancer of the ovary, it correlates with the serum concentration of CA-125. In a study undertaken in 14 women with advanced ovarian cancer, TK was even elevated in 3 patients with normal serum CA-125.

– In cancer of the breast, it has been shown that the average values of TK in serum were higher in patients (n = 8 women) with metastasised tumours (39.7 ± 7.1 U/l) than in those (n = 75) with a localised form (12.1 ± 1.3 U/l), compared with control subjects (n= 30) (2.8 ± 0.4 U/l).

– In cancer of the bladder, a study of 56 patients showed that serum TK concentrations were significantly elevated in those patients compared with healthy subjects, with no overlap of values. Serum TK activity was correlated with the stage of the tumour (I-III) and the degree of metastatic invasion (T1 – T2), but not with the grade of the tumour (G1 – G4). After surgery, serum TK fell by 66% in 1 week, reaching the values in healthy patients after 1 month. In this study, the values remained normal for at least 6 months, the time at which patient monitoring was stopped.

– After chemotherapy, the rise in TK activity is thought to be associated with Tumour Lysis Syndrome, a complication occurring during the treatment of certain tumours, particularly acute leukaemias and cancers in children. This syndrome is characterised by hyperuricaemia, hyperkalaemia, hyperphosphataemia and hypocalcaemia secondary to the massive lysis of tumour cells. It can result in acute renal failure and metabolic acidosis, which are life-threatening.

Serum TK activity increases during certain viral infections (infections by adenoviruses and herpes viruses: *Herpes simplex*, EBV, CMV and VZV): a rise can justify an early suspicion of their onset. Furthermore, the appearance of a mutation of the TK gene in HSV is one of the causes of the resistance of herpes viruses to anti-virals.

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THYROGLOBULIN

DEFINITION

Thyroglobulin (Tg) is a homodimeric glycoprotein with a molecular weight of 660 kDa which is exclusively synthesised by the follicular cells of the thyroid. It is mainly secreted and stored in the lumen of thyroid follicles, where it constitutes 90% of the colloid proteins. It is excreted in small quantities into the blood, where its half-life is between 2 and 4 days.

INTRODUCTION

The secretion of thyroglobulin is controlled by TSH (Thyroid Stimulating Hormone or thyrostimulin). It stores iodine and is the precursor in the thyroid hormone synthesis pathway.

Although Tg is concentrated in the colloid, a small quantity is nevertheless excreted into the serum. The Tg concentration in serum correlates with the volume of thyroid tissue, even allowing its weight to be estimated. If TSH is normal, a concentration of 1 to 2 ng/ml of Tg represents the production of one gram of normal tissue and if TSH is < 0.1 mUI/ml, a TS concentration of 0.5 ng/ml is expected. The serum concentration of thyroglobulin rises when TSH rises (thyroid failure, pregnancy, antibodies against TSH stimulation receptors, injection of recombinant TSH), after a needle biopsy of the thyroid gland or surgery, in the presence of thyroid gland nodules, cancers and inflammatory episodes (autoimmune, viral or radiation-induced origin). It also increases in all endogenic hyperthyroidisms. The thyroglobulin concentration in serum has no diagnostic value (it does not, for example, allow differentiation between benign and malignant thyroid nodules), but is the principal suggested tumour marker for monitoring differentiated cancers of the thyroid (follicular or papillary). Since in fact the thyroid is the only organ which synthesises thyroglobulin, finding Tg in patients after thyroidectomy allows the detection of recurrence or metastasis.

INDICATIONS FOR MEASUREMENT

■ PRINCIPAL INDICATION

In differentiated papillary or follicular thyroid cancer, a Tg measurement is made prior to treatment, in order to determine the capacity of the tumour to secrete thyroglobulin. In the days following treatment (partial, subtotal or total thyroidectomy, with or without subsequent radiotherapy), the decline in Tg concentration allows the extent of the excision to be assessed. In the subsequent years, Tg measurements are useful in order to detect recurrence, even in patients treated with thyroid hormones. L-T4 may in fact be administered to suppress thyrotrope activity (hormone therapy to suppress TSH or HTS-TSH). Tg is then in principle undetectable and screening may be made more sensitive by stimulating its secretion. This can be done by stopping the LT-4 (a window of 72 hours stimulates endogenous TSH) or by injecting recombinant TSH (rh-TSH or Thyrogen®). The Tg peak is generally expected 3 days after stimulation by rh-TSH and is usually of smaller amplitude than in endogenous stimulation.

■ SECONDARY INDICATIONS

– Diagnosis of thyroiditis (inflammation of the parenchyma releases thyroglobulin into the serum), particularly in patients treated with amiodarone who develop hyperthyroidism, in order to assist in deciding whether to treat with corticoids.

– Suspicion of thyrotoxicosis factitia (hidden ingestion of thyroid hormones).

– On discovering hypothyroidism in a newborn baby, testing for athyreosis (absence of thyroid tissue development).

INFORMATION

■ SAMPLE

Preferably dry tube (serum); plasma collected on heparin or EDTA possible. Discard haemolysed or hyperlipaemic samples.

A fasting sample is not necessary.

■ QUESTIONS FOR THE PATIENT

Have you had a recent thyroid operation or biopsy? Needle biopsy on thyroid cysts or nodules and, according to certain authors, thyroidal palpation, result in a rise in serum Tg for a few days.

Are you taking any of the following treatments? These drugs may interfere with thyroid function and/or may also result in the presence of anti-Tg antibodies risking interference with measurement of Tg: LT4 [modalities and dates of treatment], amiodarone, lithium carbonate, IL-2, alpha-interferon, and GM-CSF.

Possible pregnancy?

■ SAMPLE STORAGE AND TRANSPORT

Storage and transport of serum: 48 hours at + 4°C; several months at -20°C.

The sample must be kept in the serum bank (1 year); avoid repeated freezing/thawing.

ASSAY METHODS

Immunometric assay.

European Standard: CRM 457.

NORMAL EXPECTED VALUES

Values vary according to the method used and the clinical context.

– Subjects less than 40 years old, non-smokers, adequate iodine intake, euthyroid status (TSH: 0.5-2 mUI/l), without thyroid pathology or anti-thyroid antibodies (anti-Tg and anti-TPO): Tg = 3 - 40 ng/ml

– Subjects whose iodine intake is insufficient: Tg = 3-70 ng/ml

– After thyroid surgery: Tg 1 to 2 months after lobectomy: < 10 ng/ml (TSH < 0.1 mU/ml); Tg 1 to 2 months after subtotal thyroidectomy: < 2 ng/ml (TSH < 0.1 mU/ml).

INTERPRETATION

PHYSIOLOGICAL VARIATIONS

Thyroglobulin is always detectable in a healthy subject. Its level rises under the effect of oestrogens and during pregnancy (3rd trimester).

In newborn babies, the serum concentration rises for a few days due to the action of neonatal TSH peak, then falls by approximately one-half over the following days. This elevated value observed in children decreases slowly until puberty, when it reaches adult levels.

Age	Tg ng/ml
0 - 8 days	20 - 200
0 - 3 years	15 - 100
3 - 11 years	6 - 50
11 years - puberty	4 - 45
adult	2 - 40
Umbilical cord	15 - 100
3rd trimester of pregnancy	5 - 50

ANALYTICAL INTERFERENCES

Tg is the main thyroid antigen. Anti-Tg antibodies are detected in 20 to 40% of patients with differentiated thyroid cancer. There is, certainly, considerable variability in the affinity and quantity of autoantibodies, but their interference with thyroglobulin measurement is a major, serious unresolved problem for Tg assay. Technically, the autoantibodies interfere with sandwich methods, either by preventing the Tg from binding to the antibodies in the solid phase, or by inhibiting the binding of the 2nd antibody (marked). In all cases, their presence leads to a false low Tg value if LIA measurement is used; the value may also be falsely elevated in RIA measurement.

To circumvent this problem, an overload (or recuperation) test may be performed. This consists of adding the Tg reference standard to a serum sample and calculating the percentage of Tg found. The test validates the measurement of Tg for values between 80% and 120% of the quantity added. However, its interpretation is difficult and poorly standardised, and the test is now subject of controversy. Current recommendations are to systematically measure anti-Tg antibodies at the same time as the Tg: their presence renders the Tg result non-interpretable.

MONITORING IN THYROID CANCER

Differentiated thyroid cancers are most often of the papillary type (50% to 70% of all thyroid cancers): they are well-differentiated and frequently localised (solitary cold nodule) in a young subject, and curable by a simple lobectomy (nodule < 1.5 cm) followed by hormone therapy to suppress TSH (HTS-TSH) and minimise the risk of relapse. If the tumour is larger than 1.5 cm, treatment is radical (total thyroidectomy, radiotherapy and I-131, followed by HTS-TSH). Follicular forms (15%) occur in older subjects and are generally more serious, with haematological dissemination, and in most cases require radical treatment (total thyroidectomy, local radiotherapy and iodine 131, plus HTS-TSH). The interpretation of a Tg measurement should take account of the Tg concentration prior to therapy (which reflects the capacity of the tumour to secrete Tg), the dynamics of the post-surgical decrease, the presence of anti-Tg antibodies, the mode of treatment of the cancer (total or partial surgery) and the efficacy of HTS-TSH.

When TSH is stable under LT-4, any variation in the Tg concentration reflects a change in the tumour weight.

If the tumour is a "low secretor" (normal Tg prior to surgery), a recurrence may be observed in spite of low or undetectable Tg concentrations.

If the pre-operative Tg concentration is elevated, recurrence is almost always associated with a renewed rise in Tg. If thyroglobulin remains detectable, in spite of the HS-TSH, monitoring by measuring Tg and TSH does not require stimulation; recurrence is suspected in the presence of a rise in the Tg concentration. If Tg is undetectable and TSH stable during HTS-TSH and, in the absence of anti-Tg antibodies, sensitisation of Tg measurement is desirable in order to detect recurrence (therapeutic window of L-T4 or injection of rh-TSH). The amplitude of the response to stimulation varies with the mode of stimulation employed (rh-TSH injection results in a smaller amplitude) and with the tissue differentiation during recurrence (ability to produce Tg and sensitivity to TSH).

In patients who have anti-Tg antibodies, detection of recurrence by means of Tg is unreliable. Patients do not respond, or only respond partially, to stimulation tests; the alternative is to use the anti-Tg antibody measurements themselves to detect recurrence. The disappearance of the anti-Tg antibodies is in fact common after 1 to 2 years and their reappearance is a reason for suspecting a recurrence of the tumour.

Generally speaking, the majority of patients with Tg which becomes undetectable after treatment have a very low probability of recurrence after 15 years. The Nicoloff study (Spencer & Nicoloff, *Thyroid* 1999), shows in fact that, for papillary cancers in stages TNM I and II, post-operative Tg is stable at less than 2 µg/l under L-T4. Recurrence was only seen in 6% of cases, on average after 8 years, and were almost always detected through a rise in Tg. In patients who have been treated by total thyroidectomy and THS-TSH, elevated Tg after stimulation (Tg > 10 µg/l) is more often a sign of recurrence (local or metastatic). One should be aware, however, that in general terms the early detection of recurrence does not seem significantly to improve patient survival rates. This important factor is the subject of several current studies.

- **In thyroiditis,** inflammation of the parenchyma releases thyroglobulin with rising serum concentration (sometimes up to 500 ng/ml). Furthermore, amiodarone hyperthyroidism can result from an iodine overload mechanism or one of inflammatory damage to thyroid cells caused by the drug. In a patient with hyperthyroidism under amiodarone, an elevated Tg concentration in serum associated with an inflammatory biological scenario is an argument in favour of the use of corticoid treatment.
- **In cases of thyrotoxicosis factitia** (hidden ingestion of thyroid hormones), the patient has a biological status of hyperthyroidism, iodine fixation is nil (established by a scan) and Tg is not measurable (the parenchyma is resting due to exogenic drug absorption).
- **In neonatal hypothyroidism,** Tg allows a differentiation to be established between agenesis or athyreosis (Tg nil) and ectopias (Tg detectable).

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THYROXIN BINDING GLOBULIN

DEFINITION

Thyroxin binding globulin (TBG) is a glycoprotein belonging to the antiprotease family, with a molecular weight of 54 kDa. It has a biological half-life of approximately 5 days.

Synthesised in the liver, it is glycolysed and may contain up to nine residues of sialic acid, essential for maintenance of the tertiary structure of the protein. Sialylation is stimulated by oestrogens and leads to a prolongation of its half-life and an increase in plasma concentration, which is the reason for the high TGB concentrations during pregnancy in women receiving oestrogen therapy.

From a physiological standpoint, TBG possesses, with two other proteins, transthyretin (or thyroxin-binding prealbumin) and albumin, the ability to bind to thyroid hormones. In contrast, however, to these other proteins, TBG is characterised by a high affinity, although a low capacity, for T4 (thyroxine). T3 (triiodothyronine) also binds to TBG but with an affinity 10 times lower than that of T4.

This is why 65% of T3 and 70% of T4 circulating in the blood are bound to TBG. The thyroid hormones bound in this way are not metabolically active but constitute a reservoir of thyroid hormones. In reality, only free T3 and T4 are available to target cells.

INDICATIONS FOR MEASUREMENT

Measurement of TBG is indicated in the following cases:

– Total thyroid hormone concentrations (T4 and T3) in disagreement with the TSH concentration or the clinical symptoms.

– Discordance between total T4 and free T4 concentrations.

– Significant increase or decrease in total T4 concentrations.

– Possibility of a congenital TBG deficiency.

The most useful parameter supplied by a measurement of TBG is the T4/TBG ratio, which correlates with the free T4 concentrations. In practice, it allows a differential diagnosis between euthyroidism, hypothyroidism and hyperthyroidism.

INFORMATION

SAMPLE

TBG measurement can be performed on serum or plasma in EDTA or heparinised tubes.

SAMPLE STORAGE AND TRANSPORT

Samples can be stored for 24 hours at +4°C; for longer periods, they must be stored frozen at –20°C.

Transport at –20 °C.

ASSAY METHODS

Measurement methods are immunoenzymatic or radioimmunological.

USUAL VALUES

There is no difference between the reference values for men and women. On the other hand, serum or plasma TBG concentration depends on age. Using the radioimmunological method that we apply, the reference values as a function of age are shown in the table below.

Reference values for TBG (mg/ml) as a function of age

Age (years)	Mean (mg/l)	Standard deviation
1 - 10	31.5	14.0
11 - 16	24.1	6.5
17 - 60	20.5	4.5
> 60	19.9	6.0

During pregnancy, TBG concentrations reach a plateau at approximately 50 µg/ml from the 30th week.

Values of the T4/TBG ratio in euthyroidism vary between 3.1 and 5.2.

UNITS OF MEASUREMENT

Results are generally expressed in µg/ml or mg/l. Conversion of mg/l to nmol/l requires multiplication by 17.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

TBG is detectable in the foetus from the 10th week of pregnancy; its concentration rises steadily until full term. This rise in TBG is responsible for the rise in T4 during the 2nd and 3rd trimesters. After birth, TBG reaches a maximum during the first year of life and then falls progressively until adulthood; it subsequently remains constant.

TBG rises in pregnant women between the 10th and 30th weeks, then remains stable until the birth.

PATHOLOGICAL AND IATROGENIC VARIATIONS

A large number of pathological conditions and drugs can interfere with TBG concentrations (see table) and thus with the quantities of T3 and T4 bound to it, making it difficult to interpret the results for total T4 and T3.

Pathological conditions and drugs associated with variations in TBG

Increase in TBG	Decrease in TBG
Oestrogens	Androgens
Tamoxifen	Glucocorticoids at high doses
Oral contraceptives	Acromegaly
Intermittent acute porphyria	Nephrotic syndrome
Chronic infectious hepatitis	Serious systemic diseases
Biliary cirrhosis	Genetic factors
Genetic factors	
Perphenazine	
HIV infection	

Note that congenital disorders which affect TBG may manifest themselves either by a partial or total deficit, or by an increase in this protein.

Values of the T4/TBG ratio in the course of thyroid pathologies are shown in the following table.

	Hypothyroidism	Hyperthyroidism
T4/TBG	0.2 - 2.0	7.6 - 14.8

FOR FURTHER INFORMATION

■ Reed Larsen P., Davies T.F., Hay I.D., *The Thyroid Gland*. Dans : Wilson J.D., Foster D.W., Kronenberg H.M., Larsen P.R., eds. Williams Textbook of Endocrinology, Philadelphia, London, Toronto, Montreal, Sydney, Tokyo, 1998 : 389 - 515.

■ Schussler G.C., *The thyroxine-binding proteins*, Thyroid 2000 10 : 141-149.

TICK BORNE ENCEPHALITIS

DEFINITION

Tick Borne Encephalitis (TBE) is a disease an arbovirus (from the English *arthropod borne virus*) belonging to the *Flavivirus* genus of the huge *Flaviviridae* family. The virus is enveloped, measures around 65 nm in diameter and has an unsegmented positive polarity single-stranded RNA. It replicates in the cytoplasm of the infected cell. At least 14 antigenic types of virus exist (*TBEV-complex*) several of which are able to infect human beings. TBEV can be cultured on vertebral cells (Vero cells, chick embryo) and arthropod cells. The experientially infected young mouse develops fatal encephalitis.

Synonyms: Early summer meningo-encephalitis (ESME), Central Europe encephalitis; *Fruhsommer meningoenzephalitis* (FSME); *Tick Borne Encephalitis* (TBE).

INTRODUCTION

EPIDEMIOLOGY

Tick borne encephalitis occurs in well known endemic areas. The TBEV complex has two categories: one (Western), endemic to Central Europe and found in Germany, Switzerland, Scandinavia and Eastern France and the other (Eastern) endemic to Russia and the Asiatic coasts of the Japan sea. The viral cycle is maintained in nature by infection of wild rodents. The virus is mostly transmitted by bites from a tick vector (*Ixodes ricinus*), which is seasonally active from May to October. The proportion of ticks infected (up to 10%) varies considerably between regions. Forrest workers and ramblers are particularly exposed to infection. Livestock can also become infected and the animals excrete the virus in their milk, resulting in possible human infection from drinking unpasteurised milk. Its seroprevalence in France is estimated to be 2-3%.

SYMPTOMS

After an incubation period of 8 to 14 days following the infecting bite, the disease develops in 2 phases:

- **The primary phase** represents the viraemia. This is a typical flu-like syndrome which resolves spontaneously and completely.
- **The secondary phase** follows the first phase in less than 10% of cases after an asymptomatic period of approximately a week, and presents with lymphocytic meningitis or more rarely meningo-encephalitis. Severity depends on the infecting strain of virus. It is fatal in 1 to 2% of cases particularly in frail people. Total recovery, however, is mostly seen, although the convalescent period may be long with occasional residual problems (headaches, dizziness and sensory disorders). The occult or latent form of the infection is common.

INDICATIONS FOR MEASUREMENT

The diagnosis of meningitis or meningoencephalitis in an endemic area or after a stay in an endemic area, in spring or summer.

Monitoring for post-vaccination immunity.

INFORMATION

SAMPLE

CSF for direct testing of virus or antibodies. Serum samples taken at 7-10 day intervals to test for antibodies.

QUESTIONS FOR THE PATIENT

Occupation?

Time spent in an endemic area?

Vaccination against TBE?

SAMPLE STORAGE AND TRANSPORT

CSF, taken if possible at the start of the neurological phase, to be sent frozen at –20°C if the investigations are to be performed later.

Unhaemolysed serum stored at + 4°C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

The virus can be detected in peripheral blood during the primary phase, although is generally not tested for at this time. Testing for the virus is usually requested on CSF.

- **Isolation of the virus:** The virus can be isolated by intraperitoneal inoculation into the young mouse, inoculation into an embryonated chick egg and above all by cell culture (VeroE6 monkey cell line). Isolation is generally carried out in specialist laboratories.
- **Identification of the viral genome:** Detection of the viral genome by RT-PCR. This is tending to replace culture. Sequencing techniques provide a detailed analysis of the strains of virus.

INDIRECT DIAGNOSIS

TBE serology is generally performed by ELISA with testing for specific IgM by immunocapture. Commercial kits are available.

INTERPRETATION

Finding the virus or its genome indicates the diagnosis. It is important that the CSF is collected early after the onset of neurological symptoms as the virus is only detectable for a short period of time.

IgM antibodies generally develop around the 4^{th} or 5^{th} day after the onset of fever. The levels increase rapidly and persist for 3 to 6 months or even more. They are always found in blood and are often found in CSF.

IgG antibodies develop around the tenth day, reaching a peak at 4 – 6 weeks and persisting indefinitely after recovery. The presence of isolated IgG antibodies indicates immunity against TBEV.

The finding of IgM antibodies in CSF is strong evidence supporting the diagnosis.

Note, however, that cross-reactions commonly occur between the different flaviviruses.

TREATMENT

Flu-like forms of the disease usually do not require any treatment. Treatment in neurological forms of the disease is purely symptomatic, as there is currently no effective antiviral treatment.

Individual protection involves educating at risk people, pasteurising milk, combating rodents, preventing tick bites by using repellents and clothing protection, plus awareness of ticks that must be removed as soon as they have fixed to the body. These measures will also provide protection against *Borrelia burgdorferi* and *Ehrlichia* which use the same vector and are transmitted in the same geographical areas.

Prophylactic vaccines against tick borne encephalitis have been available for more than 30 years, although several modifications have been made to their formulations. A second generation of inactivated virus became available in 2009 (Encepur®, Novartis vaccine) with formulae for adults and children 12 years old and upwards. Encepur® uses purified inactivated virus from the European tick borne encephalitis strain: K23. The vaccine is administered as 3 injections over a period of 15 months. The recommended interval between the first and second injection is 1 to 3 months followed by 9 to 12 months between the 2nd and 3rd injection. In order to be protected during the tick bite risk period the 1st and 2nd injections should preferably be administered the previous winter and the 3rd injection immediately before springtime. Booster injections should start 3 years after the programme has finished and be repeated at 3 to 5 year intervals.

A rapid vaccination calendar, however, can be used if short-term immunity is required. The "shortened" regimen involves a first dose administered on D0, the second 7 days later and the third 21 days after the first dose. Seroconversion is generally obtained a minimum of 14 days after the second injection or at the earliest, 3 weeks after the first injection.

Recent official recommendations from the World Health Organisation (WHO) international *Scientific Working Group on Tick-borne encephalitis* (ISW-TBE) and other official bodies recommend that any adults or children who are living in, travelling to or working in at risk areas should be vaccinated.

FOR FURTHER INFORMATION

■ Rodhain F., *Fièvre jaune, dengue et autres arboviroses*, Encycl..Med. Chir, Maladies infectieuses ,8-062-A-10 ; 2001- p.1 à 18.

TIN

DEFINITION

Tin was widely used in its mineral (Sn) or organic (mostly bis tributyltin) oxide form in many industrial applications, such as the wood protection, dirt-repellent paints, processing industrial water and storage of textiles, leather and papers. In view of its harmful effects on health and the environment, its use is tending to decrease nowadays and restrictions or prohibitions on its use have been put in place in the European Union.

INTRODUCTION

It is important to distinguish tin and its inorganic derivatives (mineral tin salts) from its organic (organostannic) compounds. Tin enters the body through the lungs in the form of oxide dust or smoke. Tin mineral salts are poorly absorbed. Tin is bound 80% to red blood cells and is found mostly in the skeleton.

On the other hand the organostannic compounds are highly lipophilic and very toxic; they are well absorbed by the gastro-intestinal tract and skin (particularly the aliphatic compounds) and are found in the liver, kidneys and brain. Mineral tin salts are mostly excreted in urine whereas the organostannic forms are excreted in urine and faeces.

INDICATIONS FOR MEASUREMENT

Urinary tin measurements are the only ones which, appear to reflect exposure to organic or inorganic tin compounds, although limited data are available. Measurements are also available in whole blood.

INFORMATION

SAMPLE

Tin measurements in blood are performed on whole blood taken into EDTA or on heparinised plasma.

Blood or urine samples can be taken at any time in the day or working week.

QUESTIONS FOR THE PATIENT

What is your source of exposure to tin?

SAMPLE STORAGE AND TRANSPORT

Blood and urine samples can be refrigerated for several months. As metal contamination is the main problem with analysis, sampling materials which contain no tin must be used as these can interfere with the assay method.

It is essential that the occupational physician contacts the laboratory which is performing the analysis and the laboratory taking the sample, if these are different, in order to obtain details about the sampling and transport methods and pitfalls to be avoided. Samples must always be taken away from the working premises, ideally after a shower to reduce the risk of skin contamination.

ASSAY METHODS

The assays used are either induction coupled plasma mass spectrometry (ICP-MS), the reference method which has extremely low limits of quantification and detection or failing this, atomic absorption spectrometry (AAS).

NORMAL EXPECTED VALUES

Reference values in the general population are:

– In plasma: < 1 µg/l

– In whole blood: < 1.75 µg/l

– In urine: < 25 µg/g of creatinine.

There are no guideline values at present for occupationally exposed workers, either in blood or urine.

PATHOPHYSIOLOGICAL VARIATIONS

Blood and urine tin concentrations may be increased in exposed workers although limited data are available.

FOR FURTHER INFORMATION

- Fiche Biotox «Tin ». www.inrs.fr.

TISSUE PLASMINOGEN ACTIVATOR

DEFINITION

Tissue plasminogen activator (tPA) is a serine protease which plays a key role in the fibrinolysis mechanism. It is present in plasma, linked 95% to the plasminogen activator inhibitor (PAI-1), in platelets, as well as in numerous other tissues (uterus, lungs, salivary glands, etc.). tPA is mainly synthesised by vascular endothelial cells, but also by megakaryocytes, macrophage monocytes, cardiac fibroblasts and neurons. It is released into the bloodstream by the effects of various stimuli, including histamine, acetylcholine, adrenaline or bradykinin, as well as by stress, physical activity, venous stasis and by drugs such as DDAVP, Stanozolol (an anabolic steroid), isoproterenol (a beta 2 adrenergic agonist) and phenylephrine (an alpha-adrenergic).

tPA can also be synthesised by genetic engineering and used in human therapy as a thrombolytic agent. The variants tenecteplase and reteplase are also used for therapeutic purposes.

INTRODUCTION

Fibrinolysis is a physiological phenomenon involving the solubilisation of a fibrin thrombus by plasmin, an enzyme generated from plasminogen bound to and adsorbed by the fibrin. The main pathways for the activation of plasminogen into plasmin involve tPA and pro-urokinase.

The enzymatic activity of tPA on plasminogen in plasma is very weak. It increases 200 to 400 times if the tPA and plasminogen are adsorbed on the fibrin. Plasminogen activation therefore takes place at the surface of the fibrin clot; the plasmin formed can then break down the insoluble fibrin into fibrin breakdown products. It has also recently been shown that tPA can activate factor VII and thus have a pro-coagulant action. In addition, tPA stimulates the proliferation of endothelial cells and possesses anti-inflammatory properties.

An equilibrium between fibrinolysis and coagulation is essential for physiological haemostasis. A reduction in fibrinolytic activity encourages the development of thromboses, whilst excessive fibrinolysis can cause haemorrhages.

INDICATIONS FOR MEASUREMENT

This measurement has been suggested as a diagnostic factor in hyperfibrinolysis, which can be the reason for a haemorrhagic syndrome (rare familial cases have been described), or in hypofibrinolysis associated with an increased risk of venous or arterial thrombosis (a small number of families have been reported presenting deficient fibrinolysis activation because of insufficient release of tPA).

During the years between 1980 and 1990, DDAVP was used to assess fibrinolytic activity generated by the release of tPA in patients who had suffered one or more idiopathic deep vein thromboses (DVT's). With the same objective, tPA measurements have been made before and after tests of venous stasis, at the same time as a measurement of PAI-1. The value of these tests has not yet been demonstrated.

The measurement of tPA has also been widely used in epidemiological studies to evaluate cardiovascular risk, underlining the importance of the fibrinolysis mechanism in the occurrence of acute complications of atherosclerosis.

In the absence of any formally-agreed indication, it represents at the present time a second-level measurement, undertaken by specialised laboratories, in the presence of an unexplained haemorrhagic syndrome.

INFORMATION

■ SAMPLE

Citrate tubes with antiplatelet additives (theophylline, adenine, dipyridamole: CTAD tubes) or containing an acidified citrate solution (such as Stabilyte®) are recommended. Failing this, sample onto citrate at a concentration of 3.2% (0.109 M) at 1/10th (0.5 ml for 4.5 ml of blood); tubes citrated at 3.8% (0.129 M) are acceptable (see «*conditions générales des prélèvements en hémostase*»). EDTA tubes are not permitted (raised plasma concentrations of tPA antigen).

tPA being a labile parameter, the sampling conditions must be strictly respected:

– The sample must be taken between 8 and 10 a.m., when the subject is fasting or has only taken a light, fat-free breakfast and has rested for at least 20 minutes (circadian variations and increased plasma concentrations of tPA after physical activity).

– The subject should refrain from smoking or from taking drinks containing caffeine for at least 1 hour before the sample is taken, and from consuming alcohol for at least 18 to 24 hours.

– Sampling should be well separated from any acute thrombotic, infectious or inflammatory episode.

– Take the sample without using a cuff (or with only a lightly-tightened cuff) in order to limit venous stasis.

– It is advisable to throw away the first few millilitres of blood and therefore to perform the measurement on a second sampling tube.

■ QUESTIONS FOR THE PATIENT

Are you taking any medication? The following drugs can cause an increase in plasma concentrations of tPA: DDAVP, Stanozolol (an anabolic steroid), phenylephrine and isoproterenol (a beta 2 adrenergic agonist).

Do you have any history of venous or arterial thrombosis or haemorrhagic symptoms?

■ SAMPLE STORAGE AND TRANSPORT

The sample must be centrifuged and decanted within 2 to 4 hours of being taken.

Fast freezing to -20° C; frozen transport.

ASSAY METHODS

ELISA immunoenzymatic assay.

REFERENCE VALUES

As an indication, normal plasma concentrations range from 3 to 10 ng/ml. They vary depending on the measurement kit used.

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

A circadian rhythm is present: tPA activity is weak during the night and the early hours of the morning, but activity may triple in the course of the day.

Increased plasma concentrations of tPA with stress, intense physical exercise and anxiety.

■ PATHOLOGICAL VARIATIONS

– Pre-analytical conditions not respected: cuff too tight or left in place for too long, intense physical exercise or stress before the sample is taken, non-compliant sampling, in particular for tPA, of which the activity is highly labile.

– Interferences with ELISA assay test: presence of rheumatoid factor in the plasma.

– Raised tPA plasma concentrations are mainly observed in an inflammatory context. It is described in the course of syndromes involving respiratory distress, myocardial infarction, septicaemia or hepatic illnesses (cirrhoses), as well as in primary or secondary hyperfibrinolysis syndromes (disseminated intravascular coagulation). Rare cases of familial haemorrhagic syndrome have also been attributed to an excess of tPA.

In the literature, an elevation of tPA is often associated with an increased risk of cardiovascular disease, although this marker is not presently used to predict the risk in individual cases.

A final point is that hypofibrinolysis is found in 10 to 30 % of patients with venous thrombo-embolic histories. In a majority of cases, the reduced fibrinolytic activity is believed to be due to excessive inhibition by PAI1, or to a fibrinogen or plasminogen abnormality. In certain cases, it might result from defective tPA release. A reduction of tPA plasma concentrations would then be a (disputed) risk factor for DVT.

FOR FURTHER INFORMATION

■ Lebrazi J., Samama M.M., Bachmann F., *Système du plasminogène et son exploration*, Encycl Méd Chir (Éditions scientifiques et médicales Elsevier, Paris), Hématologie, 13019-A-30,2003: 18p.

■ Alessi M.C., *t-PA antigène*, Encycl Méd Biol (Elsevier, Paris), 2003.

■ Boneu B., Cazenave J.-P., *Introduction à l'étude de l'hémostase et de la thrombose*, 2e Ed. Boehringer Ingelheim, Reims, 1997.

TOBRAMYCIN

DEFINITION

Tobramycin is an antibiotic belonging to the aminoglycoside (or aminoside) family, characterised by its bactericidal action on numerous Gram-positive and Gram-negative bacteria. It is used in therapy either alone or in combination, in particular with β-lactamines, in infections caused by sensitive micro-organisms (mainly Gram-negative bacilli). Its principal disadvantage is its toxicity (class toxicity) for the kidneys and ears.

It is marketed in the form of 25 mg or 75 mg doses of an injectable solution.

Intramuscular (IM) injection is the preferred route of administration, using two patterns:

– two or three daily doses

– a single daily dose: in patients below the age of 65, with normal renal function and in the absence of neutropaenia, if treatment is for less than 10 days and only for infections with Gram-negative bacteria, excluding *Pseudomonas* and *Serratia*. At least identical efficacy, and sometimes better tolerance, have been demonstrated with this therapeutic pattern.

Tobramycin is also sometimes administered intravenously, by discontinuous perfusion for 30 to 60 minutes.

The usual dose is:

– in adults, babies and infants: 3 mg/kg/day

– in newborn babies of < 10 days: up to 3 or 4 mg/kg in 2 injections/day.

Dosage should not exceed 5 mg/kg/day, except in the case of life-threatening infections, if serum concentrations are monitored.

Aminosides are concentration-dependent antibiotics, characterised by a post-antibiotic effect, offering the possibility of increasing the interval between two doses, without fear of a resumption of bacterial development.

PHARMACOKINETICS

Bioavailability (parenteral administration)	100 %
Plasma peak	30 to 60 minutes after intramuscular perfusion
Steady state	24 hours (day 2 of treatment); 10 hours in children
Protein binding	Practically inexistent
Metabolism	nil
Clearance	Through the kidneys, in unchanged form
Clearance half-life	– 2 hours in subjects with normal renal function – longer in elderly subjects and those with renal failure (sometimes as much as 100 hours) – variable with body weight in intensive care or major burns patients

INDICATIONS FOR MEASUREMENT

Plasma measurements are normally performed during treatment, since the therapeutic index of tobramycin is low and its pharmacokinetics variable.

Because of the toxic risk (ototoxicity and nephrotoxicity) and the severity of the infections generally treated, these doses allow efficacy to be verified without reaching the zone of toxicity.

Measurement is indispensable if treatment is to be applied for more than 7 days in the following situations:

– patient aged over 65

– renal failure

– severe infection by Gram-negative bacteria

– new born babies and small children

– obese patients.

Two measurements are carried out:

– on-peak (maximum concentration), to monitor efficacy

– residual (valley), to verify the absence of antibiotic accumulation (toxicity).

INFORMATION

■ SAMPLE

Serum or plasma

Measurements must be performed when treatment begins, when steady state has been reached i.e. 24 hours after the start of treatment, or 24 hours after a change of dose.

– Before the next injection for a measurement of trough concentration

– 30 minutes after the end of IV perfusion (opposite arm) or 1 hour after IM injection for peak measurement.

■ ESSENTIAL INFORMATION

It is essential that all requests for drug assay include the following information: reasons for prescribing (determination of efficacy or toxicity), the time when the sample is collected, start of treatment and/or of any change in dosage (quantity, frequency and mode of administration), together with the age, height and weight of the subject when possible.

– Treatment with other nephro- or ototoxic drugs (risk of aggravation of toxicity)?

– Prior treatment with another aminoside, or with a loop diuretic such as bumetanide, furosemide or piretanide?

– Aggravation of toxicity with cyclosporin, tacrolimus, amphotericin B, polymyxin or cisplatine.

■ SAMPLE STORAGE AND TRANSPORT

Store at +4°C.

Transport of serum/plasma at +4°C.

ASSAY METHODS

Principally immunological methods: FPIA, EMIT, immunoturbidimetry, etc.

NORMAL EXPECTED VALUES

Trough concentration < 2 mg/l. A trough concentration beneath this threshold indicates that the chosen rate of administration was suitable for the patient's removal capacity.

Peak concentration: 4 to 12 mg/l. Low peak concentrations are associated with therapeutic failure.

Dosage adjustment according to serum concentrations of antibiotic: determined on a case-by-case basis, depending on the condition of the patient, the severity of the infection and the modalities of administration.

In general, an excessive trough concentration requires a longer interval between doses, while a peak concentration which is too low suggests (after confirmation) that dosage should be increased.

FOR FURTHER INFORMATION

■ Dictionnaire Vidal®.

■ Lacarelle B., Basso A., Bouquet S., Venisse N., *Suivi thérapeutique pharmacologique de la tobramycine*. In : Suivi thérapeutique pharmacologique pour l'adaptation de posologie des médicaments, Collection Option/Bio, Ed Elsevier, Paris. 2004 : 63-73.

TOLUENE AND HIPPURIC ACID

DEFINITION

Toluene or methylbenzene is a mobile colourless liquid with an aromatic smell, which is practically insoluble in water. It is an excellent solvent for many natural and synthetic substances (oils, fats, resins, etc.). It is widely used as a synthesis intermediary for many compounds including benzene and xylenes, phenol, nitrotoluene, and benzaldehyde, as a solvent for paints, lacquers, printing inks, adhesives and waxes and as an extraction solvent in the cosmetics and pharmaceutical industry. Toluene is also used mixed with benzene and xylenes as a fuel additive to improve the octane index. It is present in some petroleum products.

METABOLISM

Toluene is well absorbed by the gastro-intestinal and respiratory tract and to a lesser degree through the skin. Respiratory absorption is rapid, toluene appearing in the blood after exposure for 10 to 15 minutes. It is completely absorbed from the gastro-intestinal tract with a blood peak achieved in 2 hours. In the blood, toluene is distributed between the red blood cells, where it binds haemoglobin, and serum. It is then distributed in highly vascularised, fat-rich tissues such as the brain, particularly the white matter, bone marrow, spinal cord and also liver, adipose tissue and the kidneys. It is secreted in breast milk. Toluene is 80% oxidised in the liver by cytochrome P450 mono-oxygenases into benzyl alcohol, benzaldehyde and then benzoic acid, which is conjugated with glycine to form hippuric acid, the main metabolite removed in urine.

MECHANISM OF ACTION

Toluene mostly causes neurotoxicity by reversible interactions between the toluene itself (and not its metabolites) and membrane components (lipids and proteins) of cerebral nerve cells. Repeated interactions can impede the activity of some enzymes involved in the synthesis and/or degradation of certain neurotransmitters.

SYMPTOMS OF POISONING

■ ACUTE POISONING

Ingestion of toluene causes:

– Gastro-intestinal problems, such as abdominal pain, nausea, vomiting and diarrhoea.

– Central nervous system depression: inebriation followed by disordered consciousness. The deterioration in psychomotor functions depends on concentration and duration of exposure.

– Inhalation pneumonia.

– Ocular and upper respiratory tract irritation.

– Irritation dermatitis when sprayed onto the skin.

■ CHRONIC POISONING

The usual route of occupational poisoning is via the respiratory tract and the major toxic effect of toluene is a psycho-organic syndrome.

– Disordered memory, concentration and personality with reduced intellectual faculties. The incidence of these problems increases with level of exposure, although no dose-response correlation has been established.

– Toluene can have effects on reproductive function (risk of late miscarriage following early exposure in pregnancy); chronic maternal exposure carries a risk of intra-uterine growth retardation.

INDICATIONS FOR MEASUREMENT

Blood toluene concentrations immediately at the end of the work shift correlate well with urinary hippuric acid concentrations and air concentrations during the day. Values fall rapidly after exposure ceases. Measurement of urinary hippuric acid is useful to assess same day and work shift exposure (when > 50 ppm). Baseline values are reached 16 hours after exposure ceases. Concentrations correlate well with ambient air concentrations, particularly at moderate to high exposure levels. At low exposure (< 50 ppm) measurements are unreliable on an individual basis because of individual variations and poor specificity.

Measurement of urinary ortho-cresol at the end of the work shift reflects exposure on the same day and is more sensitive and specific than hippuric acid.

Finally, measurement of toluene in expired air during the work shift can be useful to confirm exposure, as it is specific and sensitive even at low concentrations.

INFORMATION

■ SAMPLE

5 ml of whole heparinised or EDTA blood; 20 ml of an unacidified urine specimen.

This sample should preferably be taken at the end of the work shift.

■ QUESTIONS FOR THE PATIENT

Establish whether the patient smokes, as this increases toluene concentrations, The sample should be taken more than one hour after the last cigarette.

■ SAMPLE STORAGE AND TRANSPORT

Whole blood and urine samples can be stored and transported to the laboratory at ambient temperature or at between 2 and 8°C.

■ INTERFERENCES

Urinary hippuric acid is a metabolite of other substances including ethylbenzene, styrene, dietary benzoic acid (preservative) and amino acid metabolism. Its metabolism can be increased (enzyme inducing drugs) or reduced (alcohol) by several factors.

ASSAY METHODS

Gas chromatography with flame ionisation detection (GC-FID).

Gas chromatography linked to mass spectrometry (GC-MS).

NORMAL EXPECTED VALUES

According to INRS recommendations (Biotox 2002):

– Blood toluene:
  - guideline value for France: < 1 mg/l
  - biological exposure index (ACGIH BEI) = 0.05 mg/l before the last work shift in the week.

– Urinary toluene: general population value < 0.4 µg/l

– Urinary hippuric acid:
  - general population value: < 1.50 g/g of creatinine.
  - guideline value for France: < 2.50 g/g of creatinine at the end of the work shift

– Urinary ortho-cresol:
  - general population value: ≤ 0.20 mg/l
  - biological exposure index (ACGIH BEI) = 0.5 mg/l at the end of the work shift.

FOR FURTHER INFORMATION

■ Toxicologie industrielle et intoxications professionnelles, *Toluène*, Lauwerys R. 3e édition, Masson.

■ Fiche du toluène : guide BIOTOX 2002, INRS.

TOTAL IgE

DEFINITION

Whilst as early as 1921, passive transfer experiments demonstrated that the allergic response involved a specific so-called "reagin" antibody, it was not until 1966 that a fifth class of immunoglobulins, IgE, were shown to be the support for "reagin activity". IgE is a 190 kDa monomeric immunoglobulin consisting of 2 identical light chains (κ or λ) and 2 identical heavy chains (ε) which are extensively glycosylated. It has a short plasma half-life (2 days), although this increases (3 to 4 days) when IgE reaches the increased levels characteristic of atopy. These immunoglobulins are heat labile and lose their homocytotropic activity on heating and do not activate complement.

The biological effects of IgE act through receptors which differ in their affinity, protein family (immunoglobulin and lectin superfamily) and their cell expression (see Specific IgE page).

IgE is known to play a role in the development of immediate hypersensitivity reactions but also contributes considerably to anti-parasitic immunity.

INDICATIONS FOR MEASUREMENT

High total IgE concentrations are generally found in the serum of atopic patients. Because of the many possible causes of hyper IgE-globulinaemia, measurement is not indicated for the diagnosis of atopy and testing for specific IgE against environmental allergens is preferred. Measurement in children under 3 years old, however, is of considerable informative value as high concentrations are highly suggestive of atopy. This is requested before starting anti-IgE antibody treatment.

INFORMATION

■ SAMPLE

Serum (dry tube) or possibly heparinised plasma.

A fasting sample is not essential. Measurements are not influenced by drugs (antihistaminergic agents, corticosteroids, antidepressants, etc).

■ SAMPLE STORAGE AND TRANSPORT

Store at between + 2 and + 8°C for up to one week or frozen at – 20°C for several years. Repeated freeze-thaw cycles should be avoided.

ASSAY METHODS

As IgE concentrations are extremely low in serum, highly sensitive methods have been developed. Regardless of the method used, specific IgE are sandwiched between an anti-IgE antibody fixed to a support and labelled human anti-IgE. The labelling signal rises with increasing IgE concentrations. Results are generally expressed against the second reference preparation produced by the WHO (2nd IRP 75/502). Technically, total IgE measurement does not pose problems and all commercial kits produce similar results. They differ in sensitivity at low concentrations.

EXPECTED VALUES

Total IgE concentrations vary within a very wide range between people, depending on age, ethnicity and different environmental factors (pollution, smoking, etc.). In France, the generally accepted "normal" concentration in adults is less than 150 kU/l. Values increase progressively in children reaching adult levels at around the age of 8 years old. Concentrations over 1 kU/l in cord blood are a risk factor for atopy particularly when a family history is present.

PATHOLOGICAL VARIATIONS

A high IgE concentration supports atopy, although IgE may be raised in other pathological situations:

– Parasite infestation, occasionally with the production of specific IgE to the agent responsible,

– Viral infections, such as measles, etc.

– Immunodeficiency, such as Wiskott-Aldrich syndrome, Di Georges syndrome and in hyper-IgE syndrome,

– Neoplastic disease, such as Hodgkin's disease and IgE myeloma, etc.

Total IgE concentrations offer only supporting evidence for atopy if positive, but do not exclude it if negative. Single allergen sensitised people have concentrations close to the reference range, although multi-sensitised people generally have high total IgE concentrations.

FOR FURTHER INFORMATION

■ Vervloet D., Magnan A., *Traité d'allergologie*, Flammarion Médecine-Sciences, 2003.

TOTAL T3 AND FREE T3

DEFINITION

3-5-3′ tri-iodothyronine (T3) is an iodinated amino acid (RMM = 651) produced by thyrocytes after iodination of thyroglobulin tyrosyl groups into mono-iodotyrosine (MIT) and di-iodotyrosine (DIT) followed by binding of one molecule of MIT (20%) and one molecule of DIT (20%) and by peripheral deiodination (liver, kidney, muscle and brain) of thyroxine 3-5-3′-5′ tri-iodothyronine (T4) by various 5′-deiodinases (80%).

It circulates in two forms in blood, in equilibrium:

– a free form (free T3, FT3), which is biologically active (approximately 0.3%),

– a bound form (99.7%), which is bound approximately 35 to 40% to *thyroxine binding globulin* (TBG), 25 to 30% to *thyroxine binding prealbumin* (TBPA) and 35 to 40% albumin (A).

Total T3 (T3, TT3) is the sum of the two fractions.

T3 secretion is:

– stimulated by pituitary *Thyroid Stimulating Hormone* (TSH), which itself is stimulated by hypothalamic *Thyroid releasing Hormone* (TRH);

– suppressed by T3 and T4 (thyroxine), which exert negative feedback control by binding to specific TSH and TRH receptors.

Iodine and various psychological (stress, etc.), physical (temperature, etc.) and hormonal (dopamine, noradrenaline, serotonin, cortisol, somatostatin, etc.) factors are also involved in regulating its secretion. The daily secretion rate is 25 µg and is almost stable throughout the 24-hour cycle. Its plasma half-life (T1/2) is approximately 24 hours.

INTRODUCTION

The free T3 hormone, which is physiologically active, has no preferred target organ. It acts by binding to the nuclear proteins of sensitive cells. It is metabolised through various mechanisms (successive deiodination, oxidative deamination, decarboxylation, and conjugation), into more or less active metabolites (di-iodothyronines, tri-iodoacetic acid).

It has important and varied biological activities, principally cellular and visceral. In addition to its role in iodine metabolism it is involved in accelerating general metabolism, thermoregulation, bone growth, intellectual and behavioural development and libido. It also has a role in regulating cardiac and vascular activity, bowel transit, skin and muscle trophicity and hair growth.

INDICATIONS FOR MEASUREMENT

It is not particularly useful for the diagnosis of hypothyroidism. Free T3 measurement is however useful to confirm and classify:

– hyperthyroidism with low TSH and normal FT4 (subclinical hyperthyroidism or overt T3 toxicosis);

– "low T3 syndromes" or *non-thyroidal illness* (NTI) found in clinically and laboratory euthyroid elderly people suffering from serious diseases (cancers, cirrhosis, renal insufficiency, etc.).

Measurement of FT3 or Total T3 is also useful for monitoring treatment in hyper and hypothyroidism (efficacy, adherence and overdose) and in the post-operative assessment after thyroidectomy.

Measurement of total T3 or free T3 can only be interpreted in thyroid function tests when combined with measurement of TSH. Total T3 is relatively sensitive to changes in transport protein concentrations and FT3 measurement is preferred.

INFORMATION

SAMPLE

The sample should be taken by venepuncture into a tube without additive.

Depending on the methods used, assays may be performed on plasma obtained from blood collected into EDTA or other anticoagulants.

Warning: EDTA is incompatible with immunoenzymatic methods using alkaline phosphatises (it binds zinc). EDTA and citrate must not be used with immunofluorimetric methods using rare earth metals (particularly Europium).

Avoid haemolysed, jaundiced and hyperlipaemic serum.

QUESTIONS FOR THE PATIENT

Age?

Suspected diseases and clinical features?

Current treatment as many compounds interfere, particularly: T3 and derivatives, amiodarone, aspirin, non-steroidal anti inflammatory drugs and radioactive iodine?

Recent radiological functional investigations (iodinated contrast media) and/or angiography (fluorescein)?

SAMPLE STORAGE AND TRANSPORT

After centrifugation and separation, serum can be stored for up to 2 days at room temperature, for 7 days at + 4°C and for several months frozen at – 20°C. Avoid repeated freeze-thaw cycles.

ASSAY METHODS

FREE T3

Apart from the reference methods, which are not used routinely (equilibrium dialysis and ultrafiltration), current assay methods mostly use automated non-radioactive one or two stage immunoassays with labelled antibodies *(Solid Phase Antigen Linked technique = SPALT method)* or a labelled ligand.

Depending on the signal used these involve RIA, EIA, FIA, LIA or ECLIA methods.

Because of very high antibody specificity (monoclonal or polyclonal) no cross-reactions occur particularly with FT4.

All methods however are associated with numerous causes of error, the incidence of which varies between each method.

The most well known are due to:

– Changes in circulating carrier protein concentrations:

- congenital (TBG, variant TBPA, dysalbuminaemia and analbuminaemia),
- acquired (TBG, hypoalbuminaemia and non-esterified fatty acids).

- Drugs (a very large number).

– Cross-reactions with some drugs (tri-iodoacetic acid, diclofenac and tenoxicam).

– Circulating autoantibodies, particularly in women (75%): anti-T3 (25%), anti-T3 and anti-T4 (25%).These produce antibody excess effects in one stage assay methods (SPALT).

– Heterophile antibodies (0.1 per 1000). These also cause antibody excess effects except for two stage assay methods (which are relatively insensitive to this interaction).

Rarer interferences (1 per 10,000) are caused by rheumatoid factors, anti-solid phase antibodies (SPALT method), anti-ruthenium, anti-avidin and anti-biotin antibodies.

No method "unequivocally" respects the free/bound equilibrium and the values provided only estimate the free hormone concentrations.

■ TOTAL T3

The assay is performed after releasing the bound fraction by salicylates or more often, 8-alinino-naphtalene sulphonic acid. Most assay methods use competitive techniques with mono or polyclonal antibodies and various markers: Isotopic (RIA), Enzymatic (EIA), Fluorescent (FIA), and Luminescent (LIA, ECLIA).

The assays can be subject to numerous interferences, which have varying effects depending on the method. The most common are due to:

– incomplete dissociation in people with high protein concentrations (negative bias),

– the presence of circulating antibodies in a patient (anti-T4 and/or anti-T3, heterophile antibodies),

– some drugs.

NORMAL EXPECTED VALUES

Values vary with age and physiological state (pregnancy). They depend greatly on the method used.

Total T3 = nmol/l or µg/l (T3 nmol/l x 0.651 = T3 µg/l).

Free T3 = pmol/l or ng/l (FT3 pmol/l x 0.651 = FT3 ng/l).

Age (years)	T3 (nmol/l)	T3L (pmol/l)
0 - 1	/	4 - 11
1 - 20	1.5 - 5	5 - 9.5
20 - 60	1 - 3.5	3.5 - 7.5
Above 60	0.5 - 2.5	2.5 - 7
3rd trimester of pregnancy	2 - 4	3 - 7

Blood total T3 concentrations rise in response to oestrogen during the 1st trimester of pregnancy (30%) and stabilise thereafter, whereas blood FT3 concentrations fall slightly during the 2nd and/or 3rd trimesters. Free and total T3 fall gradually with age (38% between 60 and 90 years old).

PATHOLOGICAL VALUES

Low serum TT3 and/or FT3 concentrations are seen in central hypothyroidism, severe non-thyroidal illness, (low T3 syndrome), malnutrition and some drug treatments.

High serum TT3 and FT3 concentrations are seen in hyperthyroidism, pure T3 toxicosis, thyrotrope adenomas, thyroid hormone resistance and some drug treatments. T3 concentration is normal in many cases of hypothyroidism.

FOR FURTHER INFORMATION

■ Sapin R., Schlienger J.-L., *Dosages de thyroxine (T4) et de tri-iodothyronine (T3); techniques et place dans le bilan thyroïdien fonctionnel*, Ann. Biol. Clin. 2003 ; 61 : 411-420.

■ Ingrand J., *A propos de l'exploration fonctionnelle thyroïdienne Immuno-analyse et Biologie spécialisée*, 2002 ; 17 : 165-171.

■ www.santor.net/pdf/endocrino/nacb_resume. pdf

TOTAL T4 – FREE T4 - FREE T4 INDEX

DEFINITION

3-5-3'-5' tetra-iodothyronine (thyroxine = T4) is an iodinated amino acid (RMM = 776.88) produced by thyrocytes after iodination of the tyrosyl groups of thyroglobulin into mono-iodotyrosine (MIT) and diiodotyrosine (DIT). Binding together of two DIT results in the formation of T4.

T4 is released from thyroglobulin by proteolysis and circulates in two forms in the blood, in equilibrium:

– One free biologically active form (Free T4, FT4) (approximately 0.03%)

– One bound form (99.97%) bound to different proteins: 75 to 80% to *Thyroxine Binding Globulin* (TBG), which it saturates approximately one third, 15 to 20% to *Thyroxine Binding Prealbumin* (TBPA) and 5 to 10% to albumin (A).

Total T4 (T4, TT4) is the sum of the two fractions.

T4 secretion is:

– Stimulated by pituitary *Thyroid Stimulating Hormone* (TSH), which itself is stimulated by hypothalamic *Thyroid releasing Hormone* (TRH);

– Suppressed by T4 and T3 (tri-iodothyronine) which exert negative feedback control by binding to specific TSH and TRH receptors.

Iodine and various psychological (stress, etc.), physical (temperature, etc.) and hormonal (dopamine, noradrenaline, serotonin, cortisol, somatostatin) factors are also involved in regulating its secretion. It is more active after conversion into T3 (3-5-3'tri-iodothyronine) and T4 is considered to be the precursor of T3.

Daily secretion: 80 to 120 µg, practically stable during the 24-hour cycle. Its plasma half-life (T1/2) is approximately 7 days.

INTRODUCTION

The free T4 hormone, which is physiologically active, has no preferred target organ. It acts by binding into the nuclear proteins of sensitive cells. Its activity is controlled according to requirements in human beings by complex catabolism. The major pathway, mostly in the liver and kidney, generates metabolites as a result of 5 or 5' -deiodination, the activity of which depends on the number and position of the iodine atoms in the molecule.

In euthyroid people, daily T4 secretion is converted:

– 45% into T3, which is approximately 3 to 7 times more active than T4;

– 35% into reverse T3 (rT3 or 3-3'-5'tri-iodothyronine), which is 10 to 100 times less active than T4.

T3 and rT3 are then converted into various di-iodothyronines (approximately 50 to 70 times less active than T4) and then to non-iodinated metabolites (thyronines).

– 20% into various metabolites (by conjugation, oxidation, decarboxylation and deamination). Some is converted to thyro-acetic derivatives, particularly tri-iodoacetic acid (TRIAC), through oxidative deamination. TRIAC has significant hormonal activity.

FT4 has important and varied biological activities, principally cellular and visceral.

In addition to its role in iodine metabolism, it is involved in accelerating general metabolism, thermoregulation, bone growth, intellectual and behavioural development and libido. It also has a role in regulating cardiac and vascular activity, bowel transit, skin and muscle trophicity and hair growth.

INDICATIONS FOR MEASUREMENT

FREE T4 AND TOTAL T4

Measurements of free T4 are used to confirm and classify dysthyroidism suspected from suggestive signs and accompanied by an abnormal circulating TSH:

- **Hyperthyroidism with low TSH and FT4 which is:**
  – Raised (overt hyperthyroidism),
  – Normal (subclinical hyperthyroidism or T3 toxicosis),
  – Normal or reduced (drugs, severe *non-thyroidal illness* (NTI) or "low T4" syndromes seen in clinically and laboratory euthyroid elderly patients suffering from serious diseases such as cancer, cirrhosis or renal insufficiency etc.
- **Hyperthyroidism with normal or raised TSH** associated with raised FT4 (pituitary adenoma).
- **Hypothyroidism with raised TSH** associated with FT4 which is:
  – Either low (overt hypothyroidism),
  – Or normal: subclinical hypothyroidism (with anti-TPO antibodies),
  – Or raised: thyroid hormone resistance.
- **Hypothyroidism with normal or low TSH** associated with low FT4 (pituitary or hypothalamic hypothyroidism, drugs).

FT4 or total T4 measurement is also useful in monitoring the treatment of hyper and hypothyroidism (efficacy, adherence, overdose) and in the post-operative assessment following thyroidectomy.

FREE THYROXINE INDEX (FTI)

This test is used as an indirect measurement of FT4, taking account of T4, carrier proteins and their affinities for T4. It is based on measurement of total T4 and determination of:

– TBC *(Thyroxin Binding Capacity)*, which represents the transport protein potential binding capacity assessed by saturating their binding sites by adding excess labelled exogenous T3 or T4 (T3-test, T3-*Uptake*, T4-test, T4-*Uptake*). It is expressed as the percentage of labelled hormone on the protein (or on the binder), or as the TBI *(Thyroxine Binding Index)*, an index obtained against a euthyroid person allocated a value of 1.

– TBG.

Since FT3 and FT4 assays have become available, this test (FTI) is no longer of value.

INFORMATION

SAMPLE

The sample should be taken by venepuncture into a tube without additive.

Depending on the methods used, assays may be performed on plasma obtained from blood collected into EDTA or other anticoagulants. It is then essential to confirm carefully that the anticoagulant can reliably be used.

Warning: EDTA is incompatible with immunoenzymatic methods using alkaline phosphatase (it binds zinc). EDTA and citrate must not be used with immunofluorimetric methods using rare earth metals (particularly europium). Sodium citrate reduces T4 results by approximately 10% (with some methods). Lithium heparinate produces erroneous results with some methods.

Avoid haemolysed, jaundiced and hyperlipidaemic (serum triglycerides over 1.8 g/l) serum.

QUESTIONS FOR THE PATIENT

Age?

Suspected diseases and clinical features?

Current treatment, as many compounds interfere, particularly: conventional or low molecular weight heparins, T3 and derivatives, amiodarone, aspirin, non-steroidal anti inflammatory drugs and radioactive iodine)?

Heparin treatment even with low dose, low molecular weight heparin increases FT4 by releasing free fatty acids, *in-vivo* and *in-vitro*. In practice, in order to minimise the effect of low molecular weight heparin, the sample should be taken a minimum of 10 hours after the heparin injection and serum stored for less than 24 hours at 4°C before measurement.

Recent radiological functional investigations (iodinated contrast media), angiography (fluorescein)?

SAMPLE STORAGE AND TRANSPORT

TT4 and FT4 are not particularly unstable. After centrifugation and separation, serum can be stored for up to 2 days at room temperature, for 7 days at + 4°C (only 24h if the patient is being treated with heparin) and for several months frozen at – 20°C.

Avoid repeated freeze-thaw cycles.

ASSAY METHODS

FREE T4

Apart from the reference methods, which are not used routinely (equilibrium dialysis, ultrafiltration), current assay methods mostly use automated non-radioactive one or two stage immunoassays with labelled antibodies *(Solid Phase Antigen Linked technique = SPALT method)* or a labelled ligand.

Depending on the signal used these involve RIA, EIA, FIA, LIA, and ECLIA methods.

Because of very high antibody specificity (monoclonal or polyclonal) no cross-reactions occur particularly with FT3.

All methods are associated with numerous causes of error, the incidence of which varies between the methods used.

The most well known are due to:

– Changes in circulating carrier protein concentrations:

- congenital (TBG, variant TBPA, dysalbuminaemia, analbuminaemia),
- acquired (TBG, hypoalbuminaemia, non-esterified fatty acids).

– Drugs (many).

– Cross-reactions with some drugs (tri-iodoacetic acid, diclofenac, tenoxicam).

– Circulating auto-antibodies, particularly in women (75%): anti-T3 (25%), anti-T3 and anti-T4 (25%). These produce antibody excess effects in one-stage assay methods (SPALT).

– Heterophile antibodies (0.1 per 1000). These often cause antibody excess effects except in the two stage assay methods (which are relatively insensitive to this interaction.

Rarer interferences (1 per 10,000) are caused by rheumatoid factors, anti-solid phase antibodies (SPALT method), anti-ruthenium antibodies, anti-avidin and anti-biotin antibodies.

No method "unequivocally" respects the free/bound equilibrium and the values provided only 'estimate' the free hormone concentrations.

TOTAL T4

The assay is performed after releasing the bound fraction by salicylates or more often, 8-alinino-napthalene sulphonic acid. Most assay methods use competitive techniques with mono or polyclonal antibodies and various markers: Isotopic (RIA), Enzymatic (EIA), Fluorescent (FIA) or Luminescent (LIA, ECLIA).

The assay can be affected by numerous interferences, which have varying effects, depending on the method. The most common are due to:

– Incomplete dissociation in people with high protein concentrations (negative bias),

– The presence of circulating antibodies in a patient (anti-T4 and/or anti-T3, heterophile antibodies, rheumatoid factor, etc.), which are responsible for positive or negative errors.

– Some drugs.

REFERENCE VALUES

Age	T4 nmol/l	T4L pmol/l	ITL (T4x100)/T4-test
4 to 8 days	130 - 240	12 - 30	30 - 90
0 – 1 year	80 - 180	15 - 32	25 - 80
1 – 20 years	60 - 170	12 - 28	20 - 70
Adult	60 - 160	10 - 26	16 - 60
Pregnant 1st trimester	80 - 130	9 - 21	
Pregnant 2nd trimester	100 - 165	8 - 19	
Pregnant 3rd trimester	1110 - 180	7 - 16	

T4 nmol/l x 0.7768 = T4 µg/l).

FT4 pmol/l x 0.7768 = FT4 ng/l).

FTI = an expression without units. Depending on the methodology, however, it may be expressed as nmol/l (obsolete test).

Values vary with age and physiological state (pregnancy) and depend greatly on the method used. A TSH peak during the 12 hours after birth causes a 48 hour rise in serum T4 and T3 concentrations followed by a fall, values then stabilising at higher levels than in adults between days 4 and 8 after birth. This reflects thyroid function and this period is used for systematic screening for congenital hypothyroidism. Higher values than those seen in adults are occasionally found in children and adolescents.

■ PATHOLOGICAL VALUES

– Low serum TT4 and/or FT4 values are found in central hypothyroidism, Hashimoto's thyroiditis, severe non-thyroidal illness (low T4 syndromes), malnutrition, iodine deficiency and drug treatments.

– High serum TT4 and/or FT4 concentrations are seen hyperthyroidism (auto-immune thyrotoxicosis, toxic nodular goitre, toxic adenoma, Hashimoto's thyroiditis, De Quervain thyroiditis), pure T4 toxicosis, thyrotrope adenomas, thyroid hormone resistance, reduced transport proteins (TBG etc) and drug treatments (eg amiodarone).

FOR FURTHER INFORMATION

■ R. Sapin, J.L. Schlienger, *Dosages de thyroxine (T4) et de tri-iodothyronine (T3); techniques et place dans le bilan thyroïdien fonctionnel*, Ann. Biol. Clin. 2003 ; 61 : 411-420.

■ J. Ingrand, *A propos de l'exploration fonctionnelle thyroïdienne Immuno-analyse et Biologie spécialisée*, 2002 ; 17 : 165-171.

■ www.santor.net/pdf/endocrino/nacb_resume. pdf.

TOXOCARIASIS

DEFINITION

Toxocariasis is a cosmopolitan helminth zoonosis due to accidental human infestation by ascarides larva (*Toxocara canis* and *Toxocara cati*). It is widespread in both developing and industrialised countries. The pathogenic agent responsible is a worm belonging to the *Toxocara* genus, *Ascaroidea* family, *Ascarides* order and *Nematodes* class.

Synonyms: visceral ascarides *larva migrans*.

INTRODUCTION

EPIDEMIOLOGY

Toxocariasis is the most prevalent helminthiasis in the world and mostly affects children. It occurs as sporadic or familial epidemic cases particularly in deprived areas and in communities of children. It is due to animal faeco-oral transmission (dog and cat) and the parasite is earth-borne.

Human infestation occurs by eating embryonated eggs in contaminated food (salads, raw vegetables or other vegetables from unfenced vegetable plots), hands soiled by contact with earth contaminated by animal faeces (playing in **sandpits**). An additional common method of contamination is geophagia, particularly in developing countries.

LIFE CYCLE

Toxocara canis lives in the adult state in the small intestine of the final host, the pup. The female is between 6 and 18 cm long and the male between 4 and 10 cm.

After mating, the females lay large numbers of eggs, which are excreted in the puppy's faeces and remain for very long periods of time in the ground (occasionally years) as they have a thick shell and are very resistant to changes in weather.

They embryonate in the external environment becoming capable of infestation. Once eaten by a new host they rupture and release larvae into the intestine, which remain lodged in the tissues in the case of an immunised adult dog. The cycle in gestating females continues and the larvae cross the placenta (or through breast milk) infesting the offspring. The larvae then continue to migrate in the young pup, becoming adults in the small intestine.

If human beings become infested, the eggs, which are eaten, rupture in the small intestine and the larvae migrate to the liver and then lungs and then left side of the heart, then spreading to reach the different tissues (nervous system, muscles, eyes, etc.).

SYMPTOMS

Clinical symptoms vary depending on parasite load, patient age and site of the larvae in the body. A large number of clinical presentations exist, from asymptomatic forms of the disease to severe and occasionally fatal clinical pictures. The most typical form is seen in children, whereas adults develop occult forms of the disease, which are often discovered from a routine blood count.

- **Common form of toxocariasis** involves one or more of the following clinical signs: moderate fever, asthenia, anorexia, hepatomegaly and occasionally splenomegaly, gastro-intestinal problems, pulmonary problems (cough and dyspnoea), allergic skin reactions (urticaria, erythema multiforme). More rarely patients develop neurological problems (headaches and seizures).
- **Visceral *Larva Migrans* (VLM):** this is relatively rare and affects young children living in deprived areas with geophagia and/or those who live in contact with a puppy. The clinical features are classically a combination of fever, deterioration in general health, abdominal pain, respiratory problems (chronic cough and asthmatiform dyspnoea) and hepatomegaly.
- **Ocular *Larva Migrans* (OLM):** this is rare but serious and mostly affects children and young adults, causing a sudden fall in visual acuity. Ophthalmological examination reveals endophthalmia, uveitis, retinitis, posterior pole retinal granuloma or peripheral inflammatory damage.

SEARCH INDICATIONS

Diagnosis of toxocariasis, when typical clinical features are present (VLM or OLM).

Routine testing for toxocariasis in the presence of eosinophilia in an epidemiological context or suggestive symptoms.

Differential diagnosis from other helminthiases.

Differential diagnosis between the ocular form of the disease and retinoblastoma in a young child.

INFORMATION

SAMPLE

Serum: venous blood withdrawn into a sterile dry tube for serological diagnosis.

Surgically excised organs or biopsies: sent to the histology laboratory to examine for larvae.

CSF, aqueous humour, vitreous humour: collected into a sterile dry tube. These samples are used to examine microscopically for larvae and for immunological diagnosis.

QUESTIONS FOR THE PATIENT

Clinical details?

At risk patients: children? Dog breeders?

Family history of epidemic or contact with a dog or cat?

Blood eosinophil count and total IgE measurement if performed?

Current antihelminth treatment?

SAMPLE STORAGE AND TRANSPORT

Serum: store at + 4°C for approximately 5 days and then freeze at – 20°C.

Biopsy: send within an hour of being taken.

DIAGNOSTIC METHODS

GUIDING DIAGNOSIS

- **Blood eosinophilia:** plateau (20 to 80 G/L), massive values in VLM.

- **Leukocytosis:** simultaneous (20 to 100 G/l).
- **Total IgE:** raised.
- **ESR and CRP:** raised in VLM.

DIRECT DIAGNOSIS

This is very difficult or even impossible as the parasite is not actually exteriorised.

Testing for larvae in faeces is invariably negative. The absolute diagnosis is based on finding Toxocara canis in liver, aqueous humour or CSF biopsies, although this is only very rarely performed.

INDIRECT DIAGNOSIS

Serological diagnosis is essential given the difficulty of direct diagnosis and involves testing for and confirming the presence of serum antibodies. Cross-reactions occur between T*oxocara canis* and *Toxocara cati*.

Of the methods available:

Indirect immunofluorescence, electrosyneresis and immuno-electrophoresis with *Ascaris suum* should no longer be used as these methods lack specificity.

- **Immunoenzymatic methods:** or ELISA methods, use ES antigens (excretion-secretion of *Toxocara canis larvae*). Several kits are commercially available and perform well with a sensitivity between 80 and 91% and specificity of between 86 and 93%. Cross-reactions are due to common antigens with other helminths, particularly trichinellae and anguillulae.
- **Immunoblotting or Western blot:** uses *Toxocara canis* ES antigens separated by polyacrylamide gel electrophoresis and transferred onto a nitrocellulose membrane. This is used to confirm positive or equivocal results.

INTERPRETATION OF RESULTS

Toxocariasis is very difficult to diagnose particularly in the common childhood or adult forms which are less characteristic than the clinical presentations of OLM and VLM.

The diagnosis is very often made following investigation of a fortuitous finding of eosinophilia. ***The best diagnostic method is serology.***

Serological diagnosis is usually negative in the ocular form as only low levels of circulating antibodies occur. On the other hand it is straightforward to find antibodies in aqueous or vitreous humour. A positive immunological diagnosis in aqueous humour confirms ocular toxocariasis.

The association of suggestive symptoms, a positive serological diagnosis, eosinophilia and increased total IgE provides a diagnosis of active toxocariasis.

All other situations should be interpreted with caution, as the prevalence of the seroprevalence is high and patients who have been treated and recover remain seropositive for long periods of time.

TREATMENT

Treatment involves killing the larvae. Patients often recover spontaneously, although the recovery period is often very long. Anti-helminth treatment is only prescribed if eosinophilia or clinical problems persist. There is no completely effective treatment although it appears that recovery is better when treatment is started early.

DRUGS

The compounds used are albendazole or diethylcarbamazepine.

It may be useful to add corticosteroid therapy or antihistaminergic agents to avoid allergic reactions caused by destruction of larvae.

PROPHYLACTIC MEASURES

- **General:** combating faeco-oral transmission from animal faeces, regularly worming family dogs and cats from very young ages onwards, fencing off play areas for children and family vegetable gardens.
- **Individual:** improved hand hygiene after contact with earth, avoiding contact and playing with young unknown dogs and cats (which may carry parasites and not be wormed).

FOR FURTHER INFORMATION

- Magnaval J.F., *Toxocara (larves d'ascaridiidées)*, Encycl Med Biol. 2003 Elsevier Paris.
- Gentilini M., Duflo B., *Larva migrans viscérale ascaridienne*. Dans : Médecine Tropicale, Paris : Flammarion Médecine Sciences. 1986: 237-239.

TOXOPLASMOSIS

DEFINITION

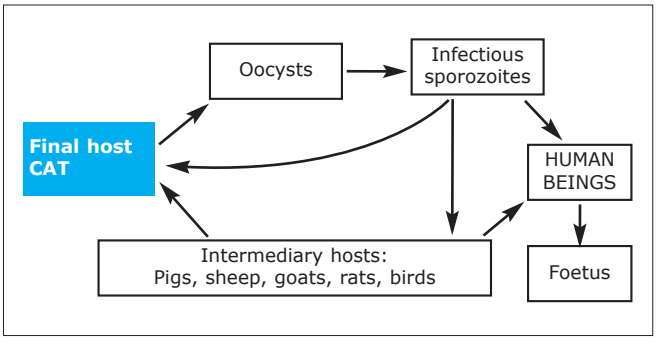
Toxoplasmosis is a disease caused by a sporozoite, *Toxoplasma gondii*. The disease can present in different forms depending on the infected host:

– ***The tachyzoite*** (*toxoplasma* form) is a 5-10 µm long by 3-4 µm wide pear-shaped, curved organism with a tapered extremity. It multiplies rapidly in macrophages and is very fragile in the external environment.

– ***The bradyzoite*** (cystozoite) has a similar structure to the tachyzoite. It replicates slowly in brain and muscle cells where the bradyzoites are grouped together in cysts.

– ***The oocyst*** measures 15 µm by 10 µm. It is resistant and matures in earth producing two sporocysts, each containing 4 **sporozoites**. The sporozoite resembles the tachyzoite.

The parasite cycle is summarised in figure 1 below.



The final host for the toxoplasma is the cat, which develops a gastro-intestinal tract infection and excretes the oocysts. These develop in the ground, producing infectious sporozoites which are eaten by the final host (short cycle) or by intermediary hosts (mammals, including human beings and birds – long cycle) in whom the bradyzoites encyst.

NTRODUCTION

EPIDEMIOLOGY

Approximately 50% of adults have antibodies to toxoplasma in France. The presence of IgG antibodies indicates old infections with persisting cysts, reflecting "immunity".

Infections occur:

- **Either by eating earth-borne oocysts** (water, food, soiled hands). The oocysts remain in the ground for several months and are killed by heat, drying and freezing.
- **Or by eating infectious cysts** contained in inadequately cooked meat. The cysts are found widely in butchery meat.

Materno-foetal transmission may occur as a result of primary toxoplasma infection developing during pregnancy. The risk of primary infection in France is currently estimated to be 2.5 per 1000 pregnancies.

Conversion of bradyzoites contained in cysts into tachyzoites, which spread through the blood stream in immunosuppressed people who have previously been infected with toxoplasma (reactivation) depends on the severity of the reduction in cellular immunity.

SYMPTOMS

- **Acquired toxoplasmosis in an immunocompetent person:** infection is usually asymptomatic. After an incubation period of a few days, the sub-acute presentation produces a picture of moderate prolonged fever with cervical lymphadenopathy and an infectious mononucleosis syndrome. It usually recovers and the cysts persist. Recovery carries with it long-lasting but non-eradicating immunity which protects against any new infection. Immunity is demonstrated by the presence of IgG antibodies.

  Complicated forms of the disease (ocular, cardiac, pulmonary or cerebral) are very rare.
- **Materno-foetal transmission: congenital toxoplasmosis:** embryofoetal disease results from the colonisation of the placenta by toxoplasma during the septicaemic phase of the disease. The risk of transplacental spread is low before the 4th month of pregnancy, although when this does occur it can cause *in-utero* death or lead to the birth of a child with serious psychomotor disorders with intracranial calcifications, hydrocephalus, seizures, autonomic and ocular disorders (pigmented chorio-retinitis). The systemic forms (liver disease with neonatal jaundice and mucosal haemorrhage) have a very poor prognosis. Foetal infection is more common during the second half of pregnancy when the consequences are believed to be less serious (pigmented chorio-retinitis, seizures and psychomotor retardation). Toxoplasmosis may be latent and asymptomatic at birth but present later in life (ocular lesions occurring after several years).
- **Toxoplasmosis in the immunosuppressed:** in AIDS patients this usually involves reactivation of cerebral cysts resulting in cortical lesions with fever, headaches, behavioural disturbance and ocular lesions. In transplant patients, conversion of bradyzoites into tachyzoites, which spread through the blood stream can result in disseminated acute toxoplasmosis, either as a result of reactivation of recipient cysts or from cysts contained in the transplant.

SEARCH INDICATIONS

Etiological diagnosis of infectious mononucleosis syndrome or lymphadenopathy.

Investigation of fever in a transplant patient or pregnant woman.

Investigation of neurological symptoms or chorioretinitis, particularly in the immunosuppressed and young children.

Investigation of foetal abnormalities.

Etiological diagnosis of congenital diseases (septicaemic form, localised form).

Determination of toxoplasma serological status in a pregnant woman, recipient and potential transplant donor.

Monitoring pregnant women.

INFORMATION

SAMPLE

Many different types of sample can be taken depending on the symptoms present: whole blood collected into EDTA or citrate, CSF, aqueous humour, bronchoalveolar lavage, brain or liver biopsy, placenta. Amniotic fluid must not be collected before the 20th week of pregnancy and at least six weeks after the date of maternal seroconversion if this can be assessed.

Antibody testing is performed on non-haemolysed serum.

QUESTIONS FOR THE PATIENT

Immune status, HIV seropositivity? Term of pregnancy, ultrasound abnormalities, type and onset of clinical signs?

SAMPLE STORAGE AND TRANSPORT

It is recommended that samples be promptly refrigerated and transported to the laboratory (a few hours if possible and within 3 days). The serum should be kept at + 4°C if the laboratory is distant to the place of sampling. CSF can be frozen for requests for toxoplasma molecular biology testing (PCR).

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

- **Histo-cytological examination:** this involves examining for toxoplasma in tissue lesions either after staining (May-Grunwald-Giemsa, histochemical staining) or after labelling with a fluorescent monoclonal antibody. This is a relatively insensitive method as usually few parasites are present.
- **Mouse inoculation:** this can be performed with any type of sample. Results are obtained after 30 to 45 days after autopsy and reveal toxoplasma in cerebral cysts. It is possible to attempt to detect parasites in peritoneal lavage fluid from day 7 after inoculation.
- **Isolation by cell culture:** this method is sensitive to bacterial contamination but can be used to identify toxoplasma in a few days by inoculating human embryonic fibroblast cells (MRC5 or equivalent). The toxoplasma are visualised after staining with Giemsa or labelling with a fluorescent antibody.
- **Detection of the viral genome:** this is performed using PCR on most sample types. The most sensitive methods target repeated gene sequences. Detection of toxoplasma DNA in amniotic fluid indicates materno-foetal infection. Real time PCR quantification of viral load is used to assess the potential severity of infection.

INDIRECT DIAGNOSIS

In non-immunosuppressed people, particularly pregnant women, the diagnosis of toxoplasmosis relies above all on demonstrating the specific humoral immune response. It is often important to combine methods using different antigens to test for several isotypes (IgG and IgM, possibly IgA and IgE) in order to provide the best possible interpretation.

- **Methods using cell antigens (toxoplasma bodies)**

Dye test (Sabin and Feldman): this is based on lysis of living toxoplasma obtained from the peritoneal fluid of infected mice by antibodies in the presence of fresh human complement. The test is read by phase contrast microscopy. It has long been considered to be the reference method but is complex and expensive and is now only performed by highly specialised laboratories.

Direct Agglutination (Fulton): this uses agglutination of a suspension of formol-inactivated toxoplasma by antibodies. The sensitivity of the method can be increased (ADS) by pre-treating the toxoplasma with trypsin. The serum is tested directly and after treatment with 2-mercapto-ethanol in order to obtain an estimate of the amount of IgM antibody possibly present from the difference in titres obtained. The test is easy to perform and read although the results may only be approximate. It is currently not widely performed.

The HS/AC test involves comparing antibody titres obtained by agglutination of formol-fixed (HS) and acetonal methanol (AC) - fixed toxoplasma, the latter being reported to develop earlier.

Agglutination following immunocapture: the ISAgA (*Immuno-Sorbent Agglutination Assay*) reaction is a variant of direct agglutination after capture of IgM and possibly IgA or IgE antibodies on a solid phase, sensitised by human anti-IgM (-IgA or -IgE) antibody. When positive, the parasites are seen, distributed uniformly as a sheet lining the base of the reaction cup. The image obtained is scored (from 0 to 12). This is a simple test to perform, which is not influenced by the presence of rheumatoid factor and is commercially available. It is highly sensitive and can be used to detect anti-toxoplasma IgM antibodies early.

Indirect immunofluorescence (IIF): this is a classical test using formol-treated toxoplasma fixed onto a slide. Depending on the labelled anti-immunoglobulin used it can detect different antibody isotypes (Remington test to detect and titre IgM). It is simple to perform although requires a trained observer to be read. The reagents are commercially available.

- **Methods using soluble antigens**

Passive agglutination: this commercially available method is simple and fast. It uses latex particles coated with parasite antigens. It is performed on a glass plate and is read by the naked eye after a few minutes. It is sensitive although is subject to the zone effect (false negative may occur if high titres of antibody are present) and does not differentiate between different antibody isotypes.

Sheep red blood cells coated with toxoplasma antigen can also be used. The reaction is performed in a microtitre plate and can be quantified.

Enzyme immunoassays (EIA): these are now very widely used with many commercially available manual and automated variants. They use soluble antigens of different types and preparation methods, which make standardising results difficult. The EIA method can be used to quantify IgG, IgA and IgM antibodies. For IgM antibodies, indirect tests are available which are revealed by labelled human anti-IgM antibodies and immunocapture tests, which avoid competition from IgG antibodies and interference from rheumatoid factor. IgM antibody quantification varies depending on the methods used to calculate the positivity indices.

- **Specific methods**

ELIFA *(Enzyme Linked Immuno Filtration Assay)*: this is a complex method, which is not commercially available and is reserved for highly specialised laboratories. It involves separating

the different parts of a complex antigen suspension by electrosyneresis in a first stage and then revealing the precipitation obtained using an enzyme-immunoassay method. The antibody response to the different antigens can therefore be analysed in detail and paired serum or other samples can be compared (maternal serum-cord serum, or aqueous humour-serum for example).

Immunoblot: this involves the same general principle as above, although the parts of the toxoplasma suspension are separated by gel electrophoresis and then transferred onto a nitrocellular strip which is placed in contact with the test serum and then revealed using peroxidase-labelled human anti-immunoglobulin antibodies.

Immune load (IL): this involves determining the number of anti-toxoplasma antibody units as a proportion of the amount of IgG by weight in a sample. In the same way as the two methods above it enables paired serum samples to be compared or a serum sample to be compared with another biological fluid (CSF, aqueous humour) taken on the same day, calculating the Desmont coefficient. For example:

$$IL = \frac{\text{(anti-toxoplasma antibodies in aqueous humour / total immunoglobulins in aqueous humour)}}{\text{(serum anti-toxoplasma antibodies / total serum immunoglobulins)}}$$

If IL is greater than 3, local antibody production is occurring.

Antibody avidity: measurement of the antibody avidity index involves measuring IgG antibodies before and after treating the antigen-antibody complex with a concentrated urea solution. A high avidity index excludes recent primary toxoplasmosis infection.

INTERPRETATION

■ ANTIBODY KINETICS

- **IgM antibodies** are the first to appear, within days after infection. The presence of IgM antibodies, however, does not always indicate recent infection as new immunocapture methods (ISAgA or second generation ELISA) detect IgM 6 months or even a year and over after the initial episode of infection.
- **IgG antibodies** appear within 2 or 3 weeks after infection. Methods using whole toxoplasma (*Dye test*, sensitised direct agglutination, indirect immunofluorescence) reveal antibodies earlier than tests using a soluble antigen extracted after lysing the parasite (ELISA, agglutination) (*figure 2*). The humoral response in primary infection is firstly against the membrane antigens and then against cytoplasmic antigens. Results should be expressed in international units (IU). Standardising results from the different methods against the international reference standard is made difficult by the problem of converting titres into IU, as the conversion process is variably reliable depending on antigen type (membrane or soluble antigen). In general, a reliable interpretation may be obtained by parallel examination of 2 serum samples taken apart (3 weeks), in the same laboratory using the same method in the same batch. In particular, a positive stable IgG antibody titre, *a priori* suggests infection dating back more than 2 months.

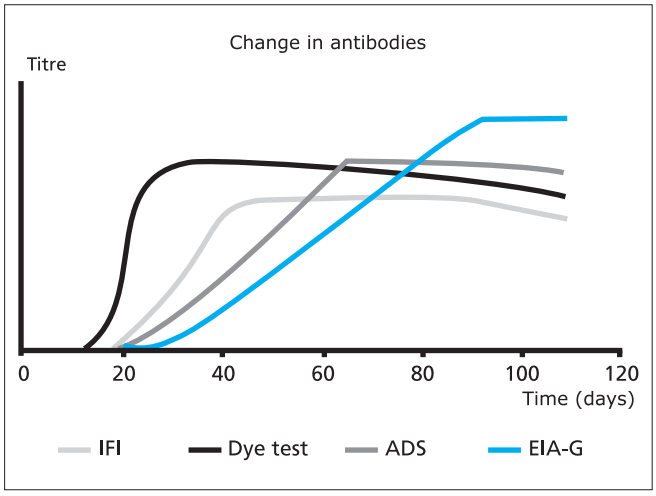


Figure 2 (from B. Fortier).

Additional diagnostic information may be obtained by examining the IgA and IgE isotypes. These have shorter kinetics than IgM and are not interfered with by rheumatoid factor or anti-nuclear antibodies. Individual variations, however, may make interpretation difficult.

■ DIAGNOSIS OF RECENT PRIMARY TOXOPLASMA INFECTION

The change in antibodies is shown on figure 3. The best indicator is demonstrating IgG seroconversion. In recent infection, the concentration of IgG antibody against membrane antigens is significantly higher than the concentration of IgG against soluble antigens. IgG antibody avidity is measured to help to distinguish primary from old infection: this test is particularly useful in pregnant women. In this situation a serum sample taken as early as possible after the start of pregnancy should be used, as available *(figure 4)*. The presence of IgM antibodies is a warning sign but must be interpreted in the context of the method used. IgM results remain positive for shorter periods of time by IIF than by immunocapture.

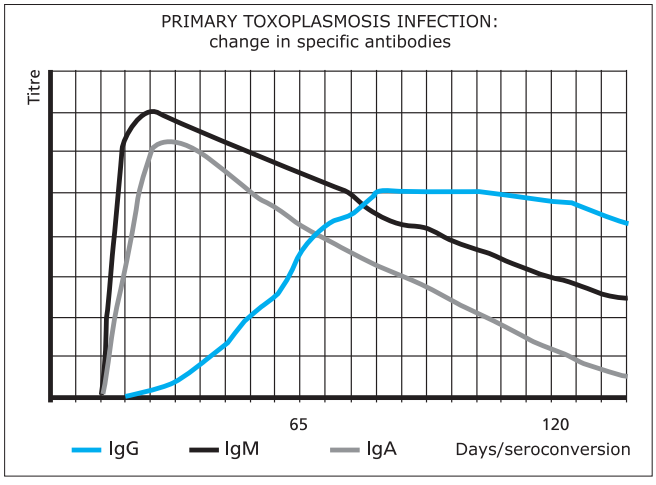
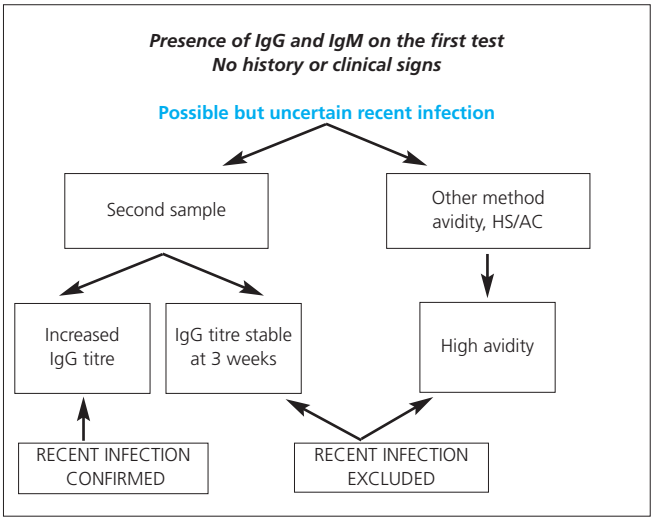


Figure 3 (from B. Fortier).



HS/AC: differential agglutination (rarely performed)

Figure 4 (from Ph. Thulliez).

■ DIAGNOSIS OF MATERNO-FOETAL INFECTION

Testing for toxoplasmosis in amniotic fluid is indicated in primary infection, which occurs or is suspected during pregnancy or after finding ultrasound abnormalities (cerebral ventricles or liver). The sample must not be taken before the 20th week of pregnancy and must be at least 6 weeks after the date of maternal primary infection if this can be established. The diagnosis is made by inoculation into the mouse followed by cell culture, and nowadays particularly using PCR. A negative result does not exclude possible late spread of the parasite across the placenta and for this reason it is important to leave sufficient time before performing the amniocentesis. Quantification of toxoplasma load in the amniotic fluid by real time PCR can help to assess the potential severity of foetal disease.

■ DIAGNOSIS AND MONITORING OF A NEWBORN CHILD SUSPECTED OF HAVING CONGENITAL TOXOPLASMOSIS *(figure 5)*

The diagnosis is based:

– on finding the parasite in the placenta, the child's blood or cord blood by PCR and/or mouse inoculation followed by cell culture;

– on differential analysis of antibodies produced by the child and those transmitted naturally by the mother: testing for IgM and IgA antibodies which do not cross the placenta, measurement of respective immune loads and different specificity of child-mother antibodies studied by ELIFA or immunoblot.

If none of these investigations *a priori* suggest infection the child should be followed up monthly. Transmission of maternal antibodies ceases after 10-12 months. In late infection the child's IgG exhibits a further rise together with an increase in immune load.

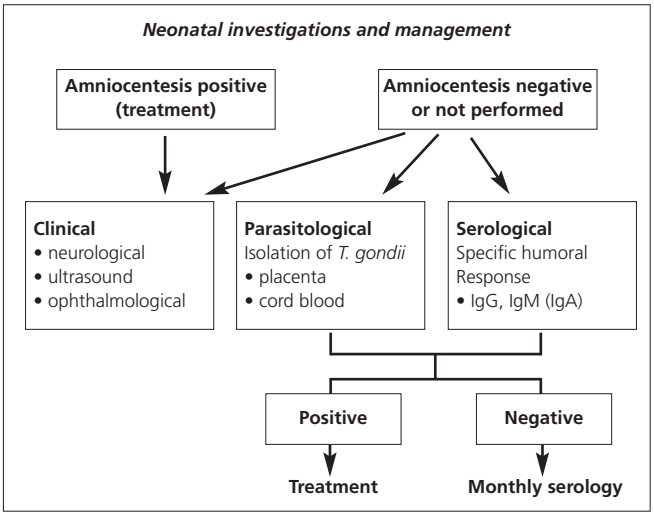


Figure 5 (from Ph. Thulliez).

■ DIAGNOSIS OF TOXOPLASMA REACTIVATION

Foci of latent toxoplasma may reactive in immunosuppressed patients and as a late recurrence following congenital toxoplasmosis. Serology is usually non-contributory and testing must be performed for markers of parasite replication (inoculation, culture, PCR) and identifying local antibody synthesis in aqueous humour or CSF by comparing the immune load to that in serum or by an ELIFA or immunoblot method.

TREATMENT

■ CURATIVE

- **Subacute toxoplasmosis:** treat with spiramycin for one month.
- **Suspected toxoplasmosis in a pregnant woman:** start treatment with spiramycin as above and wait for results of parasite testing in amniotic fluid:

  – if negative, continue spiramycin on a precautionary basis throughout the pregnancy;

  – if positive, and if ultrasound abnormalities are present, therapeutic termination of pregnancy is offered. If the pregnancy continues the spiramycin should be stopped and replaced with the association pyrimethamine-sulfadiazine combined with a folinic acid (to compensate for the adverse effects of pyrimethamine) for 3 weeks per trimester, continuing the spiramycin until birth. Pyrimethamine is contra-indicated during the 1st trimester of pregnancy.

- **In a newborn child suspected of having toxoplasmosis,** treatment depends on the laboratory results:

  – if testing for toxoplasma and toxoplasma IgM is negative, monitor until maternal antibodies become negative;

  – if the diagnosis of congenital toxoplasmosis is made or if antibodies rise again in the child, use the above association.

- **In immunosuppressed patients,** treat with pyrimethamine-sulfadiazine and then start maintenance treatment with a half-dose of the same drugs to avoid relapse. The same treatment is used for ocular toxoplasmosis (for 1 to 2 months). Corticosteroids may be used in macular chorioretinitis.

PREVENTIVE

There is no vaccine against toxoplasmosis.

– Screening and monitoring non-immunised women during their pregnancy with monthly toxoplasmosis serology from the date the pregnancy is discovered until childbirth.

– Hygiene precautions: wash hands thoroughly, brushing nails before and after handling foods, after gardening or touching objects soiled by earth and after touching animals. Raw vegetables must be carefully washed to remove all traces of earth. Hands, surfaces and utensils used must be washed whenever food is handled. Thorough cooking is essential to destroy cysts, which may be present in meat: "well cooked" meat loses its red colour and becomes pink-beige coloured at its core (reaching a temperature of more than 68°C). If a cat is present in the home, the litter must be changed daily, wearing gloves.

FOR FURTHER INFORMATION

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TPA

DEFINITION

Like Cyfra 21.1, TPA *(Tissue polypeptide antigen)* is a cytokeratin belonging to the intermediary filament group (cytokeratins, 8, 18 and 19) of the cytoskeletal family of proteins. These proteins are present in large amounts in epithelial cells and are markers of epithelial cancers. Some authors suggest that TPA is released into the circulation from dead cells within tumours during the growth phase, and others that it is a non-specific marker of cellular proliferation produced during the G1, G2 and S phases of the cell cycle and released into blood immediately after mitosis. Its secretion always reflects cellular renewal *(turnover)* and serum concentrations correlate with the extent of tumour proliferation. It is more a marker of cellular activity than of tumour mass and has a serum half-life of approximately 7 days.

In practice, it is mostly measured either alone or in combination with other tumour markers as a laboratory marker to monitor patients suffering from lung or bladder carcinomas, or possibly breast or colonic carcinomas.

INDICATIONS FOR MEASUREMENT

Determination of prognosis and monitoring treatment (detection of relapses and metastases) in patients suffering from bladder, some lung, and possibly breast or colonic cancers. It is currently not widely used.

INFORMATION

SAMPLE

Serum or plasma. A fasting sample is not necessary.

QUESTIONS FOR THE PATIENT

Type of tumour and current treatment, such as chemotherapy, radiotherapy, and surgery (types and date of treatment).

SAMPLE STORAGE AND TRANSPORT

24 hours at + 4°C; – 20°C beyond this time. Transport frozen at – 20°C.

ASSAY METHODS

Immunometric assay.

NORMAL EXPECTED VALUES

For reference: < 75 I/U. Values may vary depending on the method used.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

In pregnant women TPA increases during the third trimester of pregnancy.

PATHOLOGICAL VARIATIONS

Increased serum TPA concentrations in oncology

TPA and lung cancers:

Before treatment: TPA has no diagnostic use because of its poor sensitivity (approximately 15% in limited disease and 60% in widespread disease). The serum concentration at diagnosis correlates with extension and the stage of the disease.

Monitoring treatment: TPA is used mostly to monitor patients suffering from non-small cell lung cancers. Serum concentrations rise in approximately 25% of cases which relapse 2 to 3 months before clinical signs appear (in 40% of cases values rise after symptoms appear). TPA therefore appears to be the first indicator of surgically treatable isolated liver metastases.

TPA is also a laboratory marker for mesothelioma and asbestos-related lung cancers (early detection of asbestosis in at risk patients and use in monitoring treatment).

TPA and bladder cancers:

At diagnosis: the sensitivity of TPA for bladder cancers varies from approximately 25% for non-invasive tumours to 60% for advanced stages of disease. It is more sensitive than ACE (10 to 20%), but inadequate for screening purposes. On the other hand TPA is a useful prognostic indicator: one-year survival is 80% when initial values are normal compared to 44% when these are raised. It also predicts response to treatment as a 69% complete remission rate is found when the initial TPA is low *compared to* 41% when it is raised.

Monitoring treatment: : serum TPA concentrations correlate with clinical progression and are increased in almost all cases of metastases.

TPA and breast cancers:

TPA is a less useful marker than CA 15.3.

Before treatment: Comparative sensitivity of isolated measurement of several tumour markers in the diagnosis of breast cancer at different stages *(meta-analysis from Sölétormos G., 2001)*.

Breast cancer	stage I	stage II	stage III	stage IV
CA 15.3	6 %	17 %	35 %	70 %
CA 549	10 %	14 %	34 %	73 %
ACE	7 %	16 %	25 %	55 %
TPA	4 %	25 %	40 %	64 %

Monitoring treatment of surgically treated breast cancers:

Ability of laboratory markers to identify, predict or exclude tumour recurrence in the post-operative monitoring of women suffering from stage I or stage II breast cancer *(meta-analysis from Sölétormos G., 2001)*. NPV: negative predictive value; PPV: positive predictive value.

	sensitivity (%)	specificity (%)	NPV (%)	PPV (%)
CA 15.3	57	95	82	88
ACE	42	88	56	81
TPA	53	83	60	81

Ability of laboratory markers to identify, predict or exclude tumour relapse in the post-operative monitoring of women suffering from stage III or IV breast cancer *(meta-analysis from Sölétormos G., 2001)*.

	sensitivity (%)	specificity (%)	NPV (%)	PPV (%)
CA 15.3	55	94	89	69
ACE	45	91	81	65
TPA	43	97	91	69
CA 15.3 + ACE + TPA	69	100	100	82

Unlike what is seen in early stages of the disease, the sensitivity of CA 15.3, as a laboratory monitoring marker is improved in stages III or IV (metastatic breast cancers) by combination with ACE and TPA measurements.

TPA and other cancers:

Serum TPA is also raised in gastric (50% of cases) and ovarian cancers.

■ **Increases in benign disease**

– Benign broncho-pulmonary diseases: acute viral or bacterial, bronchial or parenchymatous infections, severe asthma, emphysema and pneumothorax. TPA values are generally under 170 I/U;

– Acute hepatitis B, chronic hepatitis, cirrhosis; inflammatory bowel diseases (ulcerative colitis, Crohn's disease);

– Renal insufficiency, mixed cryoglobulinaemia, disseminated intravascular coagulation, prostatic hyperplasia, and prostatitis.

FOR FURTHER INFORMATION

■ Sölétormos G., *Serological tumor markers for monitoring breast cancer*, Dan Med Bull 2001 ; 48 : 229-55.

■ Ugugnani M.J., *Intérêt clinique du dosage sérique du tissue polypeptide antigen*, Immunoanal Biol Spéc 1997 ; 12 : 169-72.

TRANSFERRIN
(soluble receptor)

DEFINITION

The transferrin receptor (R-Tf) is a membrane glycoprotein present on the surface of all body cells, with a predominance on erythroblast line cells (80% of R-Tf), hepatocytes and placental cells.

It enables the cell to bind iron, carried by transferrin in the circulation. The number of cell surface receptors is regulated according to intracellular iron requirements.

The soluble transferrin receptor (Rs-Tf) is the truncated circulating form of R-Tf. Serum Rs-Tf concentration is proportional to the total number of cell surface receptors.

Rs-Tf concentration is a marker of tissue iron deficiency and of bone marrow erythroblast activity.

INTRODUCTION

■ RELATIONSHIP BETWEEN CELL SURFACE AND NUMBER OF CELLS

The significance of the Rs-Tf concentration can be understood schematically by the following equation:

[Rs-Tf] ≈ [R-Tf/cells] x Number of cells

or [Rs-Tf] : serum Rs-Tf concentration

[R-Tf/cells] : number of cell surface R-Tf

■ ASSESSMENT OF BODY IRON REQUIREMENTS

Body iron requirements can be assessed using the serum iron, transferrin and ferritin concentrations and transferrin saturation capacity. These markers are limited by twenty-four hour cyclical changes (iron) and by pathological situations, such as inflammation, malignant tumours and liver disease (transferrin and ferritin).

Rs-Tf concentrations are increased in iron deficiency, when the number of cell surface R-Tf increases. When serum ferritin is below 12 µg/l and associated with reduced serum iron concentration the number of cell surface receptors and the serum Rs-Tf concentration increase.

Rs-Tf concentrations are not increased in anaemia associated with chronic inflammatory disease, as this does not involve iron depletion but tissue iron redistribution. Erythropoietin synthesis is also inhibited by inflammatory cytokines blocking growth of the erythroblast cell line.

Rs-Tf and haemoglobin concentrations are related when iron stores are normal although no relationship exists with ferritin.

■ ASSESSMENT OF ERYTHROBLAST ACTIVITY

Rs-Tf concentrations depend on the size of the erythroblast population. When erythropoietin is produced and erythropoiesis is stimulated, Rs-Tf concentrations rise. This situation may be due to peripheral haemolysis or defective bone marrow synthesis (bone marrow dysplasia or aplasia). Rs-Tf concentration can be used to monitor some treatments and adjust transfusions (aplasia, congenital erythrocytosis and thalassaemia) and is also useful to monitor the effectiveness of synthetic erythropoietin.

Rs-Tf concentrations fall when erythropoietin synthesis is inhibited (severe renal insufficiency).

INDICATIONS FOR MEASUREMENT

Assessment of iron deficiency, particularly in patients suffering from chronic diseases (inflammatory disease, cancer or infection) and anaemia.

Assessment of erythropoiesis either in the context of treatment (EPO) or in disease (renal insufficiency).

INFORMATION

■ SAMPLE

Rs-Tf may be measured in serum or plasma (EDTA or lithium heparinate).

The sample must not be haemolysed.

■ QUESTIONS FOR THE PATIENT

Are you pregnant? Geographical origin of the patient and parents (known constitutional haemaglobinopathy)? Any iron treatment or erythropoietin being taken? Presence of haematological disease with gammopathy? Presence of autoimmune disease (rheumatoid factor or rheumatoid arthritis)?

■ SAMPLE STORAGE AND TRANSPORT

If the measurement is not performed immediately the serum or plasma can be stored in a refrigerator at between + 2°C and + 8°C. Rs-Tf concentrations are stable for 3 days at between 20 and 25°C, for 7 days at between 2 and 8°C and for four weeks in samples frozen at -20°C.

ASSAY METHODS

Rs-Tf is measured either using an ELISA technique (rarely), or by immunoturbidimetry/nephelometry on latex particles (automated methods).

NORMAL EXPECTED VALUES

Reference values	Turbidimetry	ELISA
Men	2.2 to 5.0 mg/l	0.74 - 2.39 mg/l
Women	1.9 to 4.4 mg/l	0.74 - 2.39 mg/l

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

Rs-Tf concentrations in healthy people are not age or sex dependent. People living at high altitudes generally have levels 10% higher than normal reference values.

Soluble transferrin receptor concentrations are increased in pregnancy due to stimulation of erythropoiesis. Rs-Tf concentrations rise in the first two trimesters of pregnancy and in first part of the third trimester. Concentrations then fall at the end of pregnancy returning to normal *post-partum*.

	Rs-Tf range	Variation
Non-pregnant women	1.3 to 3.3 mg/l	-
1st trimester of pregnancy	1.5 to 3.6 mg/l	+ 5 %
2nd trimester of pregnancy	3.0 to 5.0 mg/l	+ 70 %
3rd trimester of pregnancy	3.5 to 6.0 mg/l	+ 21 %
1 to 2 weeks post-partum	2.4 to 4.5 mg/l	– 28 %
12 to 16 weeks post-partum	1.4 to 3.5 mg/l	– 36 %

From Jong Weon Choi, Moon Whan Im, and Soo Hwan Pai, "Serum Transferrin Receptor Concentrations during Normal Pregnancy", Clin Chem 2000 46: 725-727.

Concentrations in the newborn may be twice those in adults.

PATHOLOGICAL VARIATIONS

Rs-Tf concentrations of under 3 mg/l are associated with reduced erythropoiesis and concentrations over 8.5 mg/l indicate iron deficiency.

Defects of iron metabolism

Rs-Tf concentrations rise in iron deficiency. Deficiency is confirmed by interpreting the rise alongside erythrocyte parameters (MCV, MCH) (MCV and MCH are reduced in 90% of iron-deficient patients). Rs-Tf concentrations do not change in chronic inflammation and measurement is particularly useful in inflammatory states to distinguish inflammation from true iron deficiency. A threshold of 4.7 mg/l has been proposed to identify iron deficiency in chronic inflammation. Rs-Tf measurement does not appear to be useful in rheumatoid arthritis.

Change in parameters in clinical situations

Concentration	Haemoglobin	Serum iron	Serum ferritin	Rs-Tf
Iron deficiency (ID)	↓	↓	↓	↑
Chronic inflammatory disease (CID)	↓	↓	N ou ↑	N
ID + CID	↓	↓	N	↑

(N: normal, ↓: reduced, ↑: raised)

Rs-Tf concentrations are reduced by 10% in iron overload (haemochromatosis) and are reduced in iron supplementation to replace iron deficiency.

Disease of the erythroblast cell line

Rs-Tf concentrations are raised in patients with stimulated erythropoiesis producing an increased number of red cells and haemoglobin synthesis: secondary polycythaemia, haemolytic anaemia, malaria, beta-thalassaemia major, Minkowski-Chauffard disease, sickle cell anaemia, megaloblastic anaemia, myelodysplasia, congenital dyserythropoiesis and vitamin B12 deficiency.

Rs-Tf concentration may also be used in some haematological diseases such as chronic lymphocytic leukaemia and non-Hodgkin's lymphoma. Falls in concentrations are associated with good response to treatment. Concentrations are not abnormal in multiple myeloma, chronic myeloid leukaemia or essential thrombocythaemia. The rise seen in patients with Vaquez disease (haemochromatosis) appears to be associated more with venesection. Solid tumours are not usually associated with increased Rs-Tf although exceptions to this are prostate cancer (+ 30%) and hepatocellular carcinoma.

Rs-Tf concentrations are reduced in patients with bone marrow hypoplasia, those receiving cytotoxic chemotherapy and in patients with severe renal insufficiency. They are also reduced in acute leukaemia at the time of diagnosis and during chemotherapy.

The erythroblast count rises late (6 weeks) during treatment with synthetic erythropoietin although precedes the rise in serum Rs-Tf. A rise of more than 20% after two weeks haemodialysis is an indicator of response to treatment.

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TRANSFERRIN

DEFINITION

Transferrin is a glycoprotein with a molecular mass of approximately 80 kDa. It is produced mostly in the liver and has a half-life of 8 days. It exhibits genetic polymorphism with considerable molecular heterogeneity (variants). Transferrin has two binding sites for an iron atom.

Synonym: Siderophilin.

INTRODUCTION

Transferrin is the transport protein for iron in the body (and to a lesser extent the transport protein for zinc and copper). Each molecule can combine with a maximum of two iron atoms. Transferrin binds iron as soon as it is absorbed through the intestine and carries it to the erythropoietic bone marrow (erythroblasts) where haemoglobin is produced. Only transferrin is able to deliver iron to the cell, by binding to a specific membrane receptor, the transferrin receptor. Transferrin also carries iron to hepatocytes to be stored and mobilises iron from reserves (liver and RES cells).

Its hepatocyte synthesis is proportional to the amount of iron present in the cell. Reduced iron reserves result in increased synthesis.

Transferrin also has a role in intestinal iron absorption: the percentage of dietary iron absorbed depends on the extent of transferrin saturation.

INDICATIONS FOR MEASUREMENT

Measurement of transferrin combined with that of serum iron, with calculation of the transferrin saturation coefficient is useful in investigating iron metabolism (particularly investigating for iron overload).

When combined with the measurement of other proteins such as albumin, prealbumin, orosomucoid, and haptoglobin, transferrin measurement is used to investigate protein metabolism abnormalities in hepatocellular insufficiency, inflammatory states and malnutrition.

INFORMATION

■ SAMPLE

Serum, preferably taken from a fasting patient (transferrin has no 24 hour cycle unlike iron: beware however if ferritin measurement is combined with iron measurement).

■ QUESTIONS FOR THE PATIENT

Known or treated iron deficiency?

Full blood count results?

Drug history: iron, oral contraceptives, hormone replacement therapy and diuretics?

■ SAMPLE STORAGE AND TRANSPORT

Serum can be stored for one week at + 4°C or for 3 months at – 20°C.

ASSAY METHODS

Immunoturbidimetry, immunonephelometry.

It is recommended that calibrators standardised against International Standard CRM 470 be used.

Total transferrin iron binding capacity (TIBC) can be calculated from the equation:

TIBC (µmol/l) = transferrin (g/l) x 25

The transferrin saturation coefficient (TSat):

CS = Serum iron (µmol/l)/TTC (µmol/l)

NORMAL EXPECTED VALUES

	Transferrin (g/l)	Saturation coefficient
Newborn	1.6 – 2.8	0.55 – 0.65
Infants - children	2.0 – 4.0	0.10 – 0.30
Adult Men	2.0 – 3.2	0.20 – 0.40
Adult Women	2.0 – 3.2	0.15 – 0.35

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

– Serum transferrin does not vary during the 24-hour cycle.

– Age related: more specific age and sex-related reference values have been established in order to interpret protein profiles including transferrin measurement.

– Pregnancy: transferrin concentrations may reach 4.4 g/l at the end of pregnancy.

■ PATHOLOGICAL VARIATIONS

Main causes of changes in serum transferrin concentration

	Fall		Rise	
	Iron Metabolism	Other	Iron Metabolism	Other
Trf	Iron overload	Inflammatory states Infections Hepatocellular insufficiency Protein losses Cancer Malnutrition Atransferrinaemia	Iron deficiency anaemia	Pregnancy Oral contraceptives
SC	Iron depletion Iron deficiency anaemia	Pregnancy Inflammation (or N) Infections (or N)	Iron overload	Hypersideraemic anaemia

■ HYPOTRANSFERRINAEMIA

– Iron overload in genetic or secondary haemochromatosis (repeated transfusions or cutaneous porphyria).

– Acute or chronic inflammatory reactions and infections. Together with albumin and prealbumin, transferrin is one of the three proteins which fall in the inflammatory reaction. This is due to increased protein catabolism in favour of the synthesis of those proteins which rise (CRP, haptoglobin and orosomucoid).

– Hepatocellular insufficiency: cirrhosis, viral hepatitis, toxic or drug-induced hepatitis.

– In alcoholic liver disease which may be associated with a polyclonal rise in IgA, the IgA/ transferrin ratio has been proposed to monitor progression of cirrhosis (this ratio is normally less than 2).

– Malnutrition states: in malnutrition the fall is less sensitive and less specific than falls in albumin and prealbumin.

– Renal (glomerulonephropathies), gastro-intestinal or cutaneous (burns) protein losses.

– Congenital atransferrinaemia is very rare.

HYPERTRANSFERRINAEMIA

– Iron deficiency: inadequate intake in young children, the elderly and pregnant women; deficiency due to reduced gastric or intestinal absorption; chronic minor gastro-intestinal or genital bleeding.

– Oestrogen exposure due to induced synthesis: endogenous (pregnancy: 40 to 70% rise during the second and third trimesters) or iatrogenic (oral contraceptives: 30% rise, oestrogen replacement therapy in menopausal women or treatment of prostate cancer).

– Other drugs: thiazide diuretics (hydrochlorothiazide, bendroflumethiazide, hydroflumethiazide, chlorthalidone and indapamide).

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TRICHINELLOSIS

DEFINITION

Trichinellosis is a parasitic disease which is widespread throughout the world and affects human beings and animals. It is due to a helminth ***Trichinella spiralis*** which belongs to the nematode class, *Trichinelloidea* family and *Trichinella* genus.

The adult is a very small worm, the male measuring 1.5 mm and viviparous female between 3 and 4 mm.

The larva (L1) is 1 mm long, encysted and rolled in a spiral in muscle fibres.

Synonyms: trichinellosis = trichinosis.

INTRODUCTION

SAMPLE

Trichinellae are parasites of omnivorous and carnivorous mammals.

The disease is contracted by eating raw or poorly cooked meat (pork, boar, horse, etc.) containing infesting *Trichinella spiralis* larvae.

It is a cosmopolitan disease, endemic in regions where large amounts of pork are eaten (Eastern Europe and USA) or epidemic as a result of eating contaminated horse or boar meat (in France). Horse meat is the main source of infestation in Europe, responsible for recent epidemics.

LIFE CYCLE

The life cycle is indirect and takes place in a single unique host, an omnivorous or carnivorous mammal, including human beings.

After eating-parasite infested meat, the infesting larvae are released following digestion and rapidly enter intestinal epithelial cells where they undergo 4 successive mutations, becoming adults in 24 to 36 hours. The adult's mate and 4 to 5 days after infestation the females lay their larvae (approximately 1500 larvae per female) which migrate through the lymphatic or blood circulation to striated muscle fibres. The larvae become established in the striated muscle where they grow and become capable of infesting. They survive for years within the muscle cell, which they convert into a feeder cell. Over approximately 3 weeks the muscle cell is converted by the larvae into a cyst and ingestion of the cyst results in infestation of a new final host.

SYMPTOMS

The severity of the disease depends on the number of larvae ingested.

- It has many **asymptomatic or clinically silent forms.**
- **Symptomatic forms** of the disease progress in 4 phases:

Incubation phase: this is the transformation of larvae into adults in the gastro-intestinal tract and is often silent.

Invasion phase: this is the migration of larvae into the circulation. Predominant symptoms are fever, which may be as high as 40°C and signs of food poisoning, such as abdominal pain, diarrhoea and vomiting.

Established disease stage: this is the establishment of larvae in muscle cells and involves a plateau of fever (40-41°C), deterioration in general health, asthenia, and allergic periorbital facial oedema, myalgia and muscle cramps.

Chronic phase: this is the larva cyst formation phase and is characterised by apyrexia and improvement in general health, although fatigue, myalgia and allergic features may last for several months.

The clinical signs generally resolve spontaneously and disappear a few days to a few weeks, with persisting asthenia.

Neurological and cardiac complications, which are often fatal, may occasionally occur.

SEARCH INDICATIONS

Systematic diagnostic screening in an epidemic.

Diagnosis in the presence of suggestive clinical signs and/or laboratory evidence (eosinophilia).

Differential diagnosis with other helminthiases.

INFORMATION

SAMPLE

Blood samples: for a full blood count, serological diagnosis and measurement of muscle enzymes (a sample taken without a tourniquet is recommended, from a patient who has not had recent muscle exercise).

Muscle biopsy: taken at the level of the triceps, in the deltoid.

QUESTIONS FOR THE PATIENT

Clinical signs?

History of infesting food and/or epidemic?

Current antiparasitic treatment?

SAMPLE STORAGE AND TRANSPORT

Serum: a few days at + 4°C, then frozen at -20°C for up to a year.

DIAGNOSTIC METHODS

NON-SPECIFIC DIAGNOSIS

- **Eosinophilia:** very early, peaking 2 to 4 weeks after infection and returning to normal over 2 to 4 months.
- **Leukocytosis:** also occurs very early.
- **Muscle enzymes:** raised from the second week of infestation (particularly CPK, but also aldolase and LDH).

SPECIFIC DIRECT DIAGNOSIS

- **Fresh:** a biopsy fragment is examined directly under the microscope after being crushed between 2 glass slides to examine for living encysted larvae. This is only possible from 3 weeks after infestation.
- **Histological examination:** revealed muscle lesions.

INDIRECT DIAGNOSIS

Serum antibodies develop late and can be detected in 50% of cases, 2 to 3 weeks after infestation and in 95% of cases 8 weeks after infestation. Antibodies may last for several years.

There appears to be a correlation between antibody titres and the extent of infestation.

The methods used (commercial or in-house) are:

– immunoenzymatic reactions (ELISA),

– indirect immunofluorescence (IIF),

– latex particle agglutination,

– electrosyneresis and immunoelectrophoresis,

– immunoblotting or Western blot.

Many cross-reactions occur with other tissue nematodes (Toxocara and Filaria) and some autoimmune disease antibodies, particularly with IIF and ELISA, hence the importance of Western-blot confirmation.

INTERPRETATION

- **Non-specific laboratory signs** are the first to develop and may guide the diagnosis.
- **Serological diagnosis** is useful from approximately 14 days after infestation. Beware of false positives (cross-reactions) and false negatives (consider a late repeat sample to identify seroconversion).
- **Direct parasitological diagnosis** is the unequivocal diagnosis and involves examining for larvae in a muscle biopsy. This is only possible several weeks after infestation. It is a fairly aggressive method which is only useful if extensive infestation is present. In practice its use is limited mostly to confirming severe cases of the disease.

TREATMENT

ANTI-PARASITE CHEMOTHERAPY

The effectiveness of treatment depends mostly on the time before it is started, as nematocides act on the intestinal adult stages of the trichinella.

Benzimidazole derivatives which diffuse widely act on the L1 larvae:

– albendazole 15mg/kg per day for 15 days,

– thiabendazole,

– flubendazole.

An alternative is corticosteroid therapy combined with benzimidazole derivatives, which can prevent neurological and cardiac complications.

PROPHYLAXIS

General and individual prophylactic measures are used:

– monitoring meat in the abattoir and all imported meat,

– serving meat that has been cooked at an appropriate temperature for the correct length of time,

– freezing meat at -20°C for 8 to 15 days.

FOR FURTHER INFORMATION

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TRICHLORACETIC ACID - TRICHLORETHANOL TRICHLORETHYLENE

DEFINITION

Trichloroethylene is a trihalogenated, unsaturated aliphatic hydrocarbon and its metabolites trichlorethanol (TCE) and trichloroacetic acid (TCA), are short-chain, unsaturated, trichlorinated derivatives which, although highly toxic, are in widespread use as solvents in industrial applications (in paints, for grease removal, etc.).

Trichloroethylene is a colourless liquid which is relatively insoluble in water. It has an ether-like odour and is highly volatile at room temperature although it does not cause explosions.

It is toxic in the central nervous system where it tends to build up in fat-rich tissues. Trichloroethylene is readily absorbed via the lungs, digestive tract and skin. Its metabolites persist for several days in the blood and urine:

– The half-life of trichlorethanol in blood and urine is about 10 hours.

– The half-life of trichloroacetic acid in blood and urine is 70-100 hours.

Exposed persons should be monitored closely.

Trichloroethylene can be used for recreational intoxication, notably by inhalation.

INTRODUCTION

After exposure, a small fraction (10%) of the absorbed trichloroethylene (10%) is excreted in unchanged form via the lungs and kidneys. Most of it (80%) undergoes a series of oxidation reactions in hepatic microsomes (the cytochrome P-450 pathway) which essentially generate trichloroacetaldehyde (which is itself rapidly converted into chloral hydrate) and then trichlorethanol (30-50%) and trichloroacetic acid (10-30%). These detoxification products are cleared in the urine, either free or in conjugated form (trichlorethanol).

Acute poisoning poisoning is associated with CNS depression (loss of consciousness, narcosis, mental confusion or coma) and cardiac symptoms of variable intensity (extra-systole, fibrillation and arrhythmia). Depending on the dose absorbed, kidney and liver complications may also be observed.

Chronic poisoning mainly manifests as central and peripheral neurological signs (tiredness, headache, dizziness, sleep and memory disorders plus nausea) and psychological perturbation (impaired performance in neuropsychiatric tests, irritability and anxiety). In some cases, there are symptoms involving the skin (dermitis, eczema) and blood (anaemia, thrombocytopaenia).

Hepatic and renal complications are rare, and are usually seen in drug addicts.

Trichloroethylene is mutagenic and probably carcinogenic. It is classified in Group 2A by the International Agency for Research on Cancer (IARC).

INDICATIONS FOR MEASUREMENT

MONITORING OF WORKERS AT RISK

The blood concentrations of trichloroethylene in the hours after the beginning of the shift measure recent exposure and are useful when it comes to checking exposure levels because the results are very specific.

The concentration of TCA at the end of the working week and those of urinary TCE at the end of the shift and the end of the working week reflect exposure over the previous week and the same day, and they correlate fairly closely with atmospheric concentrations. Most TCE is excreted within 24 hours of absorption, and most of the TCA is eliminated during the second and third days after exposure, although excretion continues for about ten days. In consequence, the concentration of TCE in the urine reflects recent exposure (on the same day and on the previous day) and that of TCA reflects exposure in the last week. Assaying TCE and TCA in parallel gives the best measure of the amount of trichloroethylene metabolised.

MONITORING ACUTE AND CHRONIC POISONING

INFORMATION

SAMPLE

Both whole blood and urine can be assayed.

After venous puncture, whole blood is drawn onto heparin or EDTA with no gel.

Whole blood: At the end of the work shift, at the end of the working week.

Urine: End of the shift, end of the week, no additive (20 ml) in a plastic bottle.

QUESTIONS FOR THE PATIENT

Suspected diseases, clinical data, environment details, current medications?

ASSAY METHODS

Colourimetric methods: Fujiwara-Ross reaction (only for derivatives with a CX3 group in which X is a halogen, i.e. TCA and TCE but not trichloroethylene). This assay is non-specific; it lacks sensitivity and requires preliminary depletion of protein from the serum or plasma. Conjugated derivatives (trichlorethanol) do not react directly and have to be hydrolysed first.

Gas-phase chromatography with head space injection and detection by electron capture or flame ionisation, possibly coupled to mass spectrometry.

The TRANSURI-TRICHLO method, based on reactive strips developed by the French Research and Safety Institute for occupational accidents prevention (INRS), measures TCA and TCE at the same time.

REFERENCE VALUES

UNEXPOSED SUBJECTS

Only trace concentrations of trichloroethylene, trichlorethanol and trichloroacetic acid are found in normal blood and urine.

■ EXPOSED SUBJECTS

– Trichloroethylene, no official figure but, for reference: In the blood at the end of the week or before the next shift, <20 µg/l; at the end of the work shift, <200 µg/l.

ACGIH BEI (Biological exposure index) (updated in 2008):

– Free blood trichlorethanol: 0.5 mg/l at the end of the shift and the end of the week.

– Urinary trichloroacetic acid: 15 mg/l at the end of the shift and the end of the week.

– Trichloroacetic acid + trichlorethanol in the urine, <300 mg/g creatinine at the end of the shift and the end of the week.

■ COMMENTS

Alcohol consumption inhibits the conversion of trichloroethylene into trichlorethanol and trichloroacetic acid. This results in more being excreted in unchanged form via the lungs, and lower concentrations of its metabolites in the urine.

The same phenomenon is observed after simultaneous exposure to trichloroethylene, tetrachloroethylene and toluene.

Differences are seen between different races with higher excretion rates observed in Caucasian subjects.

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TRISOMY 21

(serum markers)

DEFINITION

Maternal serum markers for trisomy 21 are proteins or steroids secreted into the maternal blood by the foeto-placental unit, maternal concentrations of which are statistically different from the normal population when the foetal cells carry an additional chromosome 21.

INTRODUCTION

The following can be used as maternal serum markers for the risk of foetal trisomy 21:

In the 1st trimester of pregnancy:

– *Pregnancy-Associated Plasma Protein-A* (PAPP-A), secreted by the trophoblast,

– the free fraction of the β chain of human chorionic gonadotrophin (free β hCG subunit) secreted during pregnancy by the syncytiotrophoblast.

In the 2nd trimester of pregnancy:

– Alpha-foetoprotein (AFP), secreted mostly by the foetal liver,

– total hCG or the free β hCG subunit,

– unconjugated estriol (uE3), a steroid, which comes strictly from the feto-placental unit and originates from DHEA secreted by the foetal adrenal glands,

– inhibin A, secreted by the placenta during pregnancy (in Anglo-Saxon countries).

INDICATIONS FOR MEASUREMENT

There are 3 possible approaches to screening:

– "combined 1st trimester screening" with the maternal serum markers for the 1st trimester (PAPP-A and free βhCG) combined with 1st trimester ultrasounds,

– "sequential integrated 2nd trimester screening" with the maternal serum markers for the 2nd trimester (AFP, hCG or free βhCG, uE3) combined with ultrasound measurements in the 1st trimester,

– screening using serum maternal markers alone for the 2nd trimester.

If the calculated risk of trisomy 21 is greater than ≥ 1/250, the patient must be offered sampling for foetal kariotype (chorionic villous puncture or amniotic fluid sample).

INFORMATION

■ SAMPLE

The assay is performed on serum or plasma depending on the method used. Methods using fluorescent rare earth metals (Europium, Samarium) are incompatible with the plasma EDTA because of EDTA chelates the fluorescent marker.

The blood sample must be taken:

– for the first trimester between 11 weeks of the pregnancy + 0 day and 13 weeks of the pregnancy + 6 days.

– for the second trimester, between 14 weeks of the pregnancy + 0 days and 17 weeks of the pregnancy + 6 days (France).

The sample can be taken at any time during the day.

■ ESSENTIAL DOCUMENTATION TO ATTACH WITH THE SAMPLE

Clinical details sheet:

– ultrasound findings (name of ultra sonographer, date of ultrasound, measurements of nuchal clarity and cranio-caudal length);

– date of birth of mother;

– number of foetuses;

– patient weight/smoking habit;

– further information: evanescent twins, oocyte donation, and chronic renal insufficiency;

– sample date.

For France, consultation certificate and consent to the tests signed by the prescriber and patient.

■ SAMPLE STORAGE AND TRANSPORT

The sample must be centrifuged promptly and the separated serum or plasma frozen at - 20°C until assay if the assay is not to be performed immediately. Transport at - 20°C.

ASSAY METHODS

The proteins AFP, hCG or free βhCG and PAPP-A are immunometric assays (sandwich), which all are available on automated analysers. Unconjugated estriol is assayed by a competitive assay, which is also available on some automated analysers.

The reagents must carry the CE mark.

EXPECTED VALUES

Individual values are expressed as multiples of median (MoM) for the term in question and then corrected, taking account principally of any twin pregnancy, the mother's weight and smoking habit.

The calculation algorithm uses multiples of median after correction *(cf above)*, maternal age and any ultrasound measurements. The calculation is divided by a software appropriate for the reagents used and carrying the CE mark.

In addition to calculating trisomy 21 risk, the risk of neural tube closure defects can be assessed in the 2nd trimester of pregnancy from the AFP value expressed as the multiple of median.

PATHOPHYSIOLOGICAL VARIATIONS

■ TRISOMY 21

There is a correlation between a pregnancy with a foetus carrying trisomy 21 and a statistically significant difference in serum maternal markers for the term of the pregnancy: increased hCG or free β-hCG and/or reduced PAPP-A or AFP and unconjugated estriol.

There are an average of 800,000 pregnancies annually in France. The serum tests are performed in approximately

80% (approximately 620,000 women are tested). Using only the 2nd trimester serum markers, 7% of patients lie within the at risk range, the proportion screened is 75% and the positive predictive value (PPV) is 1/110 (110 amniocenteses to identify 1 trisomy 21; the serum test, nevertheless, performs twice as well as maternal age only). Second trimester markers combined with 2nd trimester ultrasound has a sensitivity of 85% to detect trisomy 21. This "stepwise" screening approach which was used in France until 2009 had the major disadvantage of generating a large number of amniocenteses: 90,000 annually (NB: "stepwise" screening: amniocentesis is indicated if the mother is over 38 years old, for increased nuchal clarity and if the serum screening test is positive).

Since the combined first trimester screening strategy was introduced the positivity rate has fallen from 7 to approximately 3%: the number of samples taken for fetal kariotypes has therefore been significantly reduced and the diagnosis of T21 is made earlier.

■ NEURAL TUBE CLOSURE DEFECTS

An AFP of 2.5 MoM or more suggests a risk of foetal damage, particularly a neural tube closure defect (NTCD) or abdominal wall closure defect, which is investigated by targeted ultrasound.

– With in-utero foetal death (IUFD) the mother's body is flooded with AFP (AFP has a ½ life of 21 days). If the AFP is abnormally high and combined with low hCG and estriol levels, foetal viability must be checked. It is also important to consider an evanescent twin or embryonic reduction in calculating the risk as high AFP associated with fetal lysis can mask a positive trisomy 21 risk.

■ OTHER ABNORMALITIES

It is important to investigate abnormal changes in serum markers:

– very low hCG or β hCG: this may be due to a normal pregnancy but also to a pregnancy in the process of miscarriage or with trisomy 18.

– Very low PAPP-A: risk of trisomy 18 or obstetric risk (pre-eclampsia).

– Very low estriol: given the complexity of the biosynthetic pathway for estriol, several enzyme abnormalities may produce this picture (for example some antibiotics), although these have no consequences on the course of the pregnancy. A large fall in serum unconjugated estriol concentration however, may also be a warning sign for the Smith-Lemli-Opitz syndrome, which is an association of numerous somatic and neurological malformations. In this case the biochemical abnormality lies immediately downstream from cholesterol synthesis (7-dehydrocholesterol is measured in amniotic fluid). Another disease to consider is X linked ichthyosis (which has no ultrasound signs).

MSM 2T	Thresholds	To Consider ?	MAN.
High AFP Normal hCG	AFP ≥2.50 MoM (Morzine consensus 2005)	- (Evanescent twins?) - NTCD, abdominal wall malformation… - Haemorrhage	Targeted ultrasounds
High hCG	≥2.50 MoM ≥10 MoM	(Mat CRI?) Placental dysfunction, Hypertension of pregnancy, T16 confined to the Choriocarcinoma	Risk +: kariotype Fetal and palcental ultrasound Uterine, dopler hCG and free βhCG/total hCG kinetics
High AFP and hCG	AFP and hCG ≥2.50 MoM	(Twin pregancy?) Triploidy	Ultrasound
High AFP, low hCG, low uE3		IUFD, SM	
Low hCG ± low AFP	≤0.20 MoM or AFP and hCG ≤0.50 MoM	T18, triploidy	Ultrasound

www.biomnis.com - Médecine Fœtale et Echographie en Gynécologie 2007 (70,69) et 2008 (75).

Legend:
MAN: further management
Values in grey between brackets should be confirmed before interpretation
CRI: chronic renal insufficiency
NTCD: neural tube closure defect
IUFD: in utero fetal death
SM: spontaneous miscarriage

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TROPONINS

DEFINITION

Cardiac troponins are proteins involved in the regulation of cardiac contraction. They form a macro-molecular complex with actin and tropomyosin. Three troponin subunits exist, I, T and C which have different roles:

– ***Troponin T (TnT)*** is present in cardiac and striated muscle. It has 3 isoforms coded for by different genes. TnT binds to tropomyosin and TnC.

– ***Troponin I (TnI)*** is involved in the control of muscle contractility. It binds to tropomyosin, actin, TnT and TnC. TnI also has 3 isoforms with specific peptide sequences.

– ***Troponin C (TnC)*** has binding sites for calcium and magnesium, which are required for muscle contraction. It has only one isoform which is common to all striated muscles and is of no use in cardiology.

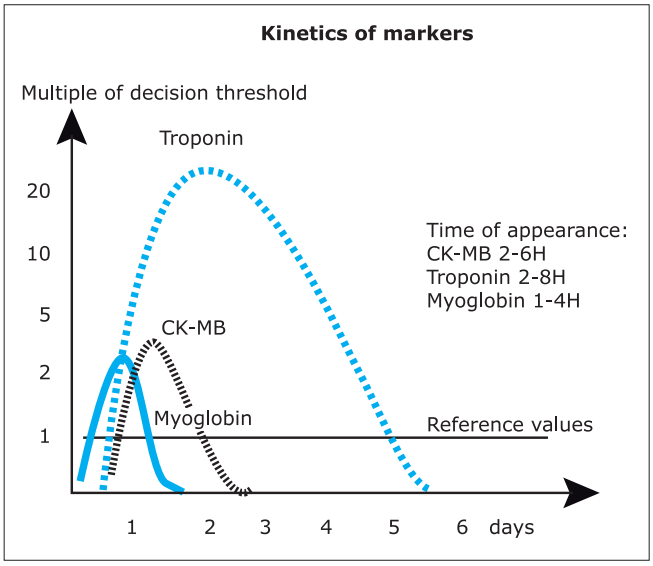
Only the troponin I (cTnI) and T (cTnT) cardiac isoforms are of use in cardiology. They are currently the most sensitive and specific markers for myocardial injury and represent the "gold standard" for the diagnosis and prognosis of acute coronary syndromes (ACS).

PATHOPHYSIOLOGY

In the absence of cardiac necrosis, plasma Tn concentrations are very low or zero. Their rise in myocardial injury makes them good, specific cardiac markers.

Within the cell, troponins are divided into two pools; in the contractile apparatus (92 to 97%) and in the cytoplasm (3 to 8%). Proteolysis of Tn I and T by the caspase, calpaine-I and metalloprotease enzymes begins in the ischaemic phase. Improved knowledge of this early release resulted in the development of highly sensitive Tn assays to improve the diagnostic efficacy of this marker. Following a myocardial infarction (MI), at least three forms of Tn may be found in the circulation: free forms, binary forms (I-C) or even ternary forms (I-C-T), with a plasma peak at around 25 hours. The circulating forms differ, depending on the time since the onset of necrosis, free forms followed by complex forms and as time goes on, increasingly small and numerous Tn fragments.

The heterogeneity of circulating forms of troponin poses a challenge to laboratory specialists, as measurements need to recognise the free Tn I or T forms and also both the binary or ternary complexes of cytosolic or intracytoplasmic Tn, and the intact.



Measurement of circulating forms of troponin is useful in the early diagnosis of cell necrosis, as it becomes positive within 2 to 3 hours after the onset of necrosis. Release of TnT is biphasic, with an initial peak 12 hours after the onset of necrosis, returning to normal after an average of 10 days. The length of the rise is associated with the size of the infarction: in a small necrosis TnT remains positive up to 7 days, whereas it may remain elevated for up to 21 days after widespread necrosis. This finding makes it a retrospective diagnostic marker for chest pain. TnI has very similar kinetics to those of TnT, other than the fact that it appears to be monophasic (within no initial peak) and that its rise appears to be smaller than that of TnT.

INDICATIONS FOR MEASUREMENT

Diagnostic and prognostic marker for acute coronary syndromes;

Diagnostic and prognostic marker of any cardiac necrosis, outside of an ischaemic setting. Plasma Tn concentrations are increased in myocarditis, myopericarditis, congestive heart failure, traumatic cardiac injury, electric shock, toxic cardiac damage (carbon monoxide, cocaine and some cardiotoxic anti-cancer agents such as anthracycline) and also in septic shock and meningeal haemorrhage.

Some groups use Tn to monitor reperfusion following an MI.

cTnI and cTnT have similar diagnostic and prognostic value. The choice of one or other is therefore above all organisational (analyser dependent).

INFORMATION

■ SAMPLE

Serum or plasma obtained from blood drawn into heparin (heparinised plasma appears to be preferable). Assay results in plasma or serum vary depending on the circulating forms of troponin (cTnT values in heparinised plasma are on average < 15% to those obtained on serum). Most methods do not recommend citrated or EDTA samples (EDTA appears to interfere with cTnI measurement but not with cTnT measurement).

Two consecutive samples at an interval of 1 to 3 hours in a patient with chest pain showing plasma troponin concentrations below the threshold are required to exclude a diagnosis of MI.

■ ESSENTIAL INFORMATION

In the context of MI diagnosis, state the time of onset of chest pain and duration.

■ SAMPLE STORAGE AND TRANSPORT

Centrifuge within 2 hours after taking the sample.

The serum or plasma can be stored for 24 hours at +4°C and for several months at –20°C.

ASSAY METHODS

Different assay kits are available for cTnI but assay standardisation is still difficult. For cTnT, only one kit is available (with four test generations) and new generation assays are appearing both for cTnI and for cTnT (highly sensitive Tn).

The difficulties of cTnI assay standardisation are due to the different antibodies (Ab) used in the kits which recognise different epitopes and therefore differ in their recognition of forms which have undergone post-translational modifications and the complexed forms; in addition the kits have different detection thresholds. Although a reference material has been proposed (SRM2921), not all problems have been resolved. In reality, commercial tests correlate well, although intermittent variations are seen which differ depending on the time since the initial episode.

At present, high sensitivity assay kits are available with Ab which target the same 20-40 and 41-49 cTnI epitopes, which have not been phosphorylated, oxidised or proteolysed giving optimum probability of measuring all circulating forms.

EXPECTED VALUES

In healthy people, cTnT and cTnI are undetectable.

American (ACC) and European (ESC) recommendations on Tn measurements.

Tn results should be produced with a *turnaround time* (TAT = time between sampling and the result) of < 60 minutes and threshold values < 99th percentile of a reference population (on a minimum of 120 healthy people) with a coefficient of variation (CV) < 10% at the 99th percentile.

INTERPRETATION

■ DIAGNOSIS AND PROGNOSIS OF ACUTE CORONARY SYNDROMES (ACS)

The diagnosis of ACS is mainly clinical. ACS is caused by the rupture of an atheromatous plaque with thrombosis which obstructs the lumen of the coronary artery, producing a clinical picture consisting mainly of pain. If the ischaemia continues beyond 20 minutes, irreversible myocardial damage may occur (necrosis) with the release of Tn. Amongst the ACS, obstructive thromboses of an epicardial vessel characterised by acute prolonged ischaemic, intense chest pain and changes in the ECG ST segment can be distinguished. These situations do not raise any diagnostic problems (Tn measurements are not essential) and are a medical emergency. The second major group is rupture of a plaque with a non-occlusive thrombus (no rise in the ST segment = ST-): necrosis is not a constant finding and clinical features vary. Tn is particularly useful in these situations.

In a clinical context of ischaemia, a rise in Tn indicates myocardial necrosis and provides a 99th percentile certain diagnosis of ACS.

Prognostically, Tn correlates with angiographic and clinical severity (more coronary thrombus and more significant thrombosis, more deaths and more long-term MI if the Tn is positive).

Finally, therapeutically, there is a large amount of data in the literature which shows that in a context of ACS, when the Tn is positive, it identifies a group of patients who will benefit particularly from anti-GP IIbIIIa treatment. International recommendations therefore, are to treat patients with an ST-Tn+ ACS with these compounds.

Note, however, **that a negative Tn does formally exclude ACS:** a thrombus is not necessarily associated with myocardial necrosis and the measurement must be performed at the correct time (ischaemia causes actual damage after approximately 20 minutes). Studies have also shown that in Tn negative cases, the incidence of cardiovascular events is still 8% at 6 months and 15% at one year.

If the pre-test probability of ACS is high and the Tn is negative, it is therefore useful to perform other tests, particularly an exercise test, before discharging the patient (reducing the risk of sudden death).

Conversely, **a positive Tn is not synonymous with ACS:** other circumstances exist in which the Tn is raised, independent of ischaemia/necrosis: these are heart failure, left ventricular hypertrophy, chronic renal failure, diabetes, sepsis and even situations of cerebral or gastro-intestinal haemorrhage (take care not to be too quick to prescribe antithrombotic agents!).

■ NEW SO-CALLED HIGHLY SENSITIVE ("HS") TROPONIN MEASUREMENTS HAVE BEEN DEVELOPED

These allow earlier detection of Tn, from the 2nd hour after the onset of pain, as they detect the cytosol pool, which makes up 3 to 8% of Tn in cardiac myocytes.

Studies have shown that hs Tn provides an earlier diagnosis: currently Tn is measured on admission to hospital (T0) and at T6h; hs Tn produces the same results at T0 and T3h, in terms of clinical events. If the hs Tn is negative, a patient can be discharged earlier from the emergency services and if positive it usually identifies patients at higher risk of progression.

It should be noted that hs Tn measurements are influenced by age and sex: the 99th percentile is higher in patients over 60 years old (which is probably physiological, i.e. age related cardiac "degeneration") and for similar age bands, Tn is higher in men than women. Should we not adjust our threshold values? This question has not yet been answered.

■ OTHER CARDIOVASCULAR DISEASE AND OTHER DISEASES

Raised troponin may indicate myocardial distress and appears to be linked to patient's prognosis in most clinical situations of cardiac necrosis outside of the context of ischaemia: heart failure, myocarditis, myopericarditis, anti-cancer drug related cardiotoxicity, traumatic cardiac injury, etc.

In addition, rises in Tn have revealed a new disease induced by intense stress (death of a relative, abuse, etc.), the Takotsubo syndrome or stress cardiomyopathy, which have various clinical features: when the heart contracts a small part is deprived of oxygen representing a myocardial reaction induced by the autonomic nervous system, causing injury with Tn release.

Tn is often found, in 35 to 75% of hospitalised patients with myocarditis, at moderately raised concentrations with a peak at 48 hours post-hospitalisation (unlike ACS). This is a slow release of Tn into the interstitial sector and not direct release into the circulation. The slow release represents stress (acute stress and also chronic disease such as hypertension, diabetes or chemotherapy treatment) on a "normal" heart which leaves cells fragile. Some of the cells can then be destroyed, releasing Tn which is detected by high sensitivity Tn assays.

Acute coronary syndromes are therefore one of many causes of an increase in Tn (cf table).

Increased troponins

Myocarditis and pericarditis	50 – 70%
Acute aortic dissection	25%
"Over-exercise"	8 – 75%
Heart failure	29 – 89%
Cardiac transplantation	50%
Renal failure	4 – 32%
Cerebrovascular accidents	17 – 65%
Severe sepsis	25 – 85%
Cardiac surgery	100%
Pulmonary embolism	32 – 47%
Myocardial injury	17 – 42%
Radio-frequency ablation	50 – 100%
Anti-cancer chemotherapy	8 – 32%
Interventional catheterisation	25 – 37%
Coronary angiography	13 – 31%
Non-cardiac surgery	5 – 70%
Babies born to a hypertensive mother	5 – 78%

FOR FURTHER INFORMATION

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TRYPANOSOMIASIS

DEFINITION

The trypanosomiases are parasitic diseases caused by flagellated protozoa known as trypanosomes. There are 2 different clinical and epidemiological forms: the African form (sleeping sickness) due to the species *T. brucei gambiense* and *T. brucei rhodesiense* and the American form (Chagas disease) due to *T.cruzi*.

INTRODUCTION

EPIDEMIOLOGY

- **African trypanosomiasis:** *T. gambiense* is found in Western Africa and tropical central Africa and *T. rhodesiense* in East Africa. African trypanosomiasis is transmitted by the bite of the tsetse fly, although congenital and transfusion related human-to-human cases of infection have been reported. The fly ingests the trypanosomes as it feeds on blood during the daytime. These then undergo active multiplication in its intestine and are converted into infesting metacyclical forms in the salivary glands. The infection is transmitted from a further bite.
- **American trypanosomiasis:** *T. cruzi* is found in Latin America where the disease affects 10 to 20 million people. It is transmitted by large blood-sucking arthropods similar to bedbugs called reduviidae. These are infected from a night-time feed on blood of a carrier human or animal. Once ingested the trypanosomes multiply in the gastro-intestinal tract of the reduviidae and in a few weeks they pass out large numbers of infesting trypanosomes in their excreta. These infesting forms enter the human beings though a pre-existing bite or scratch wound on the skin but can also pass through the mucosa (conjuctiva, buccal mucosa) from soiled hands. Transmission across the placenta, from blood transfusion and from organ transplantation has also been reported as methods of human-to-human transmission.

SYMPTOMS

- **African trypanosomiasis:** Schematically, the infection progresses in 2 phases:

  – a lymph/blood system dissemination phase producing fever, lymphadenopathy, hepatomegaly and skin signs,

  – a meningo-encephalytic phase with cerebral polarisation, which is an association of sensori-motor, psychological and neuroendocrine disorders. Disturbed sleep (nocturnal agitation and daytime drowsiness) develops later. Untreated the disease is fatal, although atypical, occult and even asymptomatic forms are seen.
- **American trypanosomiasis:** the disease involves acute and chronic phases, with an asymptomatic period between these two phases. Following silent incubation a lesion appears at the inoculation site followed by gastro-intestinal, skin, neuronal, respiratory or cardiac signs. The ***acute phase*** resolves spontaneously in most cases and is followed by a chronic phase occurring several years after apparent recovery and involving isolated or combined clinical features: heart disease (dysrhythmias, heart failure and thrombo-embolic events), gastro-intestinal problems with development of mega-organs (megaoesophagus, megacolon, etc.) or more rarely, cerebral problems.

SEARCH INDICATIONS

Diagnosis of African trypanosomiasis if the patient has stayed in an endemic area.

Diagnosis of Chagas disease if the patient has stayed in an endemic area and lived in poor conditions.

Diagnosis of acute reactivation of old *T.cruzi* infections in immunosuppressed patients (AIDS and transplant patients).

INFORMATION

SAMPLE

Venous blood: collected into citrated, heparinised or EDTA tubes for direct examination and cell culture.

Venous blood: collected into a dry tube for serological diagnosis.

Fingerprick blood: for a thick film.

Bone marrow: to examine for trypanosomes on a smear.

Lymph node aspiration fluid or lymph node biopsy: removed by needle or syringe.

CSF: obtained by lumbar puncture.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

History of insect bites in an endemic area?

Immune status?

SAMPLE STORAGE AND TRANSPORT

Sterile samples (blood, bone marrow, lymph node, aspiration fluid or CSF) should be sent within an hour of sampling at laboratory temperature. Direct examination must be performed very promptly as the mobility of the parasite over time is limited particularly in CSF.

Serum can be stored for 1 week (and transported) at + 4°C.

DIAGNOSTIC METHODS

PARASITOLOGICAL DIAGNOSIS

- **African trypanosomiasis:** the unequivocal diagnosis is based on finding the parasite in biological fluids (blood, bone marrow, lymph node aspirate or CSF centrifugation pellet). Blood is tested by examining a thick blood film between a slide and cover slip and identifying the mobile parasite and by preparing MGG stained thick or thin blood films (assorted concentration methods are required if the blood parasite count is low). Parasites are examined for in a lymph node aspirate either fresh or in some cases after MGG staining. A stained smear is used to examine bone marrow.

  Culturing the parasite and gene amplification methods are difficult and are reserved for use by highly specialised laboratories.

- **American trypanosomiasis:** direct examination for the parasite in blood is performed mostly during the acute phase. Other methods are not used routinely: blood cultures may be performed on NNN medium and the parasite can be isolated by inoculation into the mouse. *T.cruzi* can also be isolated from lymph node aspiration fluid, pericardial fluid or from a skin or conjunctival sample. *In-vivo* xenodiagnosis or culture of the parasites is a highly sensitive but long method. PCR methods are sensitive and faster.

SEROLOGICAL DIAGNOSIS

- **African trypanosomiasis:** methods available are direct agglutination on a card or the *CATT tryp Test* used as a mass screening method in endemic areas, sensitised latex particle agglutination, indirect haemagglutination and indirect immunofluorescence on a *Trypanosoma gambiense* smear. Cross-reactions occur with Chagas disease, visceral leishmaniasis and malaria.
- **Chagas disease:** serological diagnosis is useful for epidemiological screening and during the chronic phase and uses indirect immunofluorescence, gel precipitation with a soluble antigen, complement fixation and ELISA methods. Cross-reactions also occur with visceral leishmaniasis and with a trypanosome, which is not pathogenic to human beings: *T. rangeli*.

  NB: in both cases the French listing of tests and tariffs only refers to haemagglutination and indirect immunofluorescence.

FOR FURTHER INFORMATION

- Raccurt C., *Trypanosomose américaine à T. cruzi ou maladie de Chagas*, Encycl Med Biol, Elsevier Paris ; 2003.

TRYPTASE

DEFINITION

Tryptase is a tetrameric serine-protease with a molecular mass of 134 kDa, mostly secreted by mastocytes. It is mediator of the immediate hypersensitivity reaction with a longer half-life than histamine (1.5 to 2.5 hours).

INTRODUCTION

Tryptase makes up 20 to 50% of mastocyte proteins. Two molecular forms have been described in plasma:

– alpha-protryptase or tryptase α: the form secreted physiologically by mastocytes. This is raised considerably in systemic mastocytoses, unlike tryptase β which is raised only a little or not at all.

– tryptase β: the form stored in mastocyte granules. This is the biologically active form and is released with histamine during anaphylactic reactions.

Tryptase release indicates mastocyte activation, regardless of the immunological or pharmacological mechanism involved.

INDICATIONS FOR MEASUREMENT

- **Peranaesthetic anaphylactic reactions,** mostly very severe, grades 3 and 4. Combined with clinical signs and the clinical history, tryptase may point towards a precise diagnosis and enable the implicated allergen to be avoided.

  In most cases, tryptase concentrations increase from the 10th minute with a peak at around 30 minutes and 2 hours depending on the patient. Concentrations correlate with severity of the reaction and return to normal the following day.

  In fatal reactions a large rise in tryptase has been proposed as a diagnostic test for anaphylaxis, although there is no absolute threshold to define the death as being due to allergy. High tryptase concentrations are also seen post mortem in patients dying from non-allergic causes. Testing for specific IgE combined with tryptase measurement therefore provides important information.

  The rise in tryptase in moderate reactions is small and must be compared to a baseline value. A doubling in value is a positive indicator of allergy.

  In relatively non-severe, grade 1 reactions, tryptase is generally little if at all raised. A rise in plasma histamine associated with positive skin tests and/or specific IgE are used for the differential diagnosis.

- **Systemic mastocytosis**

  Very high tryptase concentrations (up to over 200 µg/l) are seen with the current assay method, which recognises both forms of tryptase. This is an aid to the diagnosis and monitoring the progression of this disease.

- **Hospital challenge test**

  In challenge tests with hymenopter venoms, tryptase rises particularly in patients with severe hypotension, from the first minute with a peak at around 15 minutes followed by a small fall in the next hour.

  A rise in tryptase is also only seen in severe reactions with oral challenge tests to medical drugs or foods and is proportional to severity with a peak ranging from 15 minutes to 3 hours after the reaction.

INFORMATION

SAMPLE

Serum or plasma (except plasma collected into fluoride which produces false positive results).

It is recommended that tryptase kinetics be followed-up by taking a sample as soon as possible after the anaphylactoid shock, then 1 to 2 hours later and then at 8 hours.

The diagnostic sensitivity of tryptase can be increased by a combined measurement with histamine, particularly in relatively non-severe reactions.

QUESTIONS FOR THE PATIENT

Date and time the sample was taken after onset of symptoms?

Medicinal products administered?

SAMPLE STORAGE AND TRANSPORT

Serum: 2 days at + 20-25°C, 5 days at + 2°C and + 8°C, and for several months at -20°C or - 70°C.

Whole blood: a moderate fall in tryptase beyond 24 hours.

Freeze-thaw cycles do not appear to affect results.

ASSAY METHODS

Automated fluorimetric method using two monoclonal antibodies, which recognise the 2 forms of tryptase.

NORMAL EXPECTED VALUES

Large inter-individual variability in normal values is seen.

On the other hand, concentrations remain stable over time in the same person.

Healthy people have detectable concentrations ranging from 1.9 to 13.5 µg/l.

PATHOLOGICAL VARIATIONS

The positivity threshold is difficult to define because of inter-individual variability. A large rise in tryptase to over 25 µg/l is recognised as a positive factor for the diagnosis of anaphylaxis by the Société Française d'Anesthésie Réanimation (French national society of anaesthesia and intensive care). An increase of twice the baseline tryptase concentration can also be used as positivity threshold. Normal tryptase concentrations, however, do not completely exclude a diagnosis of anaphylaxis.

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TSH

DEFINITION

TSH is a 28 kDa glycoprotein hormone formed by the non-covalent binding of 2 sub-units: α (92 amino acids) and β (112 amino acids). Binding is required to express the biological function of the hormone.

TSH exists in many isoforms, which have different biological activities.

INTRODUCTION

The synthesis and secretion of TSH are regulated by two main antagonistic factors:

– hypothalamic TRH (TSH *releasing hormone*), which has a stimulatory action,

– thyroid hormones (T4 and T3), which exert negative control.

Other factors are also involved including:

– dopamine and glucocorticoids: TSH ↘

– noradrenalin: TSH ↗.

TSH has a half-life of approximately 40 minutes.

TSH is secreted in a 24 hour cycle with a nocturnal peak (at around 0200 hours in the morning) and a nadir (in the afternoon); 12 pulses with a median amplitude of 0.5 mU/l are seen over 24 hours in healthy people.

Posture, physical exercise and external temperature do not cause significant changes in TSH concentration.

INDICATIONS FOR MEASUREMENT

TSH is the most sensitive and earliest indicator of primary thyroid dysfunction: primary dysthyroidism can be excluded if the TSH concentration is normal. It is the most sensitive indicator of primary thyroid dysfunction as a minimal change in free T4 produces a greatly amplified TSH response. TSH, however, has a long latent period before responding to changes in free T4 of at least eight weeks for a full response. This must be taken into consideration when monitoring thyroid replacement treatment or the effectiveness of treatment of hyperthyroidism.

INFORMATION

SAMPLE

It is highly recommended that the sample be taken into a dry tube with no additive: heparinised plasma is acceptable (EDTA interferes with methods using alkaline phosphatase).

Samples should be taken from the person not fasting, in the morning (between 0800 and 1200 hours).

QUESTIONS FOR THE PATIENT

Expected dysthyroidism?

Pregnancy? If yes, give term.

Medical history (including glucocorticoids, dopamine or biotin)?

Autoimmune disease?

SAMPLE STORAGE AND TRANSPORT

Stability: a few days at + 4°C, several months at – 20°C, after one freeze/thaw cycle. Transport at +4°.

ASSAY METHODS

Sandwich immunometric methods.

These are the so-called 3rd generation methods, which have an operating limit of detection of 0.01 – 0.02 mU/l.

N.B: The TRH stimulation test (with 200 to 250 micrograms IV.) is reserved for identifying central hypothyroidism (pituitary or hypothalamic).

Calibrators are standardised against an international standard (WHO 2nd IRP 80/558).

NORMAL EXPECTED VALUES

Baseline TSH in a healthy adult is between 0.2 and 4 mU/l (figures vary depending on the reagents used).

Note:

– a physiological peak occurs at birth (around 100 mU/l), returning to "adult" levels after approximately 5 days.

– Low values may be seen in the 1st trimester of pregnancy (5% of cases) coincident with the rise in hCG ("TSH-like" activity).

PATHOLOGICAL VALUES

– Primary hypothyroidism: TSH > 4 mU/l

– Primary hyperthyroidism: TSH < 0.2 mU/l

– Pituitary TSH adenoma (with hyperthyroidism): raised TSH

– Pituitary failure (with hypothyroidism): low TSH

– Thyroid hormone resistance syndrome: raised TSH.

N.B: subnormal values may be seen in severe depression.

FOR FURTHER INFORMATION

Piketty M.L., *TSH.* In : Encyclopédie médicobiologique (chapitre Biochimie) ELSEVIER 2003.

TULARAEMIA

DEFINITION

Tularaemia is an anthropozoonosis due to a bacterium called ***Francisella tularensis***. The *Francisella (F) tularensis* species contains several biovars. *F. tularensis* biovar *tularensis* (or type A) and the biovar *palearctica* (or *holarctica* or type B) are the main biovars which infect human beings. The biovars *novicida* and *mediasiatica* have very rarely caused human infections. *F. tularensis* is a small immobile, obligate anaerobic Gram-negative bacillus.

INTRODUCTION

F. tularensis biovar *tularensis* is found in North America and the *palearctica* biovar is commoner in Europe and Asia. The main reservoirs of the bacterium are rodents (primarily hares, rabbits, field mice, squirrels, beavers, voles and muskrats) and some insects (mostly ticks, taons and more rarely mosquitoes). The bacterium can cross, healthy or damaged skin and also respiratory and gastro-intestinal mucosa. Tularaemia is transmitted to human beings mostly through the skin by direct contact with a diseased or dead carrier animal (field mice or voles) from skinning (hares) and also from a cat or even a dog bite or scratch and by bites from carrier arthropods. Other less common means of transmission include droplet inhalation or eating or drinking contaminated food or water. Two seasonal peaks are usually described, summer from insect bites and autumn-winter during the hunting season.

Human-to-human transmission does not occur. The features of *F. tularensis* make it a potential biological terrorism agent.

SYMPTOMS

This depends on the portal of entry and biovar concerned (type A is more virulent than type B).

- **Incubation:** lasts an average of 3 to 5 days.
- **The invasion phase:** begins suddenly with a flu-like syndrome of rigors, high fever, headaches, muscle and joint pain, anorexia and asthenia and occasionally vomiting and abdominal pain.
- **The established disease phase:** there are several clinical forms (depending on the portal of entry):

The ulcer-lymph node form: this occurs after direct skin contact and affects 45% of people infected. It involves a local lesion at the inoculation site of the bacterium progressing to a necrotic ulcer with inflamed regional lymphadenopathy.

The lymph node form involves regional lymphadenopathy without any skin ulcer.

The oculo-lymph node form occurs after ocular contact and involved inflammatory conjunctivitis with painful regional lymphadenopathy.

The oro-pharyngeal form follows ingestion of contaminated food or water and involves pharyngitis or sore throat with cervical lymphadenopathy.

The pulmonary form occurs after inhaling a contaminated droplet aerosol or haematogenous spread of the organism in septicaemia patients. This involves cough, usually dry, bronchiolitis or even pleurisy and pneumonia with dyspnoea and respiratory distress.

The gastrointestinal form is a combination of abdominal pain, diarrhoea and vomiting.

The typhoid form is seen usually in the immunosuppressed and involves isolated fever, headaches, general malaise, which may be complicated by neurological problems and septic shock with coma.

Other more rare clinical features are described including skin lesions as erythema nodosum or a generalised maculo-papular rash. Tularaemia can be complicated occasionally by systemic spread of the organisms through the blood stream, producing various clinical forms: endocarditis, pericarditis, meningitis, peritonitis, hepatitis and osteomyelitis.

SEARCH INDICATIONS

Diagnosis of tularaemia in suggestive clinical circumstances or in a history of contact with a carrier animal or tick bite in an endemic area.

Diagnosis of tularaemia in a person who has been occupationally exposed to the organism.

Differential laboratory diagnosis with the *Brucella, Proteus* and *Yersinia* genera.

INFORMATION

SAMPLE

Serous fluid samples from the inoculation wound with a swab.

Lymph node aspirates or biopsies.

Other samples can be used: expectoration, pharyngeal samples, conjunctival sample, gastric aspiration-fluid and blood cultures.

Serum for serological diagnosis.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

History of contact with a reservoir potential carrier animal or tick bite, date and place?

History of disease in the patient's close contacts?

Is the patient occupationally exposed to the organism?

Current antibiotic treatment?

SAMPLE STORAGE AND TRANSPORT

Biopsy and lymph node aspirate samples must be kept at room temperature or stored at between + 2 and + 8°C.

Serum samples can be stored for 1 week at + 4°C, then frozen for up to 1 year at – 20°C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

- **Isolation** of the organism from samples provides the unequivocal diagnosis, although this is difficult to perform, as the culture is slow and poorly sensitive. *F. tularensis* is a highly contagious

bacterium belonging in laboratory category 3. Suspect samples and cultures must be handled in the laboratory with maximum precautions.

- **Identification** is based on culture features (nutrient requirements, appearance and culture time over 48 hours) and also on biochemical features (weakly positive, catalyse and oxidase and urease negative, and sugar fermentation studies). The *tularensis* species can be identified by slide agglutination with a specific anti-serum. The biovars are distinguished on their biochemical features and on their virulence in the rabbit. Finally, molecular biological methods can be used to identify the genus and species.
- **Gene amplification:** *F. tularensis* can be detected in blood, lymph node biopsies or skin wounds using PCR methods based on amplification of the gene coding for a 17 kDa membrane lipoprotein or the gene coding for the 16 S ribosomal RNA fraction.

INDIRECT DIAGNOSIS

Antibodies generally develop around day 10 (occasionally later), reaching a maximum titre after 3 to 4 weeks. They may last for several years. The most widely used method is bacterial agglutination. An antibody titre of 160 or greater is significant, although more often diagnosis seeks to establish sero-conversion or increased antibody titres in two serum samples taken at a 15-day interval. ELISA and indirect immunofluorescence methods are also available. Cross-reactions occur with *Brucella* sp., *Proteus* OX19 and the *Yersinia* genus. The Western blot method can be used to test for the presence of anti 17 and 43 kDa lipoprotein antibodies, which are specific for *F. tularensis*, although no serological method can identify the serovar responsible.

TREATMENT

CURATIVE

The bacterium is sensitive to aminoglycosides, cyclines, phenicol derivatives and fluoroquinolones. Current treatment involves administration of a fluoroquinolone (15 days) or a cycline (21 days). A combination of antibiotics can be used in the early phase of the disease until lymphadenopathy has disappeared.

PREVENTIVE

This is difficult in view of the large number of reservoirs and carriers of the disease.

Vaccination: a live attenuated vaccine is used in the USA. This is reserved for people who have been exposed.

Other preventive measures: avoid all contact with potentially infected animals in endemic areas, incinerate their cadavres and do not each their meat, avoid tick bites in endemic areas. Doxycyline antibiotic prophylaxis can be offered after suspected contact.

FOR FURTHER INFORMATION

- Centre national de référence pour Francisella tularensis : Unité des zoonoses bactériennes (laboratoire d'études et de recherche en pathologie animale et zoonoses), Agence française de sécurité des aliments de Maisons-Alfort.
- Laboratoire associé : Service de Bactériologie, centre hospitalier universitaire de Grenoble.
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TYPE 1 BONE COLLAGEN TELOPEPTIDES

DEFINITION

These are reticulated peptides produces by degradation by the C- and N-terminal ends of type 1 collagen during osteolysis.

Synonyms: CTx = Crosslaps®, NTx.

PHYSIOLOGY

The main form of collagen in bone tissue is type 1. Type 1 collagen is formed initially by the interlinking of 3 molecules of procollagen without bonds between the chains. Cleavage of the unreticulated C and N-terminal ends and the formation of covalent bonds between the chains successively stabilises type 1 collagen.

Healthy bone tissue undergoes constant remodelling with a physiological equilibrium between synthesis and resorption. During the resorption phase, degradation of type 1 collagen releases reticulated fragments from its C and N terminal ends (NTx and CTx). Bone resorption varies physiologically by sex and age, within the 24-hour cycle, and as a function of physical exercise, dietary calcium intake, vitamin D reserves, hormone production (mostly PTH, androgens and oestrogens) and after a bone fracture.

INTRODUCTION

All circumstances that promote bone remodelling are associated with a rise in CTx and NTx. High levels of bone remodelling or a chronic/prolonged balance in favour of osteolysis renders bone tissue fragile. During the menopause oestrogen deficiency is associated with a relative rise in bone destruction. This effect is particularly marked during the 5 years before the menopause.

INDICATIONS FOR MEASUREMENT

■ OSTEOPOROSIS

Markers of resorption are not of diagnostic value as the correlation between BMD and NTx and CTx concentrations is poor.

Raised markers of resorption in menopausal women may help depending on the clinical and radiological context in the decision on treatment.

Markers of bone remodelling can be used to monitor the effectiveness of resorption inhibitor treatment: a fall in CTx and NTx concentrations of approximately 30 to 50% 3 to 6 months after starting treatment is a factor indicating good adherence and effective treatment.

■ SECONDARY INDICATIONS (specific situations)

Paget's disease.

Hyperparathyroidism.

Identifying an osteolytic process.

INFORMATION

The following recommendations should be followed: always use one and the same marker, either serum or urinary, to monitor treatment (measurement before starting and kinetics on treatment to ensure adherence and effectiveness of the treatment chosen).

■ SAMPLE

– Serum: taken in the morning, fasting before 0900 hours, if possible at the same time for successive measurements.

– Urine: 1st or 2nd pass of the morning, fasting before 0900 hours. Do not use samples if haematuria is present. 1 ml for CTx; 5-10 ml for NTx.

■ QUESTION FOR THE PATIENT

Treatments? Oestrogens, androgens, rH-PTH, corticosteroids or heparin?

Vitamin D deficiency?

Intercurrent diseases (recent prolonged confinement to bed)?

Recent fracture?

■ SAMPLE STORAGE AND TRANSPORT

Serum should not be haemolysed, and should be centrifuged within 3 hours and frozen within 4 hours of sampling.

Urine, frozen within 4 hours after collection.

ASSAY METHODS

Serum: ELISA or chemoluminescence kit.

Urine: competitive ELISA kit between an antibody bound to the wells and a soluble test antigen against an anti-CTx antibody.

EXPECTED VALUES

The principle is to maintain peri-menopausal levels after the menopause. Increased bone resorption must not be "accepted" after the menopause.

Serum CTx

– Men EIA: 300-7200 pmol/l or by electrochemoluminescence: 0.16-0.44 µg/l,

– Women EIA: 780-2900 pmol/l or by electrochemoluminescence: 0.16-0.44 µg/l.

Urinary CTx

– Men: 20-400 µg/mmol of creatinine,

– Women: 20-408 µg/mmol of creatinine.

Urinary NTx

– Women: 5-41 nmol/mmol of creatinine,

– Men: 3-51 nmol/mmol of creatinine.

INTERPRETATION – DIAGNOSTIC VALUE

■ **Interpretation must take account of and patients must be asked about circumstances in which a rise in resorption markers would be expected:**

– confinement to bed for more than 2 days,

– within 6 months of a fracture,

– during corticosteroid or heparin therapy,

– in vitamin D deficiency,

– in bone metastases, Paget's disease and multiple myeloma.

■ **Intra-individual variability factors:**

Intra-individual variation is estimated to be 25-55%. The major factors contributing to variation are:

– the circadian rhythm (the sample should preferably be taken at the same time in the morning),

– dietary status (the sample should be taken fasting),

– level of physical activity (avoid sample if patient confined to bed for more than 2 days before measurement),

– excessive intake of alcohol or smoking.

■ **A significant rise is expected in the following diseases** (after estimating intra-individual variability):

–Paget's disease in the resorption phase,

– hyperthyroidism.

■ **Assessment of the efficacy of bone resorption inhibitor therapy:**

The following reductions are significant:

– greater than > 50% for serum CTx and NTx after 3 month interval,

– greater than > 25% to 30% for urinary NTx after 6 month interval.

FOR FURTHER INFORMATION

■ Cormier C., Kindermans C. et Souberbielle J.-C., *Marqueurs du remodelage osseux : données récentes sur leur intérêt et leurs limites dans l'exploration des ostéoporoses*, Ann Biol Clin, 2001, 60 : 343-346.

UNESTERIFIED FATTY ACIDS

DEFINITION

Free unesterified fatty acids (UFAs) account for less than 5% of all fatty acids in the circulation. They constitute a lipid reserve and are bound to albumin in the blood.

Synonym: Free fatty acids.

INTRODUCTION

The UFA concentration depends on carbohydrate and lipid metabolic activity and it rises on the secretion of catecholamines, corticosteroids, growth hormone and glucagon, and it drops when insulin is being secreted.

It is currently believed that UFAs may be involved in the pathogenesis of type 2 diabetes. A fatty diet can lead to high concentrations of UFAs in the blood and this can cause health problems. In the muscles, free fatty acids out-compete glucose as an energy substrate and are preferentially oxidised, entailing increased production of acetyl CoA which, in turn, down-regulates glycolysis enzymes. In the liver, gluconeogenesis is stimulated which can raise blood glucose levels and pave the way for diabetes.

INDICATIONS FOR MEASUREMENT

High UFA levels may be a risk factor for type 2 diabetes.

They may also be a marker for stavudine-induced lipodystrophy.

INFORMATION

SAMPLE

Serum or plasma + lithium fluoride (not heparinised plasma).

Sample to be taken after the subject has been fasting for 12 hours.

Reject haemolysed samples (1 gram of haemoglobin raises the reading by 40 µmol/l).

QUESTIONS FOR THE PATIENT

– Smoking? Coffee consumption? Both habits cause fluctuations in UFA concentrations.

– Recent diet? Excessive carbohydrate consumption?

– Prolonged fasting?

– Recent drugs?

– Amphetamine derivatives and heparin raise the UFA concentration.

– Aspirin, fibrate, neomycin, propranolol and insulin reduce the UFA concentration.

SAMPLE STORAGE AND TRANSPORT

The tube should be centrifuged and the plasma or serum frozen as soon as possible (within an hour of blood drawing). Frozen samples can be stored for two months.

ASSAY METHODS DISPONIBLES

Colourimetric assay based on two enzymes, acetyl coenzyme A synthetase and acetyl coenzyme A oxidase. The hydrogen peroxide released is measured in a colourimetric reaction in the presence of a chromogen.

NORMAL EXPECTED VALUES

130-445 µmol/l, i.e. 37-126 mg/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Increased UFA: Prolonged fasting, major stress.

Decreased UFA: High carbohydrate intake.

PATHOLOGICAL VARIATIONS

Increased UFA concentration: Diabetes, hyperthyroidism, hyperadrenocorticism, pheochromocytoma and myocardial infarction. An increase in UFA concentration has been linked to a risk of sudden death in cardiovascular disease. An UFA concentration of over 407 µmol/l doubles the risk of sudden death in men.

Monitoring the increase in free fatty acid levels can provide information about insulin resistance and the installation of type 2 diabetes.

A high UFA concentration is also a marker for stavudine-induced lipodystrophy.

A drop in UFA concentration is seen in hypothyroidism and islet cell adenoma (secreting insulin).

FOR FURTHER INFORMATION

■ Girard J., *Contribution of free fatty acids to impairment of insulin secretion and action: mechanism of beta-cell lipotoxicity*, Med Sci (Paris), 2003 Aug-Sep; 19 (8-9): 827-33.

■ Jouven X., Charles M.A., Desnos M., Ducimetiere P., *Circulating nonesterified fatty acid level as a predictive risk factor for sudden death in the population*, Circulation, 2001; 104: 756-61.

URINARY FREE CORTISOL

DEFINITION

Urinary free cortisol or unconjugated urinary cortisol is a minor cortisol metabolite representing only 1% of daily cortisol production in healthy people. It reflects the fraction of plasma cortisol which is not bound to proteins and considered to be biologically active. In addition it is an excellent marker as it correlates with cortisol production.

When cortisol production increases, urinary free cortisol increases relatively more: if for example cortisol production doubles, urinary free cortisol is increased by a factor of 4 or 5. It is therefore a very sensitive and specific parameter, particularly for the diagnosis of hypercortisolism (Cushing's syndrome).

Synonyms: Free using compound F, Urinary Free Cortisol (UFC).

INDICATIONS FOR MEASUREMENT

Urinary free cortisol is an excellent test for the positive diagnosis of hypercortisolism or a Cushing's syndrome. Measurement may be performed at baseline or in a dynamic test (dexamethasone, suppression test).

INFORMATION

■ SAMPLE

20 ml of unacidified 24 h urine. Because of the 24 hour variations in cortisol secretion it is essential that a full 24 hour collection is obtained (record urine volume). Creatinine is always measured in conjunction to confirm the quality of the collection.

■ QUESTIONS FOR THE PATIENT

Current treatment? Exogenous glucocorticoids interfere either with the assay method or suppress the adrenal axis and therefore reduce cortisol secretion. The type and duration of corticosteroid therapy must also be recorded.

■ SAMPLE STORAGE AND TRANSPORT

Samples may be stored for five days at + 4°C or at – 20°C beyond this time.

ASSAY METHODS

High performance liquid chromatography. Radio-immunological assay provided that a purification stage is included in the procedure.

UNITS AND REFERENCE VALUES

Units: µg/24 hours. These are converted into nmol/24 hours by multiplying µg/24 h by 2.758.

NORMAL EXPECTED VALUES

Values may vary depending on the method used.

With the radioimmunological method following a purification stage, usual values vary between 10 and 50 µg/24 hours in women and between 20 and 50 µg/24 hours in men.

Values are less than 30 µg/24 hours in children.

PATHOLOGICAL VARIATIONS

Raised urinary free cortisol provides good diagnostic evidence for hypercortisolism (Cushing's syndrome). Concentrations are invariably raised often above 200 µg/24 hours.

A reduction suggests hypocortisolism. Measurement of urinary free cortisol however is not a good diagnostic criterion for adrenal insufficiency as concentrations may be within physiological limits even if production is reduced.

FOR FURTHER INFORMATION

■ Hazard J., Perlemuter L., *Endocrinologie*, 4th Ed. Paris, Abrégés Masson, 2000.

■ Schlienger J.L., André G., Chabrier G., Reiss D., Simon C., *Explorations fonctionnelles en endocrinologie et métabolisme*, 2nd Ed, Paris : Expansion Scientifique française, 1993.

URINARY OESTROGENS

DEFINITION

These are a group of compounds obtained from the hepatic and renal metabolism of oestrogens secreted by the steroid-producing glands or produced by aromatisation of androgens in liver, muscle and adipose tissue.

Estradiol and estrone, produced by the gonads, are very rapidly converted, particularly in the liver into sulfo- and glucurono-conjugated derivatives. The conjugates are the main form of renal oestrogen excretion as they are water soluble, whereas unconjugated oestrogens are almost insoluble in water. A proportion of oestrogens are also converted into estriol (by the action of 16-hydroxylase), although outside of pregnancy this production is extremely limited.

A mixture of several sulfo- and particularly glucuronide, estrone, estradiol and estriol conjugates are found in urine.

INTRODUCTION

Urinary oestrogen excretion is an index of total oestrogen production, although this index is subject to several weaknesses, compared to the information provided from serum oestrogens.

Urinary excretion is always delayed by approximately 24 hours compared to fluctuations in the blood concentrations. This occurs particularly during the menstrual cycle.

They only provide a gauge to overall production and it is unrealistic to consider urine oestrogen to be representative of serum estrone or urine estradiol to be representative of serum estradiol. Both of these urinary measurements provide an overall picture of what has been produced or administered. The results are expressed against a single standard, estrone. The value is therefore expressed in estrone or estrone glucuronide (see below) equivalents.

Before puberty, neither girls nor boys secrete significant amounts of oestrogens. Some adrenal androgens are also produced however, even several years before puberty and a proportion of these are converted, mostly in the liver, into oestrogens by 3-betahydroxysteroid dehydrogenase which converts dehydroepiandrosterone into androstenedione and then by an aromatase which converts androstenedione into estrone. Overall, however, less than 5% of adrenal androgens are converted into oestrogens. Ovarian estradiol and estrone secretion increases progressively during puberty, well before the menstrual cycles begin and is reflected by increased urine oestrogen excretion. Values fluctuate according to the well-known menstrual rhythm in adult females.

In men, a small proportion of oestrogen originates from the testes (approximately 20%) and the majority as a result of hepatic aromatisation of testosterone into estradiol.

INDICATIONS FOR MEASUREMENT

These are the same as for measurement of estradiol (see corresponding page), although urinary oestrogen measurement offers no further information than blood measurements and requires a 24 hour collection for the result to be of significance.

INFORMATION

SAMPLE

Urine must be collected under standard conditions. Empty the bladder and then collect all urine passed for 24 hours until the next day at the same time.

During the collection period the urine which has collected must be stored at + 4°C.

QUESTIONS FOR THE PATIENT

Are you taking androgens or oestrogens?

Record height and weight.

SAMPLE STORAGE AND TRANSPORT

Transport at +4°C to avoid bacterial fermentation. An aliquot can be submitted provided that the total 24 hour urine volume is reported.

ASSAY METHODS

The physicochemical assay with Kober reagent is no longer used. Assays which are preceded by hydrolysis followed by extraction and then gas phase chromatography or high performance liquid chromatography separation are excessively complicated in view of the limited information provided by this parameter.

If measurement of urinary oestrogen excretion is required, however, the best compromise is immunoassay with an anti-estrone glucuronide antibody. This method avoids hydrolysis and extraction and measures a highly representative parameter of total urinary excretion as, because of its high clearance, estrone glucuronide represents approximately 70% of all urinary oestrogens.

NORMAL EXPECTED VALUES

Values expressed in estrone equivalents:

Adult men: 7-15 µg/24 hours

Follicular phase: 5-20 µg/24 hours

Luteal phase: 7-45 µg/24 hours

Post-menopausal women: 7-15 µg/24 hours.

FOR FURTHER INFORMATION

■ Wright K., Collins D.C., Preedy J.R.K., *Urinary excretion of estrone glucosiduronate, 17 -estradiol-17-glucosiduronate, and estriol-16 -glucosiduronate. Significance of proportionate differences during the menstrual cycle.* Steroids 1979, 34: 445-457.

UROGENITAL MYCOPLASMAS

DEFINITION

Mycoplasmas are the smallest organisms capable of reproducing outside a living cell. They belong to the class of Mollicutes (soft-skinned organisms) and are pleomorphic, containing DNA and RNA.

Four mycoplasma species are implicated in human urogenital pathology. These are: *Mycoplasma hominis (Mh), Ureaplasma urealyticum (Uu), Ureaplasma parvum (Up)* and *Mycoplasma genitalium (Mg)*. The first three are easy to culture, while the fourth is not.

Mycoplasmas are present in the commensal state. This particularly applies to Uu, which is found in approximately 50% of women, and Mh, in approximately 10%, while Mg is much less common (1 to 4%).

INTRODUCTION

Mycoplasma hominis (MH) is a saprophyte of the genital passages. It colonises the genital tract at birth or slightly afterwards. Its presence is frequently transient and it tends to disappear at about two years of age. In adolescents and young adults, reappearance in the lower genital tract depends on sexual contact and the number of partners. The carrier rate varies from 1 to 5% in asymptomatic men and 30 to 70% in asymptomatic women. In women, it plays no pathogenic role in cervicitis, but has been incriminated in bacterial vaginosis and may be involved in endometritis, salpingitis and *post partum* fever, as well as in 5% of pyeolonephritis cases. It appears to have no pathogenic effect in men but is believed to cause infections in newborn babies (pulmonary infections, meningitis and septicaemia).

Ureaplasma urealyticum (Uu) is also a saprophyte of the genital passages. It colonises newborn babies *in-utero* or at birth and is still present in 20% of girls before puberty; after puberty, the carrier rate depends on the number of sexual partners. It is found in the vagina of 40 to 80% of asymptomatic women and in the urethra of 5 to 20% of asymptomatic men. In men, it may be implicated in cases of chronic non-gonococcic urethritis (NGU), epidydimitis (exceptionally) and urinary lithiasis, while in women it is thought to be potentially pathogenic during pregnancy and responsible for *post partum* fever. In neonatology, it has been suggested in foetal hypotrophy, foetal death *in-utero* and chorioamniotitis.

Ureaplasma parvum is mainly a coloniser.

Mycoplasma genitalium (Mg) in men is the second most common cause of NGU, after *Chlamydia trachomatis*, and is believed to be responsible for 15 to 25% of NGU cases. *M. genitalium* is sexually transmitted and can in particular cause recurrent or persistent NGU. In women, it is the only mycoplasma responsible for cervicitis and urethritis, and may also produce upper tract infections such as endometritis and salpingitis. Sexual transmission is likely, but may be non-specific. Oral and rectal transmission also seem to be possible.

SEARCH INDICATIONS

Uu: Chronic and/or recurrent urethritis in men and urinary lithiasis; in pregnant women and *post partum* for repeated miscarriages and neonatal infections.

Mh: Suspicion of vaginosis, salpingitis or pyelonephritis in women, *post partum* fever and neonatal infections. No indications in men.

Mg: In men with urethritis, not caused by gonococcus nor *Chlamydia trachomatis*, which is not cured by conventional anti-Chlamydia treatment (doxycycline for 8 days). In women with suspected urethritis, cervicitis, endometritis or salpingitis.

INFORMATION

SAMPLES

Serology: A serum sample is required. It is not necessary for the patient to be fasting.

Culture: On a urethral specimen or a first flow of urine in men; in women, on a cervical (cells from the endocervix) or urethral sample. In newborn babies, an endotracheal or gastric fluid sample is required.

SAMPLE STORAGE AND TRANSPORT

Serum: serum can be stored for one week at + 4°C. For longer periods, freeze at – 20°C.

Urine, vaginal and urethral samples, sperm, laparoscopy liquid, etc.: Swab samples require a transport medium specific to mycoplasmas. Liquid samples are stored as such and transported at + 4°C.

DIAGNOSTIC METHODS

CULTURE

Mh and Uu: A medium containing cholesterol and yeast extracts. The liquid primary culture medium (acid pH) is incubated for 16 to 20 hours at 37% and then transferred to an agar medium incubated at 37°C under 10% of CO_2. The Mh colonies are observed after 2 to 4 hours under a low-power microscope, where they have a typical "fried egg" appearance. Colonies of Uu appear in approximately 48 hours and brown in colour and shaped like sea urchins. In a liquid medium, their growth causes an indicator to change colour, reflecting the alkalinisation of the medium due to hydrolysis of urea by Uu and hydrolysis of arginine by Mh. Semi-quantification is possible.

PCR: Direct diagnosis of Mg in urinary, urethral, cervical samples, etc. Due to the fact that its culture is time-consuming (2 to 3 weeks) and difficult (a complex acellular medium enriched with serum (SP4 medium, modified Hayflick) under CO^2 at 37°C). No kits are commercially available, but there are several types of "improvised" PCR e.g. qualitative, real-time and multiplex. Multiplex kits should become available in 2010 for the joint detection by PCR of C. *trachomatis*, Mg and *N. gonorrhoae*.

SEROLOGY

Metabolic inhibition or ELISA

INTERPRETATION

Culture

Samples	Threshold
Normal sterile samples (joint fluid, upper genital sample)	None. Isolation of Mh or Usp is a sign of infection
In men: in the urethra and sperm	U.sp ≥ 10^4 UCC/ml
in the first flow of urine	U.sp ≥ 10^3 UCC/ml
	Mh with no pathogenic capability
In women (unless pregnant): cervicovaginal	Mh ≥ 10^4 UCC/ml: vaginosis (essentially). U. sp with no pathogenic capability
In newborn babies endotracheal or gastric fluid	U.sp ≥ 10^3 -10^4 UCC/ml

**UCC: units changing colour*

If the species is considered to be pathogenic in the sample under consideration, it is essential to study the sensitivity to antibiotics, in particular to tetracyclines, due to possible acquired resistance, which has been the case in the recent past of 20% of Mh and 3% of U sp. in France (45% of U sp. in USA).

PCR: M. genitalium

Qualitative PCR is usually sufficient to diagnose Mg infection in men, in a context of non-gonococcal urethritis, if the infection is symptomatic and the urethral sample swab shows more than 5 polynuclear neutrophils (PNN/field (x1000)) or, in a centrifuged sample of first urine flow, more than 10 (PNN/field (x1000)).

The sensitivity and specificity of PCR are acceptable, but difficult to evaluate with respect to the culture. False negatives can occur (inhibitors, transport and storage); false positives are very rare. A study carried out in women on the basis of cervicovaginal samples showed that in 31 positive cultures, PCR was positive 12 times i.e. in 38.7% of cases (mediocre sensitivity). It became positive, however, in 80.7% of cases during monitoring from 17 to 39 months, raising the question of the need to carry out repeated PCR measurements in order to reach a conclusion.

Serology: In cases of mycoplasma infection, specific antibodies appear in the bloodstream at around the 7th day and persist for several months. Interpretation of the serology necessitates a comparison of results from two blood samples taken 15-20 days apart. A significant rise in the antibody level between these samples tends to confirm a diagnosis of active Mycoplasma infection, when these micro-organisms have been isolated in culture and/or in upper tract infections. Serological tests are now, however, almost solely of epidemiological interest, in studying and analysing the role of Mycoplasmas in different pathologies. They may also be useful in retrospective diagnosis of these infections (be aware of possible cross-reactions between the various species, particularly with *Mycoplasma pneumoniae*).

TREATMENT

The consensual treatment of NGU's is tetracycline for 7 days or a single dose of azithromycin, although numerous cases of treatment failure have been observed, particularly with tetracyclines. A recent study (Mena and coll., CID 2009) showed that, in men with an NGU caused by *Mycoplasma genitalium* treated with doxycycline for 7 days, therapeutic failure was observed in 55% of cases at the follow-up consultation after 7 days (PCR +) and, in those who received 1g of azithromycin, 13% of failures were observed at the follow-up consultation, with 47% of relapses at 6-8 weeks.

In late 2009, the efficacy of azithromycin with an initial dose of 500 mg at D1 followed by 250 mg/d from D2 to D5, was evaluated at 96%. If azizthromycin fails, moxifloxacin should be used at 400 mg/d for 10 days (100% efficacy).

FOR FURTHER INFORMATION

- Baseman J.B., Cagle M., Korte J.E. et *al*., *Diagnostic assessment of Mycoplasma genitalium in culture-positive women*, J Clin Microbiol 2004 ; 42 : 203-11.
- Boudry P., *Mycoplasmes urogénitaux : implications en pathologie humaine*, Louvain Med 1998 ; 117 : 128-41.
- Dupin N., Janier M., Alcaraz I., *Mycoplasma genitalium*, *Communication orale*, Journées Dermatologiques de Paris, 2004.
- Bebear C. Communication orale, RICAI 2009.

VALPROIC ACID

DEFINITION

Valproic acid is a molecule with anti-epileptic activity and it is marketed as the acid itself as well as sodium salt and sodium divalproate.

Valpromide or dipropylacetamide, the amide of valproic acid, is prescribed for the treatment of bipolar disorder. Valpromide is a pro-drug which is metabolised to yield valproic acid, the active compound.

INDICATIONS FOR MEASUREMENT

The equilibrium concentration will vary according to the dose administered and the subject's individual characteristics. It is assayed in the blood to check the dosage is effective, to preclude overdose, and to allow a better dosage adaptation.

This test is ordered if:

– Treatment is proving ineffective.

– There is evidence of overdose, e.g. loss of muscle tone, impaired reflexes, miosis, nystagmus, drowsiness, confusion, and ataxia; or in the event of acute poisoning with calm, hypotonic coma and reduced respiratory autonomy.

– When concomitant medications or special clinical circumstances might be affecting valproic acid metabolism:

a) If taken concomitantly, the following medications may reduce the concentration of valproic acid in the blood (thereby increasing the risk of epileptic seizures):

– 0ther anti-epileptic drugs: carbamazepine, phenobarbital, phenytoin, primidone,

– Mefloquine,

– Aztreonam, imipenem.

b) The following circumstances can reduce the fraction of valproic acid that is bound to plasma albumin, thereby increasing the active free fraction and entailing a risk of overdose:

– Renal failure,

– Jaundice (competition with bilirubin),

– A low blood albumin concentration (malnutrition, renal leakage, etc.),

– When taken concomitantly with other medicinal substances that strongly bind to plasma proteins, such as salicylate, non-steroid anti- inflammatory drugs, carbamazepine and phenytoin.

– Elderly subjects and pregnant women (increases in the free fraction in the bloodstream).

INFORMATION

SAMPLE

Serum or plasma. Samples must be taken with the subject fasting, ***before the drug is taken*** and always at the same time of day (residual level). Pharmacokinetic equilibrium should be reached after 5 half-lives, i.e. 3-4 days. If an overdose is suspected, take the sample when symptoms onset.

ESSENTIAL INFORMATION

Any order for a drug assay should include the reason the drug was prescribed (for efficacy or toxicity evaluations); the time at which the test sample was taken; the date of the beginning of the course of treatment and/or dates on which the dosage was changed; dosing details (administered dose, frequency, route of administration); and, whenever possible, the subject's age, height and weight.

QUESTIONS FOR THE PATIENT

– Are you taking any of the following medicinal products? Carbamazepine, phenobarbital, phenytoin, primidone, mefloquine, aztreonam, imipenem, salicylate, non-steroid anti-inflammatory drugs.

– Are you pregnant?

SAMPLE STORAGE AND TRANSPORT

Decant off the plasma or serum as soon as possible.

Storage: 24 hours at room temperature or 7 days at between 2°C and 8 °C; beyond that, store frozen at –20°C.

Transport: at +4 °C or frozen.

ASSAY METHODS

Although chromatographic methods (HPLC or gas phase chromatography coupled with mass spectrometry) are considered as "comparison methods" (in the absence of any reference method), they are not in routine use.

The usual assays are based on competitive immunological methods in homogenous or heterogeneous phase.

Only a simple total valproic acid assay is routine; free fraction assays require a specialist laboratory.

NORMAL EXPECTED VALUES

The therapeutic window is between 50 and 100 µg/ml (347-693 µmol/l). Concentrations of over 200 µg/ml (1386 µmol/l) are considered toxic.

Conversion factors: 1 µg/ml = 6.9348 µmol/l

1 µmol/l = 0.1442 µg/ml.

FOR FURTHER INFORMATION

■ Cahier BIOFORMA N°18 (janvier 2000), *Dosage des médicaments*, tome II, (Chapitre valproic acid, A. Mialon).

■ Dictionnaire Vidal®.

VANCOMYCIN

DEFINITION

Vancomycin is a glycopeptide antibiotic with an antibacterial spectrum of activity limited to aerobic and anaerobic Gram positive bacteria. It is a time-dependent antibiotic with bactericidal activity except against *Staphylococcus haemolyticus*. It is mostly used to treat severe multi-resistant Gram positive bacteria, particularly methicillin resistant Staphylococci. As a result, it is reserved for hospital use.

METABOLISM

Vancomycin is almost entirely unabsorbed (< 5%) after oral administration. It is, nevertheless, used orally in the local treatment of *Clostridium difficile* pseudomembranous colitis. It is administered intravenously for systemic action. It has an average half-life of 6 hours, although this may vary considerably particularly in children. It is 30 to 60% bound in the circulation to plasma proteins, mostly to albumin. It is not particularly metabolised by the liver (< 5%) and is principally removed unchanged in urine.

INDICATIONS FOR MEASUREMENT

Vancomycin is administered as a discontinuous intravenous infusion (slow infusion of 45 to 60 minutes, 2,3 or 4 times daily: 20 to 40 mg/kg/day in adults; 40 mg/kg/d in children or, increasingly often, as a continuous infusion with an initial dose of 2 g/day in adults without renal insufficiency). Because of the risk of toxicity (ototoxicity and particularly nephrotoxicity) and the severity of the infections for which it is generally used to treat, plasma vancomycin measurements are used to confirm that the dosage is effective but not in the toxic range.

Measurement is indicated together with serum creatinine when treatment is started, once steady state has been reached (i.e. from day 2 of treatment) and 48 hours after each change in dose to adjust the dose particularly in the following circumstances:

– in patients suffering from renal insufficiency, the elderly and the newborn: the clearance 1/2 life of vancomycin is increased;

– in dialysis patients;

– in obese patients: the clearance half-life of vancomycin is reduced;

– in pregnant women: the volume of distribution is increased;

– in burns patients: clearance is increased;

– when used concomitantly with other potentially nephrotoxic drugs such as the aminoglycosides;

– in oncology.

INFORMATION

SAMPLE

Preferably into a dry tube (serum) or heparinised or EDTA tubes. Avoid tubes with gel (risk of adsorption).

Take the sample once steady state has been reached, i.e. on day 2 of treatment or 48 hours after each change in dose:

– at any time with a continuous infusion (the most common situation);

– within half an hour before the next dose for measurement of trough concentrations;

– 45 to 60 minutes after the end of the infusion to measure the peak (contralateral arm to the infusion).

The method of administration generally recommended for this antibiotic is by continuous infusion as vancomycin is time-dependent . Because of this its bactericidal activity is improved when the contact time with the organisms on the infected site is increased.

ESSENTIAL INFORMATION

– Any request for drug measurement must include: the reasons for the request (testing for efficacy or toxicity), the sampling time, treatment start date and/or any change in dosage, dosage information (amount administered, frequency and route of administration) and the age, height and weight of the person whenever possible.

– Current treatments: particularly aminoglycosides (increased nephrotoxicity).

SAMPLE STORAGE AND TRANSPORT

Separate serum or plasma within 3 hours of sampling.

Store at + 4°C, 24 hours; or freeze at -20°C beyond this time.

Vancomycin is poorly stable at room temperature: vancomycin hydrochloride is converted into an inactive degradation product, CDP-1 (*vancomycin crystalline degradation product*). This compound can interfere with some assay methods. Vancomycin concentrations are also over-estimated in patients with renal insufficiency.

ASSAY METHODS

Mostly immunological methods: FPIA, EMIT and immunoturbidimetry.

NORMAL EXPECTED VALUES

CONTINUOUS INFUSION

The therapeutic range is theoretically between 20 and 40 mg/l. In severe infection, particularly with organisms that have a "borderline" MIC in the region of 4 to 8 mg/l for vancomycin, the preferred effective range is 35 to 45 mg/l, with close monitoring of serum creatinine and at least weekly vancomycin measurement.

DISCONTINUOUS ADMINISTRATION

– The "expected" trough concentration (30 minutes before starting the infusion) is between 5 and 20 mg/l. In order to be permanently active on the organism responsible for infection the trough concentration must be at least 4 times

the MIC of the organism. In order to be effective for example on a Staphylococcus aureus with an MIC of 2 mg/l, a trough vancomycin concentration of at least 8 mg/l must be achieved. In patients with severe infection, a concentration of 6 to 8 times the MIC may be recommended, i.e. a trough concentration of 10 to 20 mg/l. The trough concentration in Staphylococcus aureus endocarditis must be kept > 20 mg/l.

– The expected peak concentration is between 20 and 40 mg/l. In practice this is of limited use in vancomycin monitoring as it is a time-dependent antibiotic.

– Concentrations of > 50 mg/l (peak) or > 20 mg/l (trough) are associated with an increased risk in toxicity.

FOR FURTHER INFORMATION

■ Meley R., *Dosage des médicaments*, tome II, Cahier BIOFORMA N° 18. Paris, janvier 2000, chapitre vancomycine : 125-135.

■ Dictionnaire Vidal®.

VANYLMANDELIC ACID

DEFINITION

Adrenaline and noradrenaline are broken down into vanylmandelic acid (VMA) by the combined action of catechol-O-methyltransferase (COMT) and monoamine-oxidase (MAO). COMT is a cytoplasmic enzyme that is found in many tissues, notably the liver. MAO is a mitochondrial enzyme, also found in many different tissues, notably the adrenal medulla. Free VMA is excreted in the urine.

Overproduction of either adrenaline or noradrenaline results in a corresponding increase in the amount of VMA in the urine.

Synonym: VMA.

INTRODUCTION

A urine VMA assay is indicated for the diagnosis and monitoring of pheochromocytoma and neuroblastoma.

A pheochromocytoma is a catecholamine-secreting tumour of chromaffin tissue in the adrenal glands. It is benign in 90% of cases because these cells are not immature. The symptoms are caused by the excess catecholamines and present as hypertension, sweating, pallor and headache. It is advisable to investigate the possibility of other, intercurrent endocrine tumours (thyroid cancer). Laboratory tests are performed to measure the levels of amines (noradrenaline and adrenaline), methoxyamines (normetanephrine and metanephrine) and VMA. VMA levels can, in some cases, remain within the normal range.

Neuroblastoma is the most common tumour in children under five and is the second cause of death in this group, after accidents. It is a malignant tumour of relatively undifferentiated sympathetic nervous cells. The symptoms do not help a great deal with diagnosis. Dopamine is produced in inappropriate tissues, and this dopamine is broken down by dopamine hydroxylase into noradrenaline and then adrenaline. This is why assaying VMA is useful. The rate of VMA excretion in the urine is markedly elevated in more than 70% of children with neuroblastoma.

INDICATIONS FOR MEASUREMENT

Most VMA tests are ordered for the diagnosis and monitoring of tumours:

– Diagnosis and monitoring of pheochromocytoma in adults.

– Diagnosis and monitoring of neuroblastoma in children.

INFORMATION

SAMPLE

In the laboratory, the 24-hour urine collection should be acidified using 6 N HCl (1 ml per 100 ml sample) to adjust the pH of the urine to between 2 and 3. If the pH is too low (<1), the molecules of interest will not be extracted efficiently so their concentration will be underestimated.

24-hour urine collection being complicated in children, a simple urine sample can be assayed and the VMA concentration normalised with respect to the sample creatinine concentration.

QUESTIONS FOR THE PATIENT

Ask about how well the patient has complied with the dietary recommendations for the 48-hour period prior to the urine collection, i.e. reduced consumption of coffee, tea, vanilla, chocolate, tomatoes and citrus fruit; bananas should be completely excluded from the diet.

In addition, it is always advisable to ask about current medications.

If the patient takes adrenergic derivatives (bronchodilators, nasal decongestants, levodopa, nifédipine) and enzyme inhibitors (MAOI and tricyclic antidepressants, clonidine, disulfiram), the results can be overestimated then, to avoid errors in the assay, they have to stop their treatment 5 days before.

STORAGE AND TRANSPORT

VMA is unstable (due to its phenol and catechol grouping) but, if the sample is acidified, it can be stored and transported at +4°C. Urine collected on 10% disodium EDTA can be also stored at –20 °C.

ASSAY METHODS

High performance liquid chromatography is the reference method. It is used with an electrochemical detection system. Several column purification runs are necessary because 90% of catecholamines are in conjugated forms. Urine acidification with hydrochloric acid liberates free, assayable forms.

NORMAL EXPECTED VALUES

For reference:

Adults: < 8 mg per 24 hours, i.e. < 33 µmol/24 hours.

PHYSIOPATHOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

The mean concentration varies with age.

Age	mg/24 h	µmol/24h
0 – 7	< 3	< 15
7 – 15	< 4	< 20

PATHOLOGICAL VARIATIONS

In children with neuroblastoma, elevated urinary homovanillic acid (HVA) is often associated with an increase in VMA. Assaying dopamine and phenolic acids in the urine affords 95% diagnostic sensitivity with the other 5% corresponding to non-secretory forms. Predominant HVA secretion is a poor prognostic indicator. Certain rare neuroblastomas (ganglioneuroblastomas) secrete adrenaline and noradrenaline with more VMA than HVA.

In pheochromocytoma, urinary VMA excretion is elevated but this phenomenon is variable. Performing a methoxyamine (normetanephrine and metanephrine) assay in parallel to the VMA assay enhances sensitivity, bringing it close to 100%. For postoperative monitoring after surgical removal of a pheochromocytoma, the VMA concentration should return to normal; any rise thereafter points to relapse.

Minor elevations in VMA have been observed with certain other tumours, namely thyroid cancer, tumours of the small intestine and retinoblastoma.

FOR FURTHER INFORMATION

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■ Candito M., *Diagnostic biochimique du phéochromocytome et du neuroblastome*, Ann Biol Clin, 2002; 60: 15-36.

VARICELLA ZOSTER

DÉFINITION

Varicella zoster virus (VZV) is a strictly human virus belonging to the *Herpesviridiae* family (*Herpesvirinae* sub-family). It is an enveloped DNA virus 150-200 nm in size with an icosahedric capsid that is very fragile outside of the human body. It is transmitted by aerial spread (saliva droplets) or by skin lesions, which contain large numbers of viruses. It is a dermo-neurotropic virus. Primary infection causes varicella, an almost obligatory disease of childhood as it is highly contagious (more than 90% of adults have anti-VZV antibodies). Virus spreads through the neuronal system/blood stream reaching sensitive lymph nodes where it remains latent throughout life. A fall in the specific VZV immune response over time may result in reactivation of the virus, which passes through the nervous system to the epidermis producing a localised skin rash, known as shingles.

Synonym VZV: human herpes virus 3.

SYMPTOMS

PRIMARY VARICELLA INFECTION

Varicella occurs as small family or school epidemics, usually in spring. Following an incubation period of 15 to 21 days it produces a vesicular rash preceded variably by fever for one or two days and a fleeting scarlatina rash beginning on the trunk and then extending over the whole body (including the scalp and mucosal membranes). The rash lasts for around ten days and progresses in flares 2 to 4 days apart. Crusts begin to develop after 4 to 6 days and may leave scarring if scratched. The contagious period (through saliva) begins a few days (2 to 5) before the rash and reaches a peak during the vesicular period, persisting until the lesions are fully healed.

Primary infection is almost always symptomatic.

Complications are rare: particularly bacterial (*S. aureus*), neurological: lymphocytic meningitis, cerebellar ataxia (under 5 years old), Reye's syndrome (acute encelopathy with organ steatosis), myelitis and radiculitis.

A number of post-infectious neurological complications may however occur probably as a result of an auto-immune mechanism, although particular changes are seen in different situations:

– immunosuppressed children and the newborn (< 6 months old): the rash is persistent and may cause haemorrhagic lesions. The virus may spread to the lungs, liver and brain (encephalitis), with a mortality of 10 to 30%;

– adults: severe intense rash with pneumonia in 20 to 30% of cases;

– pregnant women: pneumonia in 40% of cases with a mortality rate of 10%.

Reinfection is rare and the symptoms of the disease are then usually mild.

REACTIVATION: SHINGLES

This occurs in 15% of infected people and is particularly common in immunosuppression (intercurrent diseases and treatments) but may also develop without an apparent cause, particularly in those over 50 years old (shingles in a child or young immunocompetent adult should lead to investigation for AIDS).

The rash is preceded by fever with satellite lymphadenopathy and neuralgia. The vesicular rash is very characteristic and painful, unilateral and over the territory of a nerve route, progressing over 15 days. It is usually intercostal but may also affect the conjunctiva (ophthalmic shingles which may be severe with uveitis and keratitis).

The complications include post-herpetic neuralgia at least a month after the onset of the rash lasting for up to a year together with facial paralysis. Shingles generally does not recur.

Chronic shingles may occur in the immunosuppressed together with disseminated forms with worrying organ involvement causing hepatitis, pneumonia or encephalitis.

VERTICAL MOTHER TO CHILD TRANSMISSION

This is due to maternal primary infection. As VZV seroprevalence is very high in women of child-bearing age (> 90%), the risk of varicella during pregnancy is low, 0.7/1000.

Transmission may occur in 8% of cases and the risk of embryofoetal disease is 2% before 20 weeks of pregnancy: this is **congenital varicella** (skin, ocular, mucosal, skeletal and neurological damage with microcephaly and mental retardation). The disease appears not to be serious between 20 and 38 weeks of pregnancy, although if it develops during the last week before delivery (when it is transmitted in 25 to 50% of cases) up to 2 days after delivery, the newborn child is at major risk of malignant varicella (severe potentially fatal lung disease with generalised rash) as maternal antibodies are transferred 5 to 6 days after the rash in the mother.

Shingles in pregnant women has no impact on the foetus.

INDICATION FOR LABORATORY DIAGNOSIS

The diagnosis of acute infection is very often clinically apparent and virology testing is not required. Testing however becomes essential in serious or atypical forms of the disease (differential diagnosis from herpes in the immunosuppressed).

The biological diagnosis is also useful:

– to determine the serological status of a pregnant woman or vaccination candidate;

– in antenatal diagnosis (which requires consent);

– to examine the sensitivity of strains of the virus during treatment.

INFORMATION

- **Culture/direct diagnosis of viral antigens:** fluid from the vesicle and cells from the base of the lesion by swabbing the lesions: use a virology transport medium for culture. Other samples may be cultured: ocular samples, CSF, or blood collected into EDTA. Samples must be transported promptly

at + 4°C as the virus is very fragile. If the analysis is to be delayed, the sample should be frozen at – 70°C in liquid nitrogen. In order to detect viral antigens, send the swab or swab spread on a slide for IF methods.

- **Molecular biology:**

  CSF in meningitis / encephalitis: frozen at – 20°C.

  Vesicle fluid and ocular fluid (aqueous or vitreous humour) + 4°C.

  Whole blood collected into EDTA: frozen at – 20°C.

  Possibly, various skin or biopsy samples: + 4°C.

  Amniotic fluid for antenatal diagnosis (molecular biology): + 4°C.

- **Indirect diagnosis:**

  Serum (5 ml of whole blood collected into a dry tube): may be stored for a few days and transported at + 4°C.

BIOLOGICAL DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

- **Detection of viral antigens in the sample:**

  – usually by immunofluorescence after spreading on a slide and fixing with acetone;

  – immunoperoxidase methods.

  These techniques are sensitive if the sample is rich in virus.

- **Isolation of the virus on cell culture:**

  MRC5 fibroblast cells generally inseminated from samples taken from vesicles; the diagnosis is based on finding a cytopathogenic effect after 5 to 10 days (cell ballooning) and confirmation with monoclonal antibodies (IF or immunoenzymology).

- **Detection of nucleic acids by molecular biology:**

  The PCR method is extremely sensitive and fast: VZV is tested for directly in CSF in neurological disease associated with varicella or shingles even if no rash is present.

  This test can also be used on amniotic fluid samples or ocular/skin samples particularly in immunosuppressed patients already receiving anti-viral treatment.

  NB: the viraemia is too fleeting to be commonly detected.

INDIRECT DIAGNOSIS

- **Detection of specific antibodies:**

  Usually on serum; on CSF in parallel with serum when investigating for intrathecal synthesis in encephalitis, meningitis or myelitis. Production however may only be detected one to two weeks after the onset of symptoms.

  Immunoenzymatic methods to detect IgG and IgM are the most commonly used. Antibodies develop approximately 5 days after the onset of the varicella rash and seroconversion demonstrates primary infection. In shingles, serology often shows high IgG concentration associated with variable IgM (generally lower than in primary infection). IgG is used to define the patient's immune status for VZV as it persists for many years: it can therefore be used to identify at risk people.

  If a contact history is present in a pregnant woman the patient can be reassured if serology is positive prior to any rash developing.

ANTENATAL DIAGNOSIS (FOETAL INFECTION)

The diagnosis is based primarily on finding the viral genome by PCR in amniotic fluid. Before the amniocentesis the mother must be checked to ensure that the PCR is negative in her blood to avoid iatrogenic contamination and a period of at least 4 weeks must be left between the rash and the amniocentesis.

PREVENTION

VACCINE

– Vaccine: attenuated virus has been available since the end of 2004. There are five therapeutic indications for the vaccines available.

1 - Post-exposure vaccine within 3 days following exposure to a patient with a rash in immunocompetent adults with no past history of varicella.

2 - Children with no past history of varicella and negative serology.

3 - Candidate solid organ transplant recipients within six months prior to transplantation.

4 - Any person with no past history of varicella and negative serology in close contact with immunosuppressed people.

5 - Entry to 1st year of medical and paramedical studies for students with no past history of varicella and negative serology.

CHEMOPROPHYLAXIS

Acyclovir: in newborn babies whose mother has had varicella within 5 days before delivery or 2 days afterwards; bone marrow transplantation in the maximum immunosuppression phase and in pregnant women to prevent congenital varicella only before 20 weeks of pregnancy.

TREATMENT

– Mild form: local disinfection of lesions with a hexamidine antiseptic.

– Serious disease: acyclovir IV (10 mg/kg 3 times per day for 10 days).

– Painful shingles: valacyclovir (the prodrug of aciclovir) orally for 7 days (3 g/day).

– Neonatal varicella: acyclovir IV (+ immunoglobulins) from birth in cases of primary maternal infection less than 5 days before delivery or up to 2 days afterwards (if the time between maternal rash and delivery > 5 days, monitor the child as varicella does not always develop).

FOR FURTHER INFORMATION

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VERY LONG-CHAIN FATTY ACIDS

DEFINITION

Very long-chain fatty acids (containing 22 or more carbon atoms) are broken down in peroxisomes which are intracellular organelles inside which hydrogen peroxide is generated. The successive steps of beta-oxidation in this pathway are catalysed by a series of enzymes and deficiency in any one of these causes disease (see Table 1). Other syndromes; the prototype of which is cerebrohepatorenal syndrome (Zellweger's syndrome) are due to more global deficiencies in this system.

Synonym: VLCFA.

Disease	Deficient protein
X-linked adrenoleukodystrophy	Transport protein (*ABCD1 gene*)
Neonatal pseudo-adrenoleukodystrophy	Acyl coA oxydase
Bifunctional enzyme deficiency	Enoyl-CoA hydratase/3-hydroxyacyl-CoA dehydrogenase
Pseudo – Zellweger's	Thiolase

Table 1: diseases due to deficiencies in enzymes in the peroxisomal pathway for the beta-oxidation of very long-chain fatty acids.

INTRODUCTION AND INDICATIONS FOR MEASUREMENT

X-linked adrenoleukodystrophy (ALD) is characterised by progressive demyelinisation in the central nervous system combined with adrenal insufficiency (Addison's disease). Paediatric forms manifest at between 5 and 12 years of age with gradually deteriorating symptoms, ranging from a decrease of visual acuity, loss of hearing, to cerebellar ataxia, hemiplegia and dementia.

The adult-onset form usually manifests at between 20 and 45 years of age as neurological and/or adrenal signs. The disease is due to a mutation in the *ABCD1* gene which encodes a protein that transports very long-chain fatty acids. The other conditions in Table 1, due to enzyme deficiencies, are much more rare.

Cerebrohepatorenal syndrome manifests *in utero* with developmental abnormalities and facial deformities accompanied by a broad range of symptoms including neurological and renal problems as well as growth disorders. Peroxisome function is impaired resulting in the build-up of not only very long-chain fatty acids but also the precursors of bile acids, certain dietary acids (notably phytanic acid) and plasmalogen precursors. Two other, very rare genetic disorders related to this syndrome have been described, namely neonatal adrenoleukodystrophy (in which there are no peroxisomes in the liver) and infantile Refsum's disease (in which phytanic acid builds up in the tissues). The genetic mutations responsible for these diseases have been identified.

Increased levels of very long-chain fatty acids are found in the plasma of subjects with all of the above-mentioned diseases.

INFORMATION

SAMPLE

Ideally, the subject should be fasting before giving blood (which is to be drawn onto EDTA or heparin).

SAMPLE STORAGE AND TRANSPORT

The plasma can be kept at room temperature for 2 weeks. Beyond that, freeze the sample and keep at –20°C.

ASSAY METHODS

Lipid is extracted from the plasma and the fatty acids are converted to their methyl esters. VLCFAs are then analysed using gas phase chromatography, usually coupled to mass spectrometry.

REFERENCE VALUES

Results are expressed as the ratio of C26:0 acids to C22:0 acids.

In normal subjects, this ratio is less than 0.02 and any increase over this threshold is evidence of impaired beta-oxidation of very long-chain fatty acids, which is characteristic of most hereditary peroxisomal disorders. Differential diagnosis of the various disorders described above depends on an analysis of the clinical picture together with measurements of the plasma levels of bile acid precursors and, eventually, enzyme activities, as well as tests to look for mutations in certain genes.

FOR FURTHER INFORMATION

■ Wanders R.J., Waterham H.R., *Peroxisomal disorders: biochemistry and genetics of peroxisome biogenesis disorders*, Clin Genet 2005; 67: 107-133.

VIP

DEFINITION – INTRODUCTION

VIP is a neuropeptide, which stimulates gastro-intestinal motility and exocrine pancreatic and intestinal secretions. Patients should be investigated for raised plasma concentrations when they are suspected of having a pancreatic neuro-endocrine tumour (VIPoma) or in the context of type 1 multiple endocrine neoplasia or Wermer's syndrome (MEN1 or OMIM 131100), or in watery diarrhoea with hypokalaemia (Verner-Morrison syndrome). MEN1 is an association of a VIP-secreting endocrine pancreatic tumour (VIPoma) with parathyroid, anterior pituitary, adrenocortical and diffuse neuro-endocrine tissue tumours (gastro-intestinal or thoracic).

Pancreatic tumours may involve the exocrine pancreas or endocrine tissue. Most exocrine pancreatic cancers are adenocarcinomas generally affecting those over 50 years old and carrying a poor prognosis. Endocrine pancreatic tumours present clinically by excess secretion of the hormone in question: hypoglycaemia with insulinoma, Zollinger-Ellison's syndrome with gastronoma or profuse water diarrhoea with hypokalaemia ("pancreatic cholera" or Verner-Morrison syndrome) with VIPoma.

Synonym: Vaso-active intestinal peptide.

INDICATIONS FOR MEASUREMENT

Suspected VIPoma suggested by profuse diarrhoea (up to 10 litres per day) and persistent water diarrhoea associated with hypokalaemia.

INFORMATION

SAMPLE

Plasma EDTA containing aprotinine, taken from a fasting patient.

SAMPLE STORAGE AND TRANSPORT

Centrifuge immediately at + 4°C for 15 minutes at 760 g, separate into a dry polypropylene tube and freeze at – 20°C within an hour of sampling.

Transport: frozen at – 20°C.

ASSAY METHODS

Radio-immunoassay.

NORMAL EXPECTED VALUES

For reference: < 65 ng/l.

PATHOLOGICAL VARIATIONS

- **Raised plasma VIP concentrations in watery diarrhoea associated with hypokalaemia.**

  VIP is raised in 90% of cases of VIPoma. Normal serum VIP concentrations do not exclude the diagnosis as the neuropeptide may not be secreted constantly.

  Differential diagnosis: other neuro-endocrine tumours associated with diarrhoea, such as the Zollinger-Ellison syndrome and carcinoid tumours.

  The diagnosis of VIPoma must be confirmed with imaging (endoluminal, pancreatic endoscopy or scintigraphy). The tumour is ectopic in approximately 10% of cases lying in the neuronal ganglia, jejunum, oesophagus, adrenal medulla or bronchi; metastases are found in more than half of the cases, mostly in the liver or lymph nodes.

  The emergency treatment is rehydration; a somatostatin derivative is then added to reduce VIP secretion followed by surgical excision of the tumour.

- **Increases in other diseases**

  In 3% of non-neoplastic chronic diarrhoea.

  Chronic renal insufficiency.

  Pre-eclampsia.

  Meningococcal septicaemia.

  Pheochromocytoma.

FOR FURTHER INFORMATION

■ Blondon H., *Hépato-gastro-entérologie – Vasoactive intestinal peptide (VIP)*. In : Kamoun P., Fréjaville J.-P., Guide des examens de laboratoires, 4e Ed. Médecine-Sciences, Flammarion, Ed. Paris, 2002 : 926.

VIRAL HAEMORRHAGIC FEVERS

DEFINITION

The viral haemorrhagic fevers (VHF) are caused by RNA viruses belonging to 4 families: *Flaviviridae, Bunyaviridae, Filoviridae and Arenaviridae*. They are responsible for thousands of cases of infections worldwide and produce a common clinical picture characterised by a haemorrhagic syndrome of variable severity.

INTRODUCTION

EPIDEMIOLOGY

The geographical distribution of these viruses is variable (*see table*) and can be widespread or well localised. In France, the North-East of the country is involved with 4 localised epidemic foci within in the Ardennes forest, in Lorraine, in Franche-Comté and in a part of the Vosges. The Picardy region is an endemic area. The predominant responsible virus is *Puumala* but in some cases the Seoul virus has been implicated. Affected individuals are usually young men living in a rural situation.

The mode of transmission to man varies according to the virus and can involve invertebrate intermediate hosts (mosquitoes and ticks) or vertebrates (rodents) as a reservoir of the virus. No animal host is known for Marburg and Ebola viruses.

– ***Transmission by mosquitoes:*** For the amaril virus, Dengue Haemorrhagic Fever and Rift Valley Fever.

– ***Transmission by ticks:*** For certain Flaviviruses (Omsk, Kyasanur, andAlkhurma) and also for Crimean-Congo Haemorrhagic Fever.

– ***Transmission by rodents:*** For viruses of the *Arenavirus* family as well as for the Hantaviruses. Accidental transmission to man occurs via the respiratory route through inhalation of dust that has been contaminated by rodent excreta.

SYMPTOMS

Asymptomatic infection is common. The clinical syndrome of viral haemorrhagic fevers (VHF) has features in common, where the incubation period lies between 3 and 21 days and is followed by a flu-like febrile syndrome of sudden or progressive onset. There is a rapid deterioration in the general condition and the development of haemorrhagic manifestations, whose extent depends on the disease. The bleeding can be superficial, cutaneous or mucosal, and often limited (petechiae, ecchymoses epistaxis, gingival bleeding and conjunctival haemorrhage) and/or internal (haematemesis, haematuria and melaena). Sometimes there is no bleeding at all. Cardiovascular involvement can occur and is characteristic of the disease. It may result in shock syndrome. Other complications include dehydration, liver disease (hepatic coma), renal involvement and neurological problems. Mortality rate depends on the virus; it is often greater than 10% but can be very high as for Ebola virus (80%). Haemorrhagic fever with renal syndrome (HFRS) combines haemorrhagic fever with renal failure. The Hantavirus pulmonary syndrome (HPS) starts like a VHF or HFRS but proceeds to cough, dyspnoea and then acute pulmonary oedema, which is sometimes fatal. There are no haemorrhagic signs.

SEARCH INDICATIONS

Diagnosis of VHF in someone with appropriate symptoms who has returned from an endemic area.

INFORMATION

SAMPLE

This is determined by the clinical context:

– Serum: If there is fever with a haemorrhagic syndrome.

– Whole blood and pleural fluid: Where there is a pulmonary syndrome or respiratory symptoms.

– Whole blood and CSF: If there is meningo-encephalitis.

– Other samples: Stool, urine, skin biopsy and *post-mortem* organ biopsies.

QUESTIONS FOR THE PATIENT

Clinical symptoms?

Possibility of a recent stay in endemic area?

Possibility of a mosquito or tick bite?

Possibility of exposure in wooded areas, in barns or other buildings which might harbour rodents?

Possibility of transfusion in an endemic country?

SAMPLE STORAGE AND TRANSPORT

As samples are potentially infective, they are transported in conformity with national and international legal safety provisions, such as triple packaging (UN norm 6.2). The package must have an information form, which furnishes identification data on the patient and clinical details with the date of the onset of symptoms, travel abroad in the last month and the exact address and identity of the referring doctor. For direct diagnosis the specimens should be sent to specialised laboratories which have the required safety facilities suitable for P3 or P4 isolation. Samples should be packed in ice for short journeys but in dry ice for longer journeys. Serum should be kept at + 4°C while waiting for transport. Transport of samples to a reference laboratory must happen with in 24 – 48 hours.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

Only specialised laboratories can perform viral isolation. The procedure is long and difficult and, in general, it involves the use of whole blood taken during a period of viraemia. Detection of the viral genome using RT-PCR is possible, using whole blood, urine, respiratory secretions, broncho-alveolar lavage, pleural fluid and biopsy material.

SEROLOGICAL DIAGNOSIS

The available techniques are: ELISA for detection of IgM and IgG (IgM is detected by immunocapture), indirect immunofluorescence and, for some viruses, seroneutralisation. Serological results must be interpreted carefully and should be confirmed by The National Reference Centre.

TREATMENT

Treatment is symptomatic in the absence of specific therapy, except for the use of ribavarin in treatment of Lassa Fever. As soon as a diagnosis is suspected precautionary measures should be put in place to prevent passage of infection via biological fluids. Prevention has an essential role. This includes measures against mosquitoes and also rodents. There are few vaccines. Some are subject to trials and others are in the process of being validated.

FOR FURTHER INFORMATION

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- Georges A.J., Georges-Courbot M.C., *Fièvres hémorragiques virales*, Encycl. Med. Chir. Elsevier, Paris, 8-063-A-10 ; 2005.
- Virus des fièvres hémorragiques (hors arbovirus), Revir. Chapitre 38 ; 2000: p137 - 139.

Family	Genus	Name of virus	Disease	Geographical distribution
Flaviviridae	*Flavivirus*	Amaril	Yellow Fever	Tropical America, sub-Saharan Africa
		Dengue (4 serotypes)	Dengue	Asia, America, Africa, Oceania
		Omsk	Omsk Haemorrhagic Fever	Siberia
		Kyasanur	Kyasanur Forest Disease	Indian sub-continent
Bunyaviridae	*Phlebovirus*	Rift	Rift Valley Fever	Africa
	Nairovirus	Crimea-Congo	Crimea-Congo Haemorrhagic Fever	Africa, Southern Eurasia
	Hantavirus	Hantaan	HFRS	China, Korea, Caucasus
		Seoul	HFRS	Widespread
		Puumala	Epidemic nephropathy	Western Europe
		Sin Nombre	HPS	USA
Filoviridae	*Filovirus*	Ebola	Ebola Haemorrhagic Fever	Sudan, Gabon, Rep of Congo, Ivory Coast
		Marburg	Marburg Haemorrhagic Fever	Uganda, Zimbabwe, Kenya, Angola
Arenaviridae	*Arenavirus*	Machupo	Bolivian Haemorrhagic Fever	Bolivia
		Junin	Argentinian Haemorrhagic Fever	Argentina
		Sabia	Brazilian Haemorrhagic Fever	Brazil
		Guanarito	Venezuelan Haemorrhagic Fever	Venezuela
		Lassa	Lassa Fever	Guinea, Liberia, Nigeria, Benin

VITAMIN A

DEFINITION

Vitamin A is a fat-soluble long chain alcohol found mostly in nature as fatty acid esters. Of the sixteen possible isomers, two are of practical importance: all-trans retinol (biologically the most active form) and the 11-cis isomer or neovitamin A (which is also biologically active). Vitamin A2 or 3-dehydroretinol has the same metabolism but only 30 to 40% of the activity of vitamin A.

Dietary sources of vitamin A are retinol esters from animals and carotenes or provitamins A from plants. The former are mostly found in fish liver oils, particularly cod liver oil, tuna, halibut and marlin liver oil, the liver of some mammals, milk, butter, cheese and eggs. Provitamins A (main compound: beta-carotene) are found in spinach, carrots, cabbage and in the pigments of some fruits (apricot, orange, marrow), but also in kidney, liver, spleen, milk, butter and full fat cheeses.

Daily requirements in human beings vary by age, sex, and undoubtedly with physical activity. They are in the region of 100 /day (NB: 1 µg of retinol = 3.3 IU = 6 g of beta-carotene). These doses can prevent the symptoms of deficiency, although they should be doubled or even tripled to ensure optimal intake. Requirements increase in pregnancy, breast-feeding and growth periods.

Synonyms: retinol, axerophthol.

INTRODUCTION

METABOLISM

Retinol is absorbed from the intestine as vitamin A alcohol, vitamin A esters or carotenes, which are then converted to vitamin A alcohol in the intestinal wall. Vitamin A is absorbed under the same conditions as fats. Once absorbed by the intestinal epithelial cells, beta-carotene is oxidised and hydrolysed into two molecules of retinal in turn producing retinol or retinoic acid. Long chain vitamin A esters are carried in the general circulation by the thoracic duct reaching a plasma concentration peak approximately 4 hours after administration. Blood concentrations fall gradually as vitamin A is stored, mostly in the liver küppfer cells (90% of the body's stores). Retinol is then released into the circulation by hydrolysis. In practice, until the liver is saturated, administration of vitamin A produces a rise in hepatic reserves rather than raised blood vitamin A concentrations. In the blood, vitamin A circulates bound to a specific protein (*retinol binding protein* or RBP), which is synthesised by the liver. The retinol-RBP complex binds to transthyretin (pre-albumin) and transports vitamin A to its target tissues.

Blood vitamin A concentrations therefore depend not only on vitamin A intake but also on sufficient production of RBP: any fall in the synthesis of retinol binding protein impacts on plasma retinol and conversely any vitamin A deficiency is associated with a fall in circulating RBP.

Twenty to 60% of absorbed retinol is degraded into different metabolites from retinoic acid. These are then removed in equivalent amounts in faeces and urine. Some metabolites excreted in bile undergo entero-hepatic cycling. The half-life of vitamin A is approximately 4 to 5 months.

BIOLOGICAL ROLES

After binding to a target cell membrane receptor, the retinol is internalised and then bound by an intracellular transport protein, *Cellular Retinol Binding Protein*. Vitamin A is contained in all tissues, particularly liver, kidney and pigmented retinol epithelium.

Vitamin A (as retinal) is involved in visual mechanisms acting through rhodopsin; in the skin, retinol and retinoic acid (a vitamin A derivative) are involved in normal epithelial cell differentiation promoting the production of mucopolysaccharides and mucus secretion or differentiation of cancer cells. Retinol also has an effect on growth and finally retinoic acid plays an important role in reproduction in both spermatogenesis and gestation and foetal development.

The symptoms of vitamin A deficiency in adults are hemeralopia (difficulty adapting visually to darkness, or night blindness), xerophthalmia with corneal opacification, which can progress untreated to blindness. In addition to the predominant ocular effects, resistance to infections is reduced and skin problems are seen (dry skin, follicular hyperkeratosis and sweat gland atrophy). Children also suffer growth retardation and develop recurrent bronchial infections.

In acute vitamin A poisoning (with doses of 100 times over the recommended doses), the main symptoms in adults are headache, dizziness and muco-cutaneous scaling. Overdose in infants causes raised intracranial pressure with bulging fontanels.

Chronic poisoning (doses of over 10 times the recommended dose for several months) produces systemic signs (asthenia, anorexia and insomnia), skin signs (dry scaly skin, erythema, eczema, pruritis, alopecia and fragile nails), neurological effects with visual disturbance and headache, joint damage with bone pain and radiological abnormalities showing increased bone resorption and long bone hyperostosis.

INDICATIONS FOR MEASUREMENT

– Investigation for vitamin A deficiency. Deficiency may be suggested by the clinical signs described above.

– Investigation for hypervitaminosis A, suspected from the symptoms listed above.

INFORMATION

SAMPLE

Venous sample collected into a dry tube or one containing heparin, wrapped in aluminium foil (kept away from light) and preferably placed on ice.

The sample must be taken after the person has been fasting for 12 hours.

SAMPLE STORAGE AND TRANSPORT

Whole blood, stored away from light for 24 hrs at + 4°C (or at room temperature), away from light (wrap the tube in aluminium foil): beyond 24 hrs, centrifuge the plasma or serum cold (+ 4°C) and freeze at – 20°C.

ASSAY METHODS

High performance liquid chromatography (HPLC) or direct fluorimetry.

NORMAL EXPECTED VALUES

These can vary depending on the method and between laboratories.

For reference: in adults: Vitamin A1 (retinol): 1.06 to 3.26 µmol/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Age-related: premature infants have lower serum vitamin A concentrations than term infants because of deficient hepatic maturation for the production of RBP. Serum Vitamin A concentrations increase regularly from birth to adulthood and are identical in adults and the elderly (in the absence of concomitant disease or malnutrition).

For reference: plasma retinol by HPLC:

– Newborn term: 0.45 to 2.36 µmol/l

– Premature 24 to 36 weeks: 0.06 to 1.04 µmol/l

– Children 3 to 5 years old: 0.61 to 1.29 µmol/l

– Children 6 to 8 years old: 0.70 to 1.93 µmol/l

– Children 9 to 11 years old: 0.90 to 2.00 µmol/l

– Children 12 to 13 years old: 0.83 to 2.32 µmol/l

– Children 14 to 16 years old: 0.99 to 2.90 µmol/l.

PATHOLOGICAL VARIATIONS

Hypovitaminosis A: 3 main causes

– Deficient intake, particularly in developing countries and aggravated by zinc deficiency. In Western countries vitamin A deficiency occurs in prolonged weight loss diets or may occur secondary to RBP deficiency (concentrations of RBP in blood fall rapidly in malnutrition and in inflammatory states).

– Reduced absorption: in chronic pancreatitis, cystic fibrosis, coeliac disease, Crohn's disease, ascaridiasis, lambliasis, and schistosomiasis.

– Defective use: alcoholic cirrhosis or post-hepatitis.

Hypervitaminosis A

This is due to excessive intake (animal liver, fish liver oil or prolonged self-medication). Vitamin A accumulates in tissues and causes toxic effects *(cf. above)*. The clinical features of overdose reverse when the vitamin is stopped.

Hypervitaminosis A may also be seen in patients suffering from diabetes, hypothyroidism, hyperlipidaemia, glomerulonephritis and chronic renal insufficiency (accumulation in blood of RBP which is normally filtered, reabsorbed and degraded by the kidney).

Hypervitaminosis A prolongs the bleeding times, increases the ESR and increases transaminases, alkaline phosphatase and serum calcium.

THERAPEUTIC USE OF VITAMIN A

Vitamin A is used therapeutically to prevent and correct deficiencies and also in dermatology practice (synthetic retinoids) to treat acne, psoriasis and various disorders of keratinisation.

FOR FURTHER INFORMATION

■ Thevenin M., *Vitamine A et bêta-carotène*, Cahier de formation Biochimie, Tome III, Bioforma, Paris 1996 :197-206.

■ Leboulanger J., *Vitamine A*. In : Les vitamines. Biochimie – Mode d'action – Intérêt thérapeutique. F. Hoffmann-La Roche et Cie Ed, Neuilly/seine, 1984 : 31-44.

VITAMINE B1

DEFINITION

Vitamin B1 is a water-soluble compound made up of a pyrimidine ring connected to a thiazole ring and containing a quaternary nitrogen atom. In its usual form as the hydrochloride, thiamine exists as odourless white crystals (when pure). Thiamine circulates in plasma in the free form or as the pyrophosphate. It is found in many tissues particularly in the liver, brain, kidney and heart. Tissue reserves are limited and depend on adequate dietary intake.

The ability of the body to store vitamin B1 is limited and in some situations losses in sweat or urine need to be replaced from dietary intake.

Synonyms: Thiamine, aneurine.

INTRODUCTION

Vitamin B1 is mostly obtained from the diet in human beings. Some is synthesised by intestinal bacteria, only in the caecum. It is absorbed in the proximal small bowel and in the duodenum and then reaches the liver through the vena porta. Its half-life ranges between approximately 9 and 19 days. Physiologically, vitamin B1 is involved as a co-factor in metabolic reactions and in neurotransmission.

Vitamin B1 requirements (1 to 2 mg/d) are usually provided by foods which are rich in this vitamin: brewer's yeast, soya, grains and cereals, potatoes, fruits, egg yolk, milk, pork, liver and fish.

Intake must be increased when metabolic exchange is increased, i.e. during sustained physical activities or sports, in pregnancy and during lactation, when carbohydrate metabolism is increased because of high alcohol intake or parenteral nutrition and in various disease situations: infection (fever), hyperthyroidism and increased excretion (diuretics or diabetes).

INDICATIONS FOR MEASUREMENT

Investigation for vitamin B1 deficiency. Deficiency may be suggested by the following clinical signs:

– systemic signs: asthenia, anorexia, gastric problems,

– peripheral nerve damage: paraesthesiae, hypoaesthesia, pain on calf pressure,

– severe psychological disturbances: depression, irritability, concentration and memory difficulties,

– cardiac disease demonstrated by changed in the electrocardiogram.

INFORMATION

SAMPLE

Whole blood collected into EDTA or lithium heparinate. The sample must be centrifuged promptly (3500 g, + 4°C) and the red cell pellet obtained after removing the plasma and leukocyte layer must be analysed immediately or frozen at – 20°C.

A fasting sample is not essential.

QUESTIONS FOR THE PATIENT

Are you taking any medical treatments?

Diuretics cause urinary thiamine loss. Multivitamins containing vitamin B1 can increase blood thiamine concentrations.

SAMPLE STORAGE AND TRANSPORT

Store and transport: tube frozen at – 20°C.

ASSAY METHODS

High performance liquid chromatography (HPLC).

NORMAL EXPECTED VALUES

These can vary depending on the method and between laboratories. For reference: Vitamin B1: 66.5 to 200 nmol/l (28 to 85 µg/l).

PATHOLOGICAL VARIATIONS

DECREASE IN LEVELS

Vitamin deficiencies are becoming increasingly common with our way of life and the ageing population. The causes of vitamin B1 deficiency are:

– Usually malnutrition, intestinal malabsorption, vomiting, prolonged diarrhoea in chronic bowel disease (generally in the elderly) and chronic alcoholism. Long-term treatment with diuretics such as furosemide increases urinary thiamine excretion.

– Deficient intake: this is seen in people who have a uniform long-term diet of highly refined foods (white flower or polished rice) or from loss of vitamins when the foods are processed (preserving, sterilising fruits, vegetables or meat, storage). Vitamin B1 deficiency can also be seen in heavy tea drinkers and people who eat raw fish as these contain thiamine antagonists.

– True deficiency presents as beriberi, which has a very wide range of clinical expressions: asthenia, anorexia, polyneuritis, cardiac beriberi (peripheral vasodilatation, heart failure, salt and water retention), and cerebral beriberi (alcoholic encephalopathy). It is still seen in some countries such as India and Indonesia. Subclinical forms are the most commonly seen in the industrialised countries.

– Apart from acquired deficiencies, there are thiamine-dependent inherited diseases. They are very occasionally seen in children and are the thiamine-sensitive leucinoses, the congenital thiamine-sensitive lactic acidoses, Leigh-Feigin-Wolf subacute necrotising encephalopathy and thiamine dependent megaloblastic anaemia.

INCREASE IN LEVELS

Blood thiamine concentrations may be raised in some pathological conditions including Hodgkin's disease, polycythaemia rubra vara, some forms of leukaemia and in stomach and bowel cancers. Hypervitaminosis B1 is not toxic.

TREATMENT OF DEFICIENCIES

This involves massive prolonged administration of thiamine and must be started as soon as possible.

FOR FURTHER INFORMATION

■ Leboulanger J., *Vitamine B12*. In : Les vitamines. Biochimie – Mode d'action – Intérêt thérapeutique, F. Hoffmann-La Roche et Cie Ed, Neuilly/seine, 1984 :141-50.

■ Dauvergne A, Galinier A. *Vitamine B1*. In Cahier de formation en biologie médicale Bioforma 2007; n°38: 74-83

VITAMIN B2

DÉFINITION

Vitamin B2 is a flavine, a nitrogen-containing heterocyclic body with three hexagonal nuclei, iso-alloxazine and a 5-carbon sugar ribose, hence its name (riboflavin). It is moderately soluble in water, stable in strong acids, unstable in alkalis and sensitive to light. It is found in significant amounts in the retina, milk and urine.

Requirements in human beings (from 1.5 to 1.8 mg/d) are usually covered by the diet. Natural sources rich in vitamin B2 are wheat germ, baker's yeast, cereals, liver, goat's cheese, camembert cheese, foie gras, kidneys, eggs and oily fish.

Synonym: riboflavin.

INTRODUCTION

Vitamin B2 is mostly obtained in human beings from the diet (particularly milk and dairy products and also cereals, meat, oily fish, some fruits and vegetable). Some is synthesised by intestinal bacteria, although this is inadequate to cover needs. Following ingestion, riboflavin is absorbed in the intestinal wall where it is phosphorylated and then carried to the liver through the vena porta. Small amounts of riboflavin stores exist in the kidney, cardiac muscle and spleen. These reserves remain stable for long periods of time, even during severe prolonged deficiency. Riboflavin is then excreted mostly in the urine as free riboflavin and various metabolites. Physiologically, vitamin B2 is converted in the intestinal wall by phosphorylation into flavine mononucleotide and flavine-adenine-dinucleotide which are prosthetic groups for several enzymes (flavoproteins). These enzymes are involved in the degradation of many substrates and in enzyme reactions producing the energy required for cell needs, particularly in the respiratory chain.

INDICATIONS FOR MEASUREMENT

Vitamin B2 status is assessed from measurements in blood but also in plasma and urine, together with measurement of erythrocyte glutathione reductase activity (not discussed here).

Vitamin B2 measurement in blood is used to investigate for deficiency, which may be suggested by the following clinical signs:

– Muco-cutaneous lesions: smooth shiny lips, abnormally red with chelitis and perleche, seborrhoeic dermatitis of the face predominantly on the rings of the nose and possibly on the ear lobes, stomatitis (purple tongue with smooth papillary atrophy), occasionally hyperpigmentation of the vulva.

– Ocular features: photophobia, increased tear formation, conjunctival hypervascularisation.

The signs are usually subclinical, non-specific and often combined with symptoms suggesting other vitamin deficiencies. The symptom complex more often suggests multiple vitamin deficiencies.

INFORMATION

SAMPLE

Whole blood collected into lithium heparinate; the sample must be stored away from light and at +4°C.

A fasting sample is not essential.

QUESTIONS FOR THE PATIENT

Are you taking any medical treatments?

Chlorpromazine, phenothiazines, tetracyclines and theophylline can reduce blood riboflavin concentrations. Multivitamins containing riboflavin increase blood vitamin B2 concentrations.

SAMPLE STORAGE AND TRANSPORT

Store and transport away from light at + 4°C.

ASSAY METHODS

High performance liquid chromatography (HPLC) followed by UV spectrophotometry.

NORMAL EXPECTED VALUES

These can vary depending on the method and between laboratories. For reference: Vitamin B2: 123 to 161 nmol/l.

PATHOLOGICAL VARIATIONS

Requirements are usually covered by the diet. Deficiencies may occur, however, in some situations:

– Patients receiving long-term unsupplemented parenteral nutrition or who have undergone a gastrectomy.

– The elderly (deficient intake).

– Chronic alcoholism.

– Chronic childhood malabsorption in biliary atresia.

– Newborn infants receiving phototherapy.

– Hemodialysis (increased losses).

– Pregnant women (increased requirements).

Hereditary deficiencies involving vitamin B2 metabolism are also seen, although these are rare. They cause liver and muscle problems.

TREATMENT OF DEFICIENCIES

Vitamin B2 is used to treat people with deficiencies, particularly in the situations described above when muco-cutaneous and/or ocular clinical signs are present. Vitamin B2 is also indicated in other situations, in some gastro-intestinal disorders and in infants with developmental delay. The usual dosage is 20 to 40 mg/day. Higher doses may be given as hypervitaminosis B2 is not seen. This is often combined with other vitamins in the form of a multivitamin complex.

FOR FURTHER INFORMATION

■ Leboulanger J., *Vitamine B2*. In : Les vitamines. Biochimie – Mode d'action – Intérêt thérapeutique. F. Hoffmann-La Roche et Cie Ed, Neuilly/seine, 1984 : 87-96.

■ Galinier A, Bonnefont-Rousselot D. *Vitamine B2*. In Cahier de formation en biologie médicale Bioforma 2007; n°38 :84-97

VITAMIN B6

DEFINITION

Vitamin B6 is a generic term grouping together the 3-hydroxy-2-methylpyridine derivatives. Three compounds have vitamin B6 activity: pyridoxine, pyridoxal and pyridoxamine.

Daily requirements in human beings range from 0.3 mg/d in infants to 2.5 mg/d during pregnancy or when breast-feeding. They are increased further in periods of dietary excess, particularly in a high protein diet.

Vitamin B6 intake is obtained from meats, green vegetables, fresh fruits, potatoes and cereals. The foods richest in vitamin B6 are wheat germ and brewer's yeast. Heat and storage of protein-rich foods cause a fall in vitamin B6 and reduces its bioavailability because of conversion of pyridoxal and its phosphorylated derivative into alpha-pyridoxallysin.

Synonyms: Pyridoxine, pyridoxal, pyridoxamine, pyridoxal phosphate (PLP).

INTRODUCTION

METABOLISM

Vitamin B6 is absorbed mostly in the proximal jejunum. Pyridoxine, pyridoxal and pyridoxamine are then phosphorylated and the phosphorylated derivates are oxidised into pyridoxal phosphate or PLP, the active form of vitamin B6. This can then either be dephosphorylated, or oxidised into 4-pyridoxic acid in the liver or diffuse outside of the cell. Many tissues have enzymes to produce PLP, although production is predominantly in the liver. 75 to 80% of PLP in the body is stored in muscle, bound to glycogen. Pyridoxine bound to extra-hepatic tissues is stored as pyridoxine-5'-phosphate, accumulation of which is believed to be responsible for the neurotoxicity of high dose vitamin B6.

In the blood, erythrocytes contain the enzymes needed to cover all of the vitamin forms into PLP. Under optimal dietary conditions, PLP in plasma represents 55 to 90% and pyridoxal makes up 15% of total vitamin B6. PLP is 95% bound to albumin.

The main urinary metabolite of vitamin B6 is 4-pyridoxic acid, which represents approximately 60% of the ingested dose of pyridoxine. Other unidentified metabolites have been found in urine.

PHYSIOLOGICAL ROLES

– Role in growth and development:

Vitamin B6 as PLP is the co-factor for more than 100 enzymes (transaminases, decarboxylases, dehydratases, trans-sulphidases, etc.), which are involved in numerous cell metabolic reactions, particularly amino acid and protein metabolism. It also plays an essential role in growth and development of the body and in maintaining homeostasis.

– Role in the functioning of the central nervous system:

Vitamin B6 is a cofactor for decarboxylases involved in the synthesis of many neuromediators including dopamine, noradrenaline, 5-hydroxytryptamine and histamine.

– Other roles:

Vitamin B6 is involved in immune function, in regulating cell application, in the oncogenetic process in fatty acid and phospholipid metabolism and in the metabolism of tryptophan, homocysteine, haem and porphyrins.

INDICATIONS FOR MEASUREMENT

– Investigation for vitamin B6 deficiency or overload (far rarer) and monitoring supplementation. There is no actual specific syndrome of avitaminosis B6, although vitamin B6 deficiencies result in dermatological signs (seborrhoeic dermatitis, glossitis and stomatitis), gastro-intestinal symptoms (nausea and vomiting), and neuro-psychiatric features (asthenia, insomnia, depression, etc.), peripheral neuropathy, haematological disturbances (hypochromic, hypersideraemic anaemia and lymphopaenia) and metabolic disorders (increased synthesis of oxalic acid).

– in a metabolic abnormality of vitamin B6 or congenital enzyme defect.

INFORMATION

SAMPLE

Whole blood into lithium, heparinate or EDTA, a fasting sample is preferable.

QUESTIONS FOR THE PATIENT

Are you taking any medical treatments?

Treatments with vitamin B6 antagonists reduce plasma vitamin B6 concentrations. These include theophylline, oral contraceptives containing oestrogens, isoniazid, procarbazine, L-dopa, D-penicillamine, hydralazine and cycloserine.

Preparations containing vitamin B6 and multivitamins containing vitamin B6 increase blood vitamin B6 concentrations.

SAMPLE STORAGEAND TRANSPORT

Store away from light, for a few hours at +4°C.

If the sample is to be transported it must be kept away from light, centrifuged, separated and frozen at -20°C within 4 hours of sampling.

ASSAY METHODS

HPLC with UV spectrophotometry, microbiological and enzymatic methods (enzymatic are currently the most widely used).

NORMAL EXPECTED VALUES

These vary depending on the assay methods. For reference: Vitamin B6: 30 to 100 nmol/l (5 to 15 µg/l).

PATHOLOGICAL VARIATIONS

Vitamin B6 deficiencies are extremely rare outside of the congenital metabolic abnormalities. Epidemiological studies, however, have shown that more or less severe deficiencies of vitamin B6 are commonly seen and cause moderate hyperhomocysteinaemia, which is a recognised independent risk factor for thrombosis.

In general, vitamin B6 deficiency becomes apparent at levels below 20 nmol/l. This may occur as a result of:

– low or imbalanced intake: low calorie and/or high protein diet, chronic alcoholism, prolonged unsupplemented parenteral nutrition;

– increased requirements: pregnancy, breast-feeding, growth, convalescence, high protein anabolism, stress;

– metabolic disturbances: inate (inherited metabolic diseases) or acquired. The acquired causes include drugs which antagonise vitamin B6 (cf. above), alcohol, smoking, haemodialysis and various other diseases including some peripheral neuropathies, some sideroblastic anaemias, hepatic insufficiency, cancers and malabsorption (Crohn's disease).

■ TOXICITY

As vitamin B6 is water-soluble it is rapidly metabolised and then excreted and should have low toxicity. Cases of sensory neuropathy, however, have been reported in patients taking 2 to 7 g of pyridoxine hydrochloride daily for several months. These problems have resolved when the treatment was stopped. Daily doses of more than 50 mg are deemed potentially toxic.

TREATMENT OF DEFICIENCIES

Vitamin B6 either alone or combined with other vitamins is indicated for the treatment or prophylaxis of deficiency due to abnormal vitamin metabolism, in some neuropathies, in treatments with drugs which antagonise vitamin B6 (theophylline, isoniazid, etc.) and in the treatment of vitamin-dependent diseases: pyridoxine-dependent homocystinuria, hyperhomocysteinaemia, cystathionuria, hyperornithinaemia with choroid and retinal atrophy, xanthurenic aciduria and pyridoxine-dependent seizures.

FOR FURTHER INFORMATION

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VITAMIN B8

DEFINITION

Vitamin B8 or biotin has two isomers, the alpha isomer, which is found in egg yolk and the beta isomer, which is found in the liver. Biotin (alpha and beta) is 2-keto-3-4 imidazolido-tetra-hydrothiophane valerianic acid. Free biotin is slightly soluble in water, stable to heat but sensitive to oxygen and ultra-violet light. Requirements in human beings are estimated to be between 150 and 300 µg/day and appear to be largely covered by a normal diet. Foods that are richest in biotin are egg yolk, liver and kidney, although it is also found in peanuts, chocolate, mushrooms, dried peas and, like all of B group vitamins, in brewer's yeast and in small amounts in many animal and plant tissues. Biotin is also produced by the intestinal flora.

Synonyms: biotin, vitamin H (= *Haut*: skin, in German, as deficiency causes skin problems).

INTRODUCTION

■ METABOLISM AND PHYSIOLOGICAL ROLES

Vitamin B8 is absorbed in the small intestine and then passes into the blood and body tissues. It is found particularly in the skin, brain and liver. Vitamin B8 is then removed in urine and in faeces. Urinary excretion depends on dietary intake.

Physiologically, biotin acts as a co-enzyme for specialist carboxylases involved in the active transport of CO2 groups in the intermediary metabolism of proteins, carbohydrates and fats. It is the major agent involved in carboxylation and transcarboxylation reactions in the body. It is also believed to be involved in the production of some amino acids in human beings. It is considered to be a "protector" of the skin and mucosal membranes.

INDICATIONS FOR MEASUREMENT

Investigation for vitamin B8 deficiency suggested by fatigue and some adult or childhood skin conditions.

INFORMATION

■ SAMPLE

Serum or heparinised plasma: the sample must be kept away from light.

A fasting sample is not essential.

■ QUESTIONS FOR THE PATIENT

Are you taking any medical treatments? Antibiotics and sulphonamides may cause vitamin B8 deficiency by adversely affecting the bowel flora.

Vitamin B8 preparations and multi-vitamins containing vitamin B8 can increase blood vitamin B8 concentrations.

■ SAMPLE STORAGE AND TRANSPORT

Store and transport away from light at + 4°C.

ASSAY METHODS

Immunoenzymatic method.

NORMAL EXPECTED VALUES

These may vary depending on the method. For reference: Vitamin B8: 0.5 to 3.3 nmol/l.

PATHOLOGICAL VARIATIONS

Vitamin B8 deficiency is rare and is usually due to one of three mechanisms: reduced intake, reduced absorption or a genetic abnormality. Deficient intake occurs in specific situations such as in people who eat mostly raw eggs or dried egg white as the avidine in egg white is a vitamin B8 antagonist and in people on long-term unsupplemented parenteral nutrition. Reduced vitamin B8 absorption is seen in bowel absorption disorders, chronic alcoholism and in treatment with anti-epileptics (competitive inhibition of intestinal absorption). Two inherited metabolic diseases also cause biotin deficiency: holocarboxylase synthetase deficiency (biotin is present in normal concentrations but is ineffective) and biotinidase deficiency (low plasma biotin concentrations).

The clinical signs of deficiency are scaling dermatitis: the skin is dry, greyish in colour and ichthyiotic in appearance and the tongue papillae are atrophied. These are associated with the systemic signs including fatigue, anorexia, gastrointestinal signs, (nausea, vomiting and hepatosteatosis) and neuro-psychiatric features (drowsiness, depression, hallucinations, localised paresthesiae associated with myalgia).

TREATMENT OF DEFICIENCIES

Biotin is used at a dose of 5 to 10 mg/day in infants and 10 to 20 mg/day in adults. It is indicated for the treatment of seborrhoeic dermatitis, particularly in infants and in the Leiner-Moussu syndrome (generalised form). Biotin is also used for the treatment of acne and alopecia with or without seborrhoea. It is well tolerated and hypervitaminosis B8 has never been reported.

FOR FURTHER INFORMATION

■ Leboulanger J., *Biotine, vitamine H*. In : Les vitamines, Biochimie – Mode d'action – Intérêt thérapeutique, F. Hoffmann-La Roche et Cie Ed, Neuilly/seine, 1984 :125-9.

■ Cuvelier I. *Vitamine B8*. In Cahier de formation en biologie médicale Bioforma 2007;n°38 :141-150.

VITAMIN B12

DEFINITION

Vitamin B12 is found in the body in different forms under the name cobalamins. The two physiologically active forms are methylcobalamin and adenosylcobalamin. These are unstable as they are easily oxidised.

Vitamin B12 is a water-soluble vitamin obtained from the diet. It is essential for the synthesis of DNA and deficiency has major consequences on rapidly renewed tissues, particularly haemopoietic tissue.

Synonym: Cobalamin.

INTRODUCTION

Cobalamin synthesis by intestinal bacteria in human beings is extremely limited and it is therefore almost exclusively exogenous. The main dietary sources are animal proteins, such as liver, fish, shellfish, meat, eggs, milk and dairy products. Vegetables contain almost no vitamin B12 explaining why deficiencies occur in strict vegetarian diets.

After being ingested, dietary cobalamins are released in the stomach by gastric HCl and pepsin and then absorbed in the distal ileum by an active mechanism requiring the presence of intrinsic factor produced in the fundus. In portal blood they are bound to a transport protein, transcobalamin II (TCII), which enables them to enter the target cells where they exert their physiological role. Cobalamins act as an enzyme co-factor. Together with methionine synthase and 5-methyl tetrahydrofolate as a methyl donor, methylcobalamin is involved in the production of methionine from homocysteine; adenosylcobalamin enables propionate to be converted to succinate via methyl malonate in the mitochondria.

In plasma the cobalamins are bound to another transport protein, haptocorrin. An enterohepatic cycle appears to exist, a large proportion of the cobalamins excreted in bile being selectively reabsorbed in the intestine. Urinary and faecal losses are low, no more than 0.1% of reserves.

A normal human diet provides between 3 and 30 micrograms of vitamin B12 per day, which largely covers requirements, estimated to be between 2 and 10 micrograms per day in adults. The body has large reserves of between 3 and 5 mg, mostly held in the liver. These reserves cover requirements for 3 to 4 years.

The disease associated with vitamin B12 is deficiency is only occasionally due to inadequate intake (except in strict vegetarians) and usually due to an abnormality of absorption. As a result of abnormal DNA synthesis, deficiency preferentially affects rapidly renewing cell lines particularly bone marrow cells.

CLINICAL FEATURES

Biermer's disease (pernicious anaemia) is characterised by failure of secretion of intrinsic factor by the gastric mucosal parietal cells and is the typical picture of isolated vitamin B12 deficiency. It is an association of anaemia with asthenia, intense pallor and dyspnoea of effort, gastro-intestinal complaints with anorexia and diarrhoea and neurological signs involving abnormalities of deep sensation.

LABORATORY FINDINGS

The predominant haematological abnormalities of the typical form involve normochromic macrocytic anaemia with bone marrow megaloblastosis. Signs may be less apparent with isolated macrocytosis or normocytic normochromic anaemia when iron deficiency co-exists (a common finding in the elderly). Leukopaenia and/or neutropaenia are common.

On a blood smear

– Anisocytosis with macrocytes,

– Neutrophil hypersegmentation (more than 3% of neutrophils having 5 lobes),

– Giant platelets.

In the bone marrow

– Erythroblastosis with large erythroblasts (rich marrow),

– Basophilic erythroblast cytoplasm ("blue marrow"),

– Bone marrow megaloblastosis with asynchrony of nucleo-cytoplasmic maturation.

INDICATIONS FOR MEASUREMENT

– Testing for vitamin B12 deficiency. Deficiency may be suspected from a normochromic non-regenerative macrocytic anaemia or isolated macrocytosis without anaemia. This is generally however a late sign and may even be absent when vitamin B 12 deficiency is associated with iron deficiency. In this situation, vitamin B12 is generally measured alongside folic acid, the second dietary factor required for nucleic acid synthesis.

– In the absence of anaemia or macrocytosis, B12 measurement is also used to investigate neurological or psychiatric signs, which may reveal deficiency.

– Finally, vitamin B12 measurement is used in hyperhomocysteinaemia found following an arterial or venous thrombosis. Raised plasma homocysteine is known to be an independent risk factor for thrombosis and is often associated with vitamin B12, B6 or folate deficiency, which should therefore be investigated in this situation.

INFORMATION

SAMPLE

Heparinised or EDTA plasma or serum: fluoride must not be used: do not use haemolysed samples.

A fasting sample is not required.

QUESTIONS FOR THE PATIENT

What medical treatments are you taking?

Multi vitamins containing vitamin B12 can increase serum vitamin B12 concentrations.

Have you recently had a transfusion?

SAMPLE STORAGE AND TRANSPORT

Serum or plasma can be stored for 5 days at + 4°C. Freeze at – 20°C.

Vitamin B12 is light sensitive. Do not leave serum or plasma exposed to light for more than 8 hours.

Transport: at + 4°C.

ASSAY METHODS

Competitive immunological methods.

NORMAL EXPECTED VALUES

These can vary slightly depending on the assay methods and between laboratories. For reference: 145 to 735 pmol/l or 197 to 999 ng/l.

Conversion factor: 1 ng/l = 0.74 pmol/l.

PATHOLOGICAL VARIATIONS

- **Reduced serum vitamin B12 concentrations** indicate deficiency which has the following main causes:

Inadequate intake

Very rare: only in strict vegetarians.

Malabsorption

– Pernicious anaemia: deficiency occurs as a result of a loss of intrinsic factor secretion variably associated with the presence of autoantibodies Pernicious anaemia is often associated with other auto-immune diseases (Hashimoto's thyroiditis, haemolytic anaemia, adrenal insufficiency and type I diabetes).

– Total or partial gastrectomy due to reduced or abolished intrinsic factor secretion. Non-immune atrophic gastritis (particularly after the age of 60 years old).

– Parasitic gastro-intestinal infestations: bothriocephale and lamblia.

– Various gastro-intestinal diseases: Crohn's disease, coeliac disease, intestinal lymphoma, tropical sprue, gastro-intestinal bacterial pulling and distal ileal resection.

Various disorders

– Cystic fibrosis.

– Zollinger-Ellison syndrome.

– Congenital transcobalamin II deficiency.

NB: Normal serum vitamin B12 concentrations do not exclude tissue deficiency particularly in transport abnormalities or in congenital intracellular disorders involving this vitamin.

- **Raised serum vitamin B12 concentrations** are found in the following circumstances:

– Myeloproliferative syndrome (particularly chronic lymphoid leukaemia).

– Alcoholism.

– Various liver diseases.

TREATMENT OF DEFICIENCY STATES

Vitamin treatment is administered as oral or intramuscular cyanocobalamin: one 250 microgram tablet per day for 15 days to one month and then one tablet every 10 days or an injection of 1000 micrograms daily or every two days for 1 month and then one monthly injection.

FOR FURTHER INFORMATION

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VITAMIN C

DEFINITION

Vitamin C or 1-ascorbic acid is the lactone of a hexuronic acid closely related to the C6 sugars. It is a water-soluble vitamin, which in complete deficiency causes scurvy (a fatal disease) and in partial deficiency predisposes to various problems including infections, allergies, osteoarthritis, cardiovascular diseases, stress or cancer.

Vitamin C requirements are estimated to be between 60 and 100 mg/d. Vitamin C is only obtained from the diet in human beings and it is found mostly in fruits (citrus fruits, strawberries, blackcurrants, kiwi fruits, rose hip and acerola), vegetables (cabbage and cauliflowers) and some animal foods (beef, pork, liver, kidneys and cow's milk). Vitamin C is unstable and is partly destroyed by heat and light. The loss of vitamin C when food is cooked is mostly due to extraction of the vitamin into the cooking water, which is then discarded. Vitamin C concentrations in milk are also greatly reduced by pasteurisation.

Synonym: ascorbic acid.

INTRODUCTION

METABOLISM AND PHYSIOLOGICAL ROLES

After ingestion, the vitamin C is almost entirely absorbed in the small intestine. Plasma concentrations rise rapidly, following which vitamin C concentrations become significantly higher in leucocytes and platelets. Ascorbic acid is distributed unequally throughout all tissues. It is found mostly in the liver, adrenal cortex and pituitary gland. It is then removed in urine in the unchanged form or as dehydroascorbic acid (25%), oxalic acid (55%) and 2,3-dicetogulonic acid (2%).

Ascorbic acid and dehydroascorbic acid have the same anti-scurvy properties and are the two main oxidised and reduced forms of a reversible oxidation-reduction system. Ascorbate is a potent reducing agent, which traps and removes free radicals (the superoxide anion and OH-hydroxyl group), produced from cell metabolic activity, normally to combat pathogens. Ascorbate can prevent oxygen free radical-induced lipid peroxidation by promoting vitamin E regeneration. Vitamin C also inhibits the formation of advanced protein oxidation and glycation products. It is involved in the hydroxylation of steroid hormones and in collagen formation and is also required for gastric and duodenal mucosal iron absorption.

INDICATIONS FOR MEASUREMENT

Investigation of vitamin C deficiency, particularly in the following clinical circumstances: fatigue, anorexia, malnutrition, inflammatory bowel diseases, smoking, alcoholism, severe anaemia, dysmyelopoiesis, respiratory diseases, poor healing, dermatological conditions (follicular hyperkeratosis, dull fragile hair, bruising, haemorrhagic gingivitis), renal dialysis and in suspected scurvy.

INFORMATION

SAMPLE

Heparinised plasma (EDTA must not be used): the sample must be promptly centrifuged, separated and stored away from light.

QUESTIONS FOR THE PATIENT

Are you taking any medical treatment?

Multivitamins containing vitamin C and commercial vitamin C preparations can temporarily increase blood vitamin C concentrations.

SAMPLE STORAGE AND TRANSPORT

Samples must be centrifuged within an hour of sampling and the heparinised plasma frozen at - 20°C away from light (tube covered with aluminium foil).

Transport: frozen away from light.

ASSAY METHOD

High performance liquid chromatography (HPLC) or UV spectrophotometry.

NORMAL EXPECTED VALUES

These may vary depending on the method and between laboratories. As a guide, by HPLC: 4.6 to 14.9 mg/l or 26 to 85 µmol/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Daily vitamin C requirements are poorly covered in 10 to 30% of French people, particularly as requirements are increased in smokers, during stress, the growth period, pregnancy, lactation, ageing and in fatigue states, whether seasonal or due for example to sports exercise.

PATHOLOGICAL VARIATIONS

Vitamin C has no storage system and plasma vitamin C concentrations therefore reflect recent intake. Vitamin C deficiency is responsible for scurvy. Childhood scurvy is extremely rare but may develop between 6 and 18 months of age in an infant only being fed with artificial foods and no fresh fruit juice supplementation. The initial signs are loss of appetite and weight loss in an infant and crying when the infant is handled.

Hypovitaminosis C is now not unusual in adults and presents with non-specific symptoms (asthenia, weight loss, headache and reduced resistance to infections) and also with dry skin, dull fragile hair, poor healing and sometimes bruising or haemorrhagic gingivitis. Vitamin C deficiency is also common in many diseases: infection, anaemia, dysmyelopoiesis, haemorrhage, rheumatic diseases, allergies, renal dialysis patients, patients with chronic malabsorption and in unsupplemented parenteral nutrition.

THERAPEUTIC USE

Vitamin C supplementation at doses of between 500 mg and 2 g/day is indicated in all of the above clinical situations: haemorrhagic gingivitis (scurvy), anorexia, malnutrition, etc, and when vitamin C requirements are increased (smoking, alcoholism, growth, pregnancy, lactation and iron overload).

NB: hypervitaminosis C does not exist as the excess is removed in urine and faeces in the unchanged form and as metabolites.

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VITAMIN D (1,25 DI-HYDROXY-)

DEFINITION

1,25-dihydroxy vitamin D or calcitriol is the biologically active form of vitamin D (see vitamin D/25-hydroxyvitamin D) and is produced from the dual hydroxylation of vitamin D, firstly in the 25 position in the liver leading to the production of 25-OH vitamin D (or calcidiol) and secondly in the 1 position in the kidney leading to 1,25 di-OH vitamin D.

1α hydroxylation of calcidiol can also take place in the placenta where the 1α-hydroxylase is very active and can contribute to the production of circulating calcitriol during pregnancy. Similarly, sarcoidosis macrophages contain a 1α-hydroxylase and can be responsible for high concentrations of calcitriol in sarcoidosis.

Whilst 25-hydroxylation is not particularly controlled, the same does not apply to 1α hydroxylation, which is regulated by parathyroid hormone (PTH) and blood phosphate. PTH, hypophosphataemia and hypocalcaemia (through PTH) stimulate production of calcitriol, whereas hyperphosphataemia, hypercalcaemia and calcitriol inhibit it.

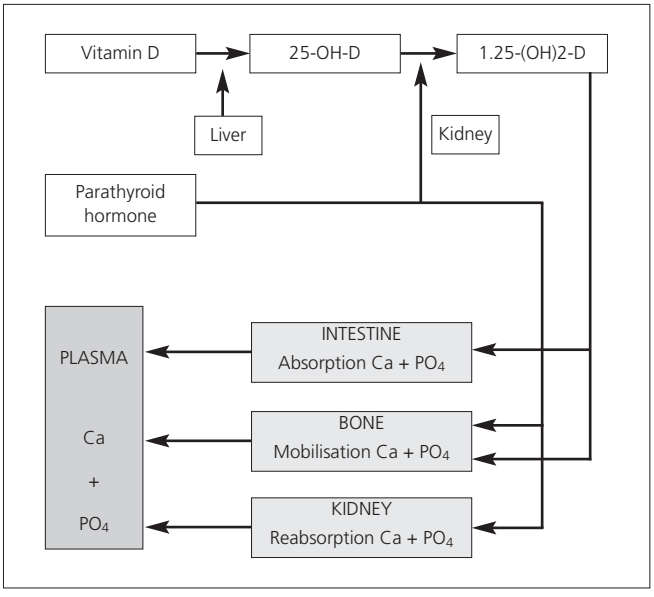
Synonym: calcitriol.

INTRODUCTION

PHYSIOLOGICAL ROLE

Calcitriol is the physiologically active form of vitamin D. It acts mostly in the intestine to stimulate intestinal absorption of calcium and phosphate and therefore increases blood calcium and phosphate. In the bone it facilitates mineralisation indirectly through the increase in blood calcium. In the kidney, calcitriol stimulates tubular phosphate and calcium reabsorption.

In parallel, calcitriol reduces PTH production acting directly on the parathyroids and indirectly by increasing blood calcium.



INDICATIONS FOR MEASUREMENT

Calcitriol or 1,25 di-OH vitamin D does not reflect vitamin D reserves but the activity of renal 1-alpha hydroxylase. In practice, measurement is indicated 2nd or even 3rd line in the investigation of some cases of hypo or hypercalcaemia (for example, hypercalcaemia in sarcoidosis or lymphoma), combined with a parallel full calcium and phosphate profile (isolated measurement is of no value). It is also useful to diagnose a rare familial genetic disease, vitamin-resistant rickets (VRR).

Calcitriol measurement should therefore only be used in situations where an abnormality of 1α-hydroxylation is suspected (defective vitamin D metabolism).

INFORMATION

SAMPLE

Serum or EDTA plasma.

The sample should be taken in the morning with the patient fasting.

ESSENTIAL INFORMATION

Current treatments: all proprietary products containing vitamin D may cause hypervitaminosis D.

Unlike 25-OH vitamin D, calcitriol concentrations are not affected by anti-epileptic drugs.

Other treatments can alter the activity of renal 1 alpha-hydroxylase including corticosteroids, insulin, growth hormone, sex hormones, calcium, phosphates and potassium.

SAMPLE STORAGE AND TRANSPORT

Freeze within 4 hours of sampling. Store and transport at – 20°C.

ASSAY METHODS

Radio-immunoassay.

NORMAL EXPECTED VALUES

For reference: 45 to 145 pg/ml.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Plasma calcitriol concentrations are extremely high (approximately 3 times adult values) during the first year of life and during the pubertal growth spurt. Concentrations are also high during childhood and in pregnancy (approximately twice the value seen in adults) firstly because of an increase in the binding protein (DBP, *vitamin D Binding Protein*) and secondly because of synthesis by the placenta. Concentrations fall during the first two weeks of breast-feeding and then return to similar values to those in controls.

PATHOLOGICAL VARIATIONS

Reduced calcitriol is only seen in chronic, severe vitamin D deficiency. Mild deficiency causes hypocalcaemia, which is compensated by hyperparathyroidism resulting in stimulation of renal 1α-hydroxylation and therefore increasing calcitriol.

The different pathological situations are associated with a rise or fall in calcitriol are shown in the table below.

Reduced serum calcitriol	Increased serum calcitriol
Chronic severe vitamin D deficiency	Vitamin D intoxication
Defective 25-hydroxylation	Primary hyperparathyroidism
Chronic renal insufficiency	Sarcoidosis
Type 1 rickets	Lymphoma
(1α-hydroxylase deficiency)	Tumour calcinosis
Hypoparathyroidism	Type II rickets
Pseudohypoparathyroidism	(calcitriol receptor abnormalities)
Hyperphosphataemia	Acromegaly
Hypermagnesaemia	
Hypercalcaemia malignancy	
(excluding lymphomas)	
Hyperthyroidism	

Diagnosis of vitamin-resistant rickets (VRR) (genetic disease): type 1 VRR is usually diagnosed in children and is caused by an abnormality of the enzyme 1-alpha hydroxylase: serum 1,25 di-OH vitamin D concentrations are undetectable (whereas 25-hydroxy D concentrations are normal). Type 1 VRR is treated with drugs containing 1-alpha hydroxyvitamin D3.

FOR FURTHER INFORMATION

■ Souberbielle J.C., Cormier C., Kindermans C. et al., *Vitamin D status and redifining serum parathyroid hormone reference range in the elderly*, J Clin Endocrinol Metab 2001; 86: 3086-90.

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VITAMIN D (25-HYDROXY-)

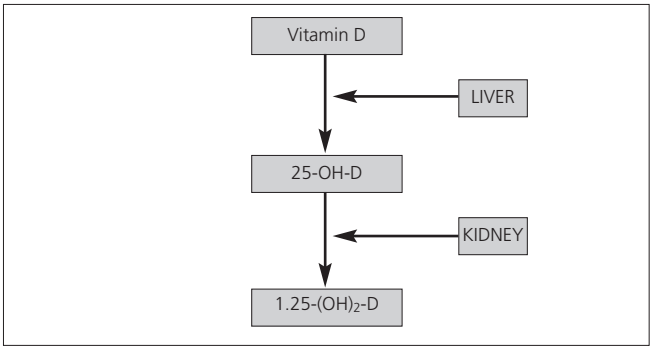
DEFINITION

Vitamin D is a generic term for all of the secosterols with anti-rickets activity. The two vitamins D2 (ergocalciferol) and D3 (cholecalciferol) differ from their plant or animal origin and have biologically similar activity in mammals. The term vitamin D is therefore used to designate either one.

Vitamin D has two origins in human beings: exogenous and endogenous. Vitamin D is provided by the diet as vitamin D2 in plant foods and vitamin D3 in foods from animal origin (it is found particularly in cod liver oil and oily fish and in small amounts in eggs, meat and dairy products). The source of vitamin D is mostly however endogenous as it is produced from 7-dehydrocholesterol in response to certain UV irradiation on the skin.

Endogenous production varies greatly as it depends on latitude, sun exposure, season and atmospheric pollution (which blocks UV in the same way as glass and perspex), social and clothing habits and skin type and state. UV absorption and therefore vitamin D production in the skin also decreases with age and is particularly less effective in people with highly pigmented skins.

Regardless of origin, vitamin D accumulates in the liver where it is initially C25-hydroxylated into the 25-D3 or 25-hydroxycholecalciferol or calcidiol and 25-hydroxyvitamin D2 or 25-hydroxy-ergocalciferol.



These compounds pass into the blood where they bind to carrier proteins (mostly DBP, *Vitamin D Binding Protein*) and then bind to the renal parenchyma undergoing a second hydroxylation either in the C1 position to 1α, 25-dihydroxyvitamin D or calcitriol, or C24 to 24,25-dihydroxyvitamin D. Calcitriol is the biologically active form of vitamin D.

Synonyms: vitamin D: ergocalciferol (vitamin D2), cholecalciferol (vitamin D3). 25-hydroxyvitamin D3 or calcidiol.

INDICATIONS FOR MEASUREMENT

Calcidiol or 25 hydroxy-D is the predominant circulating form and the best reflection of the body's vitamin D reserves. Measurement can therefore reveal deficiency regardless of cause and be used to monitor vitamin D treatment. For this reason, measurement is particularly indicated in people susceptible to vitamin D deficiency, particularly post-menopausal women and more generally, the elderly.

PATHOPHYSIOLOGY

Severe vitamin D deficiency causes osteomalacia in adults and rickets in children. Before referring to true vitamin D deficiency, however, there is an intermediary stage, which some authors have referred to as "insufficiency" or moderate vitamin D deficiency, the major consequence of which particularly in the elderly is secondary hyperparathyroidism with an increased risk of fracture. The rise in PTH concentrations however, does not always exceed physiological limits. It is therefore important to detect these cases of vitamin D "insufficiency" as vitamin D and calcium supplementation can reduce PTH and bone resorption and as a result very significantly reduce the incidence of non-vertebral fractures. American economists have also recently proposed systematic supplementation of all people over 70-75 years old.

INFORMATION

SAMPLE

EDTA or heparinised plasma or serum following the recommendations of the laboratory performing the test.

The sample should be taken in the morning, preferably fasting.

ESSENTIAL INFORMATION

Current treatments: all drugs containing vitamin D (D2 or D3) can cause a hypervitaminosis D syndrome.

Anti-epileptic treatments may cause vitamin D deficiency as they accelerate the hepatic catabolism of vitamin D.

Pregnancy?

Age and season?

SAMPLE STORAGE AND TRANSPORT

Whole blood: 3 days at room temperature,

Serum: 7 days at + 4°C. Freeze at -20°C beyond this time. Vitamin D is stable even after 4 freeze-thaw cycles.

ASSAY METHODS

– Competitive radio-immunoassay. Direct measurement after extraction but without prior chromatographic purification.

– Chemoluminescence immunoassay on an automated analyser: competition is against either the carrier protein (DBP) or an anti-serum.

NORMAL EXPECTED VALUES

The kits available are not standardised and can therefore produce very different results for the same patient. In addition, the definition of "normal values" is complicated as this must take into account other factors (age, season, etc.).

Several processes have been used by different authors who have for example proposed defining the threshold for lower "normal" values as the concentration of 25OH vitamin D beneath which bone signs develop (10 ng/ml) or above which no seasonable variation occurs (36 ng/ml).

The current accepted approach only uses the concentration of 25-OH D below which (and/or above which) adverse effects may occur on health.

The desirable 25-OH D values are therefore: 30 to 80 ng/ml (75 to 200 nmol/l).

The conversion factor is: 1 nmol/l = 0.40 ng/ml.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Plasma 25-hydroxyvitamin D concentrations vary during the year depending on sun exposure, with a peak in summer.

Published values in black women are fifty per cent lower than those in white women.

PATHOLOGICAL VARIATIONS

Hypovitaminosis D

This may occur as a result of:

– Defective endogenous production: low sun exposure, high latitude, clothing habits, skin ageing and pigmentation. Immobility and reduced ability of the skin to produce vitamin D in the elderly worsens deficiency in view of their underlying low dietary intake.

– Dietary insufficiency: vegetarian diet.

– Intestinal vitamin D malabsorption: coeliac disease, cystic fibrosis, partial gastrectomy, Crohn's disease and chronic pancreatitis.

– Urinary (nephrotic syndrome) or peritoneal (peritoneal dialysis) losses.

– Anti-epileptic treatment *(cf. above)*.

In the literature, specialists have proposed a definition of vitamin D insufficiency as concentrations < 30 ng/ml (75 nmol/l).

Clinically, profound prolonged deficiency is associated with rickets in children and osteomalacia in adults. Before bone problems due to deficiency become clinically apparent, however, suggestive biochemical changes may be seen including hypokalaemia, hypophosphataemia, raised alkaline phosphatase and hypocalciuria.

Hypervitaminosis D

This is characterised by increased serum 25OH vitamin D (> 200 nmol/l) and is mostly due to exogenous intoxication sometimes from diet (chronic excessive ingestion of fish liver preserves) or, usually, therapeutic (long term vitamin D treatment).

The symptoms of acute intoxication (serum concentrations of 25-OH vitamin D > 250 nmol/l) are: hypercalcaemia, gastro-intestinal problems, thirst, polyuria, hypertension, pre-renal renal insufficiency and hypercalciuria. When very severe this may cause altered consciousness leading to coma.

Chronic prolonged intoxication is characterised by vascular calcification, nephrolithiasis, nephrocalcinosis and irreversible renal failure.

FOR FURTHER INFORMATION

■ Souberbielle J.C., Cormier C., Kindermans C. et *al*., *Vitamin D status and redifining serum parathyroid hormone reference range in the elderly*, J Clin Endocrinol Metab 2001 ; 86 : 3086-90.

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VITAMIN E

DEFINITION

Vitamin E is a generic term for all of the tocopherols and tocotrienols derived from 6-chromanol. They are powerful anti-oxidants. Alpha-tocopherol has the most potent biological activity of these compounds and is the most abundant plasma form (approximately 88%). Vitamin E is found in many foods: sunflower, corn, olive, rape seed and peanut oils, margarines, wheat germ, offal, eggs and milk. Recommended daily intake is 3 to 12 mg/l; requirements are increased in disease and in smokers. Vitamin E deficiency is rare in the population.

Synonym: tocopherol

INTRODUCTION

After hydrolysis of the esters in the intestine and enterocyte, tocopherols are carried in the blood bound to lipoproteins. Alpha-tocopherol is approximately 70% absorbed and absorption is influenced by the presence of bile salts, pancreatic lipase and dietary fats. Following digestion, vitamin E is transported by chylomicrons and reaches the systemic circulation through the lymphatics. Following the action of lipoprotein lipase, vitamin E is bound by hepatocytes and alpha-tocopherol is then incorporated into VLDL and follows its vascular metabolism. Plasma alpha-tocopherol concentrations are correlated with total cholesterol and phospholipid concentrations. In the circulation, vitamin E is believed to be found mostly in LDL and HDL, although considerable exchange occurs between the different lipoprotein classes. Vitamin E is removed in bile, either directly or after oxidation into the quinine and then reduction to hydroquinone and glucuronide conjugation.

Physiologically, vitamin E is a major lipid anti-oxidant. In particular it prevents lipoprotein oxidation and prevents development of the atheromatous plaque. Tocopherols also have cell membrane stabilising activity and vitamin E may in addition be involved in regulation of haem synthesis. Finally, given the likely pro-carcinogenic role of lipid peroxides, vitamin E may have a protective effect against cancer.

INDICATIONS FOR MEASUREMENT

Testing for vitamin E deficiency, particularly in the following clinical situations: haemolytic anaemia, malabsorption, neuro-muscular and/or ophthalmological problems.

INFORMATION

SAMPLE

Serum or heparinised plasma: samples must be kept away from light. Fasting samples are preferable.

QUESTIONS FOR THE PATIENT

Are you taking any medical treatment?

Multi-vitamins containing vitamin E and commercial vitamin E preparations increase vitamin E in the blood.

SAMPLE STORAGE AND TRANSPORT

Serum or plasma must be stored at – 20°C within 4 hours of sampling.

Storage: separated serum/plasma should be stored away from light for up to 15 days to 1 month at – 20°C and for up to 3 months at – 80°C.

Transport: frozen away from light.

ASSAY METHOD

High performance liquid chromatography (HPLC), fluorimetry.

NORMAL EXPECTED VALUES

These may vary depending on the method and between laboratories and by age and sex. For reference, by HPLC:

- Newborn: 2.17 to 9.56 mg/l
- Children from 1 to 6 years old: 3.02 to 9.05 mg/l
- Children from 7 to 12 years old: 4.30 to 9.05 mg/l
- Adult men: 4.29 to 13.30 mg/l

NB: fluorometric results are higher as they do not specifically measure alpha-tocopherol.

Conversion factor for mg/l to µmol/l: x 2.3.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Apart from age and sex related variations, blood alpha-tocopherol is increased in pregnancy (by 30 to 70%) and reduced in smokers and after drinking alcohol (concentrations may be extremely low in chronic alcoholic patients). Serum vitamin E concentrations also correlate with those of total cholesterol and phospholipids.

PATHOLOGICAL VARIATIONS

Requirements are usually covered by diet, although sub-clinical deficiencies are not uncommon in people in apparently good health. In the long-term these may promote the development of degenerative diseases. Deficiencies are seen in different pathological situations: in malnourished children, enteropathies (congenital biliary tract abnormalities, abetalipoproteinaemia, coeliac disease, chronic pancreatitis, ulcerative colitis, cystic fibrosis), haemolytic diseases (beta-thalassaemia, major sickle cell anaemia, hereditary spherocytosis, haemolytic anaemia of prematurity), in chronic haemodialysis patients and in patients suffering from Gaucher's disease.

An abnormality of the alpha-tocopherol transport protein (or α -TTP) gene is responsible for a form of deficiency which has long been unexplained "idiopathic familial vitamin E deficiency", now called "ataxia with isolated vitamin D deficiency (AVED)" which is associated with very low or undetectable vitamin E concentrations in blood despite normal dietary intake.

Vitamin E supplementation is given in deficiency. Vitamin K status must be monitored during vitamin E supplementation as vitamin E has a negative effect on the procoagulant properties of vitamin K and the risk of bleeding is increased in patients on AVK who are being supplemented with

vitamin E. In addition, high doses of vitamin E reduce the action of cyclo-oxygenase, thromboxane-dependent coagulation and coagulations factors II and VII. It can therefore also potentiate the anticoagulant effects of aspirin. Long term vitamin E treatment therefore requires coagulation to be monitored.

FOR FURTHER INFORMATION

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- Léger C.L., Carbonneau M.A., Fouret G. *Vitamine E*. In Cahier de formation en biologie médicale Bioforma 2007; n°38:49-63.

VITAMIN K

DEFINITION

The name vitamin K, groups together a set of natural fat-soluble compounds whose structure includes a 2-methyl-1,4 naphthoquinone group bound to an aliphatic chain, which have anti-haemorrhagic properties. Vitamin K1 (phylloquinone, phytomenadione) in human beings comes exclusively from plants and vitamin K2 exclusively from animals. Both have the same vitamin activity. Recommended intake is 10 µg/kg/day in the newborn and 1 µg/kg/day in adults and is largely covered by the diet. It is found in large amounts in spinach, cabbage and tomatoes and in pig and ox liver. It is also produced in small amounts by intestinal bacteria.

Synonyms: phylloquinone, phytomenadione, anti-haemorrhagic factor.

INTRODUCTION

As applies to all of the fat-soluble vitamins, vitamin K1 is absorbed in the intestine in the presence of bile salts and then enters the blood circulation, where it reaches peak concentrations two hours after ingestion. Only the water soluble compounds pass into the circulation. Reserves are limited (a few days) and mostly contained in the liver but also in bone, muscle and skin. It is then removed in bile and urine, to a large extent as oxidated or glucuoronide conjugated derivatives.

Vitamin K acts as a cofactor for a microsomal carboxylase, which converts glutamic acid molecules into gammacarboxyglutamic acids, which bind calcium and have biological properties. Carboxylation therefore enables some coagulation factors to mature (factors II, VII, IX, X) and proteins C and S, together with osteocalcin and protein Z (which may help to reduce the action of thrombin at a site of injury).

The liver firstly produces inactive precursors following which vitamin K converts around ten molecules of glutamic acid in the NH2 terminal segment of each of the factors into gamma-carboxyglutamic acid, as this stage is required for these factors to bind to anionic cell membrane phospholipids. In order to act as a co-factor for hepatic carboxylase, vitamin K must be reduced. Anti-vitamin K agents (oral anticoagulants) prevent the reduction of vitamin K by inhibiting the activity of two enzymes, vitamin K epoxyreductase and vitamin K reductase. In vitamin K deficiency the liver produces non-functional coagulation factors, known as PIVKA *(Protein Induced by vitamin K Absence or Antagonist)*. These proteins cannot bind calcium and act by inhibiting coagulation, causing bleeding.

INDICATIONS FOR MEASUREMENT

– Investigation of vitamin K deficiency. Deficiency may be suggested by bruising, haemorrhage, in alcoholism (hepatic insufficiency) and in severe malabsorption. NB: Vitamin K deficiency can be easily tested for from a fall in the prothrombin level.

– Monitoring vitamin K concentrations after vitamin K treatment.

INFORMATION

SAMPLE

Dry tube (serum); the sample must be stored away from light.

QUESTIONS FOR THE PATIENT

Are you taking any medical treatments?

Cholestyramine inhibits the intestinal absorption of vitamin K.

Anti-vitamin K agents produce hypoprothrombinaemia by reducing vitamin K-dependent coagulation factors. Vitamin K1 can be administered to correct accidental overdose of AVK.

SAMPLE STORAGE AND TRANSPORT

Store away from light. Centrifuge and freeze at – 20°C within an hour of sampling.

Transport: frozen at – 20°C.

ASSAY METHODS

Liquid extraction followed by preparative high performance liquid chromatography and then assay by a second HPLC with fluorimetric detection after reducing vitamin K1.

NORMAL EXPECTED VALUES

These can vary depending on the method and between laboratories. For reference: Vitamin K1: 150 to 900 ng/l.

PATHOPHYSIOLOGICAL VARIATIONS

PHYSIOLOGICAL VARIATIONS

Newborn babies have hypoprothrombinaemia, which lasts for the first few days after they are born until their intestinal flora develops and sufficient dietary vitamin K is absorbed. Plasma concentrations of vitamin K-dependent coagulation factors are 20 to 40% of levels seen in adults (they are even lower in premature infants) and fall during the first three days after birth before rising gradually to normal adult levels. Breast milk is also low in vitamin K and vitamin K deficiency is commonly seen in breastfeeding: these infants should be supplemented during the first two months of breastfeeding.

Vitamin K deficiency is also common in the elderly, particularly in people suffering from osteoporosis.

PATHOLOGICAL VARIATIONS

Requirements are usually covered by diet. No deficiencies of intake are found in adults (except in long-term parenteral nutrition which is not supplemented or in reduced endogenous synthesis due to long-term antibiotic therapy). On the other hand, haemorrhage due to avitaminosis K can occur in:

– absorption-related deficiency: severe malabsorption (Crohn's disease, steatorrhoea, cystic fibrosis and extensive bowel resection) and obstructive jaundice;

– defective metabolism: severe hepatic insufficiency (hepatitis, cirrhosis, either infectious, toxic or secondary to long-term biliary tract obstruction), chronic alcoholism and anti-vitamin K treatments (oral anticoagulants);

– haemorrhagic disease of the newborn: this is due to hepatic immaturity associated with deficient intake.

■ THERAPEUTIC USE

Vitamin K treatment is used in the newborn *(cf. above)* and in people with AVK overdose.

Management of AVK (warfarin) overdose: North-American recommendations. 8th ACCP consensus, Chest 2008.

INR above the therapeutic range but < 5, no bleeding:

– reduce the dose or omit a dose of AVK and monitor INR more frequently. If the INR is just above the therapeutic range or a temporary cause is present, the dose does not need to be reduced.

INR ≥ 5 but < 9, no bleeding

– omit 1 or 2 doses of AVK, check the INR more frequently.

– alternatively: omit 1 dose of AVK and administer 1 to 2.5 mg of oral vitamin K, particularly if the patient is at risk of bleeding.

INR > 9, no bleeding

– stop warfarin treatment and administer 2.5 to 5 mg of oral vitamin K and check the INR more frequently; give more vitamin K if necessary.

Patients with severe bleeding and higher INR

– stop the warfarin and administer vitamin K (10 mg) by slow IV injection, together with virally inactivated fresh frozen plasma (FFP), concentrated prothrombin or recombinant factor VIIa concentrate. Further dosses of vitamin K should be given every 12 hours if the INR remains high.

FOR FURTHER INFORMATION

■ *Antithrombotic and thrombolytic therapy: American College of Chest Physicians evidence-based clinical practice guidelines (8th Edition),* Chest 2008;133 (6):72S-73S.

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VITAMIN PP

DEFINITION

The term vitamin PP covers two compounds, nicotinic acid or 3-pyridine-carboxylic acid and nicotinamide, the amide of nicotinic acid. They are both white crystals, which are soluble in water and alcohol, stable to light and heat and resistant to oxidation. They have identical vitamin activity. Requirements in adults are 15 to 20 mg/day and are increased during adolescence, pregnancy and lactation. 1/3 comes from free nicotinamide in the diet and 2/3 from conversion of tryptophan, which itself is obtained in the diet, in the body. Vitamin PP is found in many foods, mostly yeasts, cereals, meat, fish and mushrooms.

Synonyms: nicotinamide, nicotinic acid and niacin.

INTRODUCTION

Vitamin PP is generally completely absorbed throughout the length of the gastro-intestinal tract. It is then incorporated into different co-enzymes in which it is the active part and it is found widely in most cells in the body. Vitamin PP is the precursor of two factors, which are involved in almost all of the body's oxidation-reduction reactions: NAD and NADP.

Vitamin PP in blood is concentrated to a large extent in erythrocytes. For this reason only whole blood measurement provides a good estimate of body vitamin PP status. Vitamin PP is then removed in urine, mostly as metabolites.

The name vitamin PP comes from the discovery by Funck in 1912 of its preventative action against pellagra *(Pellagra Preventive factor)*. This disease, which is largely due to vitamin PP deficiency is characterised by gastro-intestinal, cutaneous and then neuro-psychiatric problems. Other factors, also however appear to contribute, particularly vitamin B6 deficiency and inadequate dietary protein (tryptophan deficiency).

INDICATIONS FOR MEASUREMENT

Testing for vitamin PP deficiency, which is one of the main factors involved in pellagra. Deficiency is suggested by muco-cutaneous lesions and gastro-intestinal problems in the elderly, during long-term parenteral nutrition, in chronic alcoholism and in some inherited diseases.

INFORMATION

■ SAMPLE

Whole blood collected into lithium heparinate or EDTA. The sample must be stored away from light.

A fasting sample is not essential.

■ QUESTIONS FOR THE PATIENT

Are you taking any medical treatments?

Isoniazide, carbidopa and 5-fluorouracil can reduce blood vitamin PP concentrations.

Nicotinic acid is used therapeutically for its lipid lowering effect and nicotinamide in the treatment of vitamin PP deficiency can increase blood niacin concentrations.

■ SAMPLE STORAGE AND TRANSPORT

Store away from light, for up to a few days at + 4°C.

Transport: + 4°C.

ASSAY METHODS

High performance liquid chromatography (HPLC) or microbiological method.

NORMAL EXPECTED VALUES

These vary depending on the assay and between laboratories. For reference, vitamin PP in whole blood: 38 to 58 µmol/l (4.6 to 7.1 mg/l).

PATHOLOGICAL VARIATIONS

There are many natural sources of vitamin PP in the diet and needs appear to be fairly well covered. Vitamin PP deficiency can be seen however, in chronic alcoholics, in the elderly, during isoniazid, carbidopa or 5-fluorouracil treatment with long-term parenteral nutrition and in small bowel carcinoid tumours.

Pellagra is usually characterised initially by non-specific symptoms of asthenia, anorexia, weight loss, headaches and dizziness associated with tendency towards depression. The established disease phase is characterised by the triade of "dermatitis, diarrhoea and dementia". Skin signs are seen (painful erythema, rough brown coloured skin which scales), gastro-intestinal signs (gastritis, enterocolitis with diarrhoea, glossitis, magenta coloured buccal mucosa) and neuropsychiatric disorders (peripheral nerve damage with sensory disorders of the extremities and occasionally severe pain).

In addition, in the same way as thiamine deficiency, a picture of sub-acute encephalitis resembling Gayet-Wernicke's encephalopathy may occur. High dose vitamin PP may be effective in some patients who are resistant to Vitamin B1.

Finally, vitamin PP deficiency can be seen in Hartnup's disease, an inherited disease caused by abnormal tryptophan metabolism.

TREATMENT OF DEFICIENCIES

Nicotinamide (Nicobion®) is reserved for the treatment of vitamin PP deficiency in adults. The usual dose is 500 mg to 1 g/day (1 to 2 tablets).

FOR FURTHER INFORMATION

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VON WILLEBRAND FACTOR

DEFINITION

Von Willebrand factor is a glycoprotein produced by the endothelial cells and megakariocytes, which is then stored in the platelet alpha granules and secreted into the circulation during platelet activation. It consists of 15 to 20 multimers of total molecular weights ranging from 500,000 to 20,000 000 Da. The high molecular weight multimers are essential for its biological activity.

Specific measurement of von Willebrand factor uses two types of tests: measurement of the antigen and measurement of activity. This must be combined with measurement of factor VIII coagulant, a fall in which can indirectly reflect an abnormality of von Willebrand factor *(cf. below)*.

Synonyms:
Ristocetin co-factor antigen and activity, vWF
Von Willebrand antigen factor: vWF: Ag
Activated von Willebrand factor: vWF: Act
Activated von Willebrand factor, ristocetin co-factor: vWF: RCo.

PHYSIOLOGICAL ROLE

Von Willebrand factor has two roles in haemostasis: firstly it enables coagulation factor VIII to be transported in the circulation and stabilises its coagulant activity, which is very labile, and secondly it contributes to platelet adhesion and aggregation. Factor VIII not bound to vWF is found in normal plasma with a very short half-life.

INDICATIONS FOR MEASUREMENT

The measurement of von Willebrand factor is used to screen for and diagnose von Willebrand disease, the commonest of the inherited bleeding disorders (prevalence 0.5 to 1/100).

Clinically the disease is expressed very variably and is characterised by muco-cutaneous bleeding involving bruising, epistaxis, gum bleeding, and menorrhagia in women. It may present as post-operative bleeding, which is highly suggestive after tonsillectomy, adenoidectomy or tooth extraction. The clinical picture in severe forms of the disease is similar to that of haemophilia, with tonsillar bleeding, haematomas and haemarthroses.

Laboratory findings include an increase (variable) in some haemostasis tests: bleeding time (BT), occlusion time (OT) on a PFA® *(Platelet function Analyser)*, and the activated partial thromboplastin time (APTT).

Von Willebrand factor is therefore measured in suggestive bleeding or with a prolonged BT, OT or APTT. The APTT is classically prolonged in vWF deficiencies although may be almost normal and a normal APTT does not exclude the diagnosis of von Willebrand disease.

INFORMATION

SAMPLE

Samples should be collected into citrate, concentration 3.2% (0.109 M), 1/10 (0.5 ml per 4.5 ml of blood). 3.8% (0.129 M) are acceptable. CTAD tubes (citrate, theophylline, adenine, dipyridamole) are recommended in patients receiving heparin. No other anticoagulant may be used.

The sample should preferably be taken in the morning between 0700 and 1100 hours with the patient resting, seated, for at least 5 minutes. A light low fat snack is permitted but caffeine, tobacco and physical activity must be avoided during the hour before the sample is taken. The samples must be centrifuged promptly after the sample is taken and the test performed within 2 to 4 hours of sampling (if not, freeze plasma at –20°C). Check that no micro-clots are present and discard haemolysed or lipaemic samples. For more information refer to the "General pre-analytical conditions in haemostasis" section.

QUESTIONS FOR THE PATIENT

Are you taking medical treatment?

Aspirin, non-steroidal anti-inflammatory drugs and platelet anti-aggregants (ticlopidine, clopidogrel) prolong the BT. The OT measured on a PFA® is highly sensitive to aspirin and is prolonged in patients taking IIb – IIIa anti-glycoproteins although not always changed in those taking ticlopidine or clopidogrel. The APTT is prolonged by treatment with heparins and heparin derivatives: unfractionated heparins, low molecular weight heparins, danaparoid, hirudin and derivatives, pentasaccharide, dabigatran or rivaroxaban.

Oral contraceptives increase von Willebrand factor levels.

Do you have a personal or family history of bleeding?

SAMPLE STORAGE AND TRANSPORT

The test is performed on platelet-poor plasma obtained by centrifugation (2000 to 2500 g, 15 minutes at between 10 and 20°C). If the test is to be performed later, the sample should be frozen after a second centrifugation, separating the plasma from slightly above the white leukocyte and platelet layer and centrifuging the plasma from the first centrifugation in a polypropylene plastic or PET tube.

Maximum time before test: 2 to 4 h, at laboratory temperature (never store the sample at + 4°C); can be stored for 2 weeks at – 20°C and for 6 months at – 70°C.

Transport: at – 20°C. If the sample is to be transported the plasma must be frozen at – 20°C within 2 hours of sampling. It is recommended that it be thawed promptly in a water bath at 37°C and the sample be mixed thoroughly before testing.

ASSAY METHODS

Assay of von Willebrand factor antigen (vWF: Ag): immuno-enzymatic or latex particle agglutination method (colourimetry).

Two types of method are available for vWF activity: ristocetin co-factor activity vWF:RCo (reference method) and chromogenic measurement of vWF:Act activity (concordance with vWF:Rco is 98%, or 100% if combined with PFA-100). Measurement of vWF activity is used to assess the binding capacity of vWF to platelet GpIb.

REFERENCE VALUES

Results are usually expressed as a percentage of normal or in IU/ml, 1 IU/ml = 100%.

Reference values in children from 1 month old and in adults:

– Von Willebrand factor antigen: 50 to 150% (0.50 to 1.50 IU/ml);

– Activated von Willebrand factor: 50 to 150% (0.50 to 1.50 IU/ml).

PATHOPHYSIOLOGICAL VARIATIONS

■ PHYSIOLOGICAL VARIATIONS

– Reference vWF Ag and vWF RCo values in blood group O subjects are approximately 25 to 35% lower: between 35 and 150%.

– At birth: 80 to 210%.

– During pregnancy: 95 to 1 000%.

■ INTERPRETATION

■ 1- Investigation of von Willebrand factor (vWF)

1st stage: is a vWF abnormality present?

vWF activity is classically reduced in all forms of von Willebrand disease except for the 2N form (normal activity). It is therefore the parameter of choice for the diagnosis.

Interpretation:

If activity is normal: von Willebrand disease (excluding 2N) is unlikely. Qualitative platelet studies are then indicated. The possibility of "false negatives", however, should be considered:

– Von Willebrand factor may rise transiently (to normal or abnormal) in minor forms of von Willebrand disease, inflammation or pregnancy,

– In von Willebrand disease 2B, activity rate may remain normal (particularly "New York" subtype 2B). The diagnosis of this form of the disease can be difficult. A low dose ristocetin aggregation test (RIPA) should therefore be performed routinely in all patients with a past history of unexplained bleeding regardless of vWF:RCo levels if the PFA-100® is prolonged. The RIPA is the only constant laboratory finding in type 2B.

If activity is reduced: the diagnosis is probably von Willebrand's disease (90% are diagnosed at this stage). FVIII:C and vWF:Ag should then be measured for typing.

2nd stage: is the abnormality quantitative or qualitative?

The tests to perform are vWF:Act, vWF:Ag and FVIII:C with calculation of ratios.

Measurement of von Willebrand factor antigen (vWF:Ag)

In a quantitative abnormality, the fall in vWF:Ag is proportional to the reduction in activity. In a qualitative abnormality the antigen is normal or reduced disproportionately to activity.

Assay of factor VIII (FVIII:C)

This is not always reduced and depends on vWF:Ag levels (except in the 2N form). In a quantitative abnormality the fall in FVIII:C parallels that of the fall in vWF:Ag. In a qualitative abnormality the result is the same or below the vWF:Ag level except in the 2N form (isolated ↘ with normal vWF).

Calculation of the vWF:RCo/vWF:Ag ratio:

– < 0.7 in qualitative abnormalities,

– ≈ 1 in quantitative abnormalities and in type 2N.

Calculation of the FVIII:C/vWF:Ag ratio:

– ≥ 1 in all types,

– except in 2N: < 0.5.

Summary of abnormalities by type in von Willebrand disease

Type 1: partial quantitative deficiency

– vWF:Rco or vWF:Act reduced
– vWF:Ag reduced
– FVIII:C reduced
– vWF:Act/vWF:Ag ratio close to 1
– FVIII:C/vWF:Ag ratio > 1

Type 2: qualitative defect

– vWF:Rco or vWF:Act reduced
– vWF:Ag normal or reduced
– FVIII:C: normal or reduced
– vWF:Act/vWF:Ag ratio < 0.7
– FVIII:C/vWF:Ag ratio close to 1 except 2N

Type 3: total quantitative defect

– vWF:Rco or vWF:Act very low
– vWF:Ag very low
– FVIII:C greatly reduced
– Ratio is not applicable

3rd question: what is the exact subtype of von Willebrand disease?

The investigations required are mostly a study of von Willebrand multimers and the RIPA test. Depending on the context and results a vWF-FVIII binding study to diagnose type 2N, or a vWF-collagen binding study may also be required.

Overall, the diagnosis of von Willebrand's disease is based principally on von Willebrand factor concentrations and the bleeding score. The diagnostic difficulty, however, is partly due to variability in levels and possible discrepancies between the bleeding score and von Willebrand factor measurement.

■ 2- Interpretation of levels

– vWF increased (> 150%): inflammation (fibrinogen+++), pregnancy, oestrogen-progestogens, stress, physical exercise, infection, the elderly, hepatic insufficiency; beware of possible masked forms of von Willebrand disease (repeat measurements).

– vWF reduced (< 50%): the cut off below which the diagnosis of von Willebrand disease is made is ≤ 30% *(ASH education program book 2009, Sadler and Rodeghiero)*. When the vWF is between 30 and 50%, measurements must be repeated as the molecule is fragile. This may be a blood group O person or von Willebrand disease in a patient with an acute phase response or a pregnant woman, a heterozygote carrier of a type 3 abnormality or "low Willebrand" patients.

"low Willebrand" patients can be blood group O or non-O. They have no family history of bleeding and are generally a- or paucii-symptomatic (low bleeding score). The Willebrand gene is normal. In reality this is not a disease but a risk factor.

Investigation of Von Willebrand factor Sub-types

	Normal	Types 1	Type 2A	Type 2B	Type 2M	Type 2N	Type 3	PLT-VWD*
VWF: Ag	N	L, ↓ or ↓↓	↓ or L	↓ or L	↓ or L	N or L	Absent	↓ or L
VWF: RCo	N	L, ↓ or ↓↓	↓↓ or ↓↓↓	↓↓	↓↓	N or L	Absent	↓↓
FVIII	N	N or ↓	N or ↓	N or ↓	N or ↓	↓↓	1-9 IU/dl	N or L
RIPA	N	Often N	↓	Often N	↓	N	Absent	Often N
LD-RIPA	Absent	Absent	Absent	↑↑↑	Absent	Absent	Absent	↑↑↑
PFA-100 CT	N	N or ↑	↑	↑	↑	N	↑↑↑	↑
BT	N	N or ↑	↑	↑	↑	N	↑↑↑	↑
Platelet count	N	N	N	↓ or N	N	N	N	↓
VWF multimer Pattern							Absent	Abnormal 
			Abnormal	Abnormal				

From Nichols et al. Haemophilia 2008; 14:171-232

These people have a relative risk of bleeding of x 1.9 compared to the general population. In significant bleeding the "low Willebrand" is associated with another abnormality (thrombocytopathy or deficiency of another factor). The mechanism responsible for this fall has not been identified (possible Y/C 1584 polymorphism?).

3- Role of vWD genotyping

– This investigation should not be performed in all cases, as the boundary between the 150 non-pathogenic polymorphisms and the mutations is blurred. The genotype-phenotype relationship is complex and other genes and environmental factors are involved in vWF.

Gene typing is however useful in specific cases particularly to confirm the 2B type (study of the vWF gene, exon 28), to distinguish the 2B type from pseudo-WBD (WBD gene and GPBA gene), and to study specific variants and composite heterozygotes (1/2N, 1/2A…). It is also useful in diagnosing 2N Willebrand's disease (who responds differently to DDAVP depending on the mutation).

TREATMENT

Aspirin and non-steroidal anti-inflammatory drugs are contra-indicated in patients suffering from von Willebrand disease.

If bleeding occurs, "minor measures" should be used first line. Anti-fibrinolytics (tranexamic acid) can be used for menorrhagia. Epistaxis should be treated with compression with resorbable gauzes and anti-fibrinolytic agents for any ENT bleeding.

If severe bleeding occurs, spray desmopressin or DDAVP can be used, 1 to 2 nasal sprays depending on whether the patient's weight is < or ≥ 50 kg, if a previous test has shown it to be effective, under clinical and laboratory monitoring.

Desmopressin test

This test is used in type 1 and some 2A forms but not in type 3 (desmopressin is relatively ineffective or ineffective) or in type 2B (risk of aggravating thrombocytopaenia).

It involves injecting a standard dose of 0.3 µg/kg in 30 ml of physiological saline over thirty minutes. Various samples are taken over time (up to 6 or 9 hours with extrapolation up to 12 or 24 hours). The test is positive if detectable von Willebrand factor levels are found at T12 with a moderately prolonged BT. In acquired forms of vWF deficiency, the half-life of vWF is greatly shortened and the correction lasts for a very short period of time.

It acts immediately in responders (peak at 30 minutes) with an increase in von Willebrand factor levels by 3 or 4 and the BT is corrected.

When desmopressin cannot be used (type 3; severe type 1, patients who do not respond to desmopressin and most type 2), von Willebrand factor concentrates are required: pure von Willebrand factor or von Willebrand factor + factor VIII. In emergencies, the combination vWF and F VIII form must be used first.

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WEAK D

DEFINITION

Almost 85% of people in Europe carry the D antigen (RH1). By convention, these people are described as "RH1 or Rh D positive". The 15% who do not carry the antigen are described as "RH:-1 or Rh D negative". The RH1 antigen is well developed at birth and is strictly limited to erythrocytes.

An RH1 red blood cell contains between 10000 and 30000 RH1 sites. The weak RH1 phenotype (RHW1) is characterised by a quantitative deficiency of RH1 antigen sites which, depending on the limit of sensitivity of the method used, produces a reduced reaction or failure to detect the antigen.

The term "weak D" formally called "Du" therefore groups together those RH1 phenotypes which are weakly expressed in measurement of RH status and those which appear negative in which the RH antigen cannot be identified by more complex techniques.

The definition of "weak D" depends on the reagents and methods used.

The prevalence of weak D antigen is relatively low and is in the region of approximately 1%, in the Caucasian population but is far higher in black Africans.

Weak D red blood cells are considered as being RH (D) positive as they can cause transfusion or foeto-maternal alloimmunisation in RH (D) negative people although they have very low immunogenic potential.

INDICATIONS

Weak D is tested for:

– In newborn babies born to Rh negative mothers: as it may cause maternal immunisation.

– In pregnant women: as measurement can avoid anti-D injection after childbirth.

– In blood donors: as a risk of alloimmunisation exists if the blood is given to D positive people.

This test is also conventionally performed in people with C and/or E antigen.

INFORMATION

This is identical as for standard RH1 typing.

METHODOLOGY

The different screening methods used for weak D antigen are:

– The indirect Coombs test or indirect anti-globulin test

– The proteolytic enzyme test

– Elution fixation.

The conventional method is ***the indirect anti-globulin test*** which is performed following the same practical methods as standard RH1 typing. Concomitant use of monoclonal reagents selected for their performance on RH1 normal and weak phenotypes and new methods such as filtration and the use of a proteolytic enzyme can increase the sensitivity of the test and detect weak RH1 variants in a single stage.

INTERPRETATION

The use of new methods and improvement in anti-D reagents has increased the limit of detection of weak D antigens and the prevalence of weak D antigens has therefore fallen.

Theoretically, weak D antigens should be interpreted as an example of variability in the reactivity of blood group antigens. They are not qualitatively different antigens from the D antigen but have reduced D reactivity which is transmitted as an inherited trait.

FOR FURTHER INFORMATION

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WEST NILE (virus)

DEFINITION

The *West Nile* virus is an arbovirus belonging to the *Flaviviridae* family and *Flavivirus* genus. It is a small, positive polarity, single strand icosahedric RNA virus, which was isolated for the first time in 1937 in the West Nile province in Uganda, hence its name. It infects mosquitoes, ticks and birds but also mammals that are considered to be accidental hosts (mostly horses and occasionally human beings). In human beings the virus is responsible for a sudden onset fever occasionally complicated by encephalitis. It is found in Africa, Madagascar, the Middle East, Australasia, India, the American continent and in Europe. In France it is found around the Mediterranean basin.

Synonym: West Nile virus / West Nile fever / Camargue fever.

INTRODUCTION

EPIDEMIOLOGY

The virus is transmitted to human beings by infected mosquitoes. Human-to-human infection cannot occur. Some cases of transmission have been reported through blood transfusions and following organ transplantation. The main hosts for the virus are wild and domestic birds. Migratory birds are responsible for the spread of the virus from Africa to Europe and Asia in spring. It is a ubiquitous virus found particularly in humid areas, which predispose to collections of mosquitoes. Since it was first identified in Africa the virus has been found in various regions of the world in the Middle East, India and Europe. Emergence of the virus in the United States is a recent phenomenon: an epidemic occurred in New York in 1999 (62 cases including 7 deaths). The virus has spread greatly there since: 1356 cases were diagnosed in 2008, of which approximately 50% involved encephalitis/meningitis and 44 were fatal and in 2009 when 663 cases were reported, 50% of which caused encephalitis/meningitis (source CDC, USA) and 30 of which were fatal. Human and equine cases were reported in the South-East of Continental Europe in 2008 and 2009.

CLINICAL FEATURES

Infection is usually asymptomatic or pauci-symptomatic in human beings involving an uncomplicated flu-like syndrome with a morbiliform rash. Complications involving meningitis or encephalitis occur in less than 15% of cases, particularly in children and in the elderly. These generally resolve but can be fatal in some circumstances.

INDICATIONS FOR MEASUREMENT

Diagnosis of West-Nile fever in a patient returning from an endemic area with compatible clinical signs.

Diagnosis of West-Nile fever is part of the epidemiological monitoring of the virus in France.

Differential diagnoses with other arboviral infections.

INFORMATION

SAMPLE

Peripheral blood should be taken during the acute viraemic phase.

CSF in meningitis/encephalitis.

QUESTIONS TO ASK THE PATIENT

Clinical features.

History of mosquito bite in an endemic area?

Recent transfusion?

SAMPLE STORAGE AND TRANSPORT

Samples must be transported according to national and international legal procedures for staff safety (triple bagged). Samples for direct diagnosis are sent to specialist laboratories with type P3 or P4 confinement premises. CSF must be transported as soon as possible whereas blood samples can be sent within 24 to 48 hours. Serum can be stored for a few days and should be transported at + 4°C.

ASSAY METHODS

DIRECT DIAGNOSIS

Isolation of the virus by cell culture from peripheral blood or CSF and detection of viral DNA by molecular methods (PCR, RT-PCR) performed in specialist laboratories.

INDIRECT DIAGNOSIS

Several methods are available although the most widely used in EIA with immunocapture detection of specific IgM combined with testing for IgG. Serology testing is a simple, sensitive method, which can be performed on serum and CSF, although is it occasionally difficult to interpret. Early and late serum samples are required for interpretation.

TREATMENT

There is no specific treatment for the disease and at present this is purely symptomatic. Two candidate vaccines against the West-Nile zoonosis have recently been produced. Prophylaxis is essential and involves mosquito eradication, which is already undertaken in France in the affected regions, the use of mosquito repellents and monitoring the main hosts of the virus.

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WHOOPING COUGH

DEFINITION

Whooping cough is a highly contagious respiratory tract bacterial infection. It affects human beings at all ages of life, particularly the newborn and unvaccinated infants. Its typical form is caused by *Bordetella pertussis*. According to the World Health Organisation (WHO) it affects an average of 50 to 60 million people annually throughout the world. *Bordetella parapertussis* is responsible for a whooping cough-like syndrome (parawhooping cough). *Bordetella bronchiseptica*, a pathogenic upper respiratory tract bacterium in domestic animals can rarely cause pseudo-whooping cough in human beings

Synonyms: *Bordetella pertussis*, Bordet Gengou bacillus.

INFORMATION

EPIDEMIOLOGY

Whooping cough has increased again markedly over the last ten years in industrialised countries. Most cases of whooping cough affect infants who have not yet been immunised and are infected by an adult, although it also affects children and adults who have been vaccinated previously. Human transmission occurs within communities or within the same family, with infection occurring from spray secretions from a cough, from an infected person.

SYMPTOMS

Whooping cough is defined as an association of an infectious syndrome (the bacterium binds and multiplies in target cells lining the surface of the trachea and bronchi) and a toxic syndrome (it secretes toxins which destroy the ciliated cells). Clinical features vary with age:

Classical form in the unvaccinated child

– Average incubation period 10 days.

– Invasion phase: Simple cough and rhinitis for 10 to 15 days.

– Established disease phase: Coughing fits over a period approximately 3 to 4 weeks, usually at night and occasionally followed by thick expectoration and vomiting. The coughing fits are repeated violent attacks of expiratory shaking coughing with no inspiratory period, producing facial congestion or cyanosis typically followed by a deep noisy expiratory phase, sounding like a cock crow.

– Convalescence phase: Disappearance of the coughing fits, but persistent residual cough for several weeks.

Newborn or unvaccinated infant form

Materno-foetal immune protection is almost non-existent and new-born babies and infants are therefore extremely exposed to infection. The cough occurs in fits and produces cyanosios without an inspiratory phase (asphyxiating coughing fits). The severe disease is due to respiratory (apnoea, super-infection pneumonia and atelectasis), cardiac (profound bradycardia), neurological (seizures and encephalopathy) and nutritional complications due to dehydration or malnutrition. The mortality rate is 2 to 3%. Whooping cough appears to be involved in sudden infant death.

Previously vaccinated child and adult form

The immunity acquired from vaccination or from the natural disease falls gradually over time explaining the possibility of recontracting the infection, with variable degrees of severity. It is always less severe however than the classical form and is characterised by persistent atypical cough. Because of this, adolescent or adult whooping cough is often not recognised.

SEARCH INDICATIONS

Diagnosis of whooping cough in an infant or new-born baby.

Diagnosis of whooping cough in an adolescent or adult with prolonged cough.

Diagnosis of whooping cough in an unvaccinated child.

Differential diagnosis from other whooping cough-like syndromes (Adenovirus bronchial disease, Chlamydia and Mycoplasma pneumonias) or from other causes of refractory coughs (influenza, respiratory allergies, tuberculosis, cystic fibrosis, infective pneumonias, etc.).

INFORMATION

SAMPLE

To identify the bacterium an early sample must be taken, as soon as the initial symptoms develop. The amount of sample has a huge impact on the value of the result.

Nasopharyngeal secretions: Taken by nasal aspiration or swabbing using a flexible swab introduced through the nostril up to the posterior nasal fossa.

Bronchial secretions or sputum: Collected during a coughing fit when it is difficult to perform the nasopharyngeal aspiration.

Blood: Taken into a dry tube for serological diagnosis and into EDTA for a full blood count.

QUESTIONS FOR THE PATIENT

Clinical features (type of cough)?

Age of patient?

Immune status (date of 1st injection and dates of booster if vaccinated)?

History of disease in close family members?

Current antibiotic treatment?

SAMPLE STORAGE AND TRANSPORT

Nasopharyngeal or bronchial samples must be transported as soon as possible to the laboratory (< 2 hours at room temperature for culture or within 24 hours for PCR) as no transport or storage medium is available and the organism is fragile. Serum should be stored at + 4°C.

DIAGNOSTIC METHODS

GUIDING DIAGNOSTIC FEATURES

- **Leukocytosis with lymphocytosis** in the paroxysmal phase.
- **ESR** normal or slightly raised.

DIRECT DIAGNOSIS

- **Direct examination** of a nasopharyngeal aspirate by immunofluorescence is no longer recommended because of its lack of sensitivity and specificity.
- **Culture on specific media:** Bordet-Gengou or Regan Lowe, from a biological sample inseminated promptly. Culture is highly specific although its sensitivity which is estimated to be 50 or 60% at the start of the period of the coughing fits falls rapidly thereafter. It must therefore be performed with in the first 2 weeks of the disease.
- **PCR:** Detection of bacterial DNA from a nasopharyngeal aspirate or sputum. This offers good specificity and is more sensitive than culture.

  It is however very rare to detect bacterial DNA when the sample is taken more than three weeks after the onset of symptoms.

INDIRECT DIAGNOSIS

- **Agglutination** detects antibodies which agglutinate the bacteria in the case of a vaccine antibody (whole organism vaccines) but this is far less sensitive with primary infection antibodies.
- ***The complement fixation reaction*** lacks sensitivity.
- ***ELISA*** is used for semi-quantitative detection of *B. pertussis*-specific antibodies using purified antigens (pertussin toxin or PTX, filamentous haemagglutinin or FHA).
- ***The Western Blot method (or protein immunoblot method)*** provides qualitative detection of two antibodies at the same time, one against the *B. pertussis*- specific PTX and the other against adenyl cyclase-haemolysin (AC-Hly) which is a toxin common to *B. pertussis* and *B. parapertussis*. It is however relatively common to find the anti-adenyl cyclase antibodies in isolation, which has no, defined diagnostic association.

INTERPRETATION

Although PCR has become the most widely used direct diagnostic method because of its high sensitivity, ***culture*** currently remains the method of certainty to diagnose early whooping cough. It can differentiate *B. pertussis* from *B. parapertussis* infections and is used to monitor the change in circulating *Bordetella* strains.

Serology is useful when PCR or culture cannot be performed or are negative although only provides a retrospective diagnosis. It is difficult to interpret if vaccination has occurred less than a year previously as it does not distinguish vaccine antibodies from natural antibodies. Antibody titres need to be compared on 2 serum samples taken 2 to 3 weeks apart.

TREATMENT

MEDICAL

- **Antibiotic therapy** eradicates the organism in 4 to 5 days if administered early for approximately 15 days although does not change the course of the disease. It can reduce the infectivity of the disease. The reference antibiotic is *erythromycin* although other macrolides, *josamycin, roxithromycin, clarythromycin* and *azithromycin* are also used.
- **Corticosteroids** are occasionally useful in serious forms to reduce the severity of the coughing fits.
- **Hospitalisation**

  For infants under 6 months old for constant cardio-respiratory monitoring.
- **Prophylaxis**

  By administering macrolides to people who have come into contact with an affected patient, for a period of 7 to 10 days.
- **Vaccination**

  The vaccines currently available in France are:

The conventional whole organism vaccine combined with diphtheria, tetanus, poliomyelitis and *Haemophilus influenzae* vaccines, type b: Pentacoq®.

Acellular vaccines composed of one or more purified antigens which may be combined with other vaccines. These are better tolerated than the whole organism vaccines but are probably less effective, particularly following primary vaccination.

The recommended vaccine protocol is 3 injections at one month intervals from the age of 2 months old with the whole organism vaccine followed by a first booster at the age of 18 months old with the whole organism vaccine or acellular vaccine and a second booster at between 11 and 13 years old with the acellular vaccine.

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XYLENES

DEFINITION

Xylenes or dimethylbenzenes are colourless, mobile liquids with a characteristic pleasant smell, which are practically insoluble in water. They are excellent solvents for many natural and synthetic substances (oils, fats, resins, etc.) and are widely used as solvents for pains, lacquers, printing inks, adhesives, in the preparation of insecticides and dyes, in the rubber industry and for pharmaceutical products. Isomers are used in organic synthesis to manufacture phthalic anhydride (o-xylene), isophthalic acid (m-xylene) and terephthalic acid (p-xylene). They are also found in some fuels and petroleum products.

METABOLISM

The main route of xylene absorption is through the lungs (65% of inhaled xylene, regardless of isomer, are absorbed after exposure for 10 minutes). Liquid xylenes can be absorbed through the skin. Gastro-intestinal absorption, which is probably extensive, has not been studied. Xylenes are distributed throughout blood and tissues over 6 hours and in adipose tissue over a few days. 95% of absorbed xylenes are metabolised in the liver by oxidation into methylbenzoic acids, which are then conjugated with glycine to perform methylhippuric acids (or toluric acids), the main metabolites that are 95% removed in urine. Negligible amounts of xylenes are excreted in the unchanged form in urine (< 0.01%) and are less than 6% of the amount absorbed in expired air. Removal is at a maximum at the end of the working shift. Xylenes can accumulate in the body (its metabolites do not accumulate at concentrations of up to 100 ppm).

SYMPTOMS OF POISONING

■ ACUTE POISONING

Xylene ingestion causes:

– gastro-intestinal problems: abdominal pain, nausea, vomiting and diarrhoea;

– central nervous system depression: inebriation followed by disordered consciousness or even coma with seizure with massive doses. The declining in psychomotor functions depends on concentration and duration of exposure;

– inhalation pneumonia;

– ocular and upper respiratory tract irritation;

– irritation dermatoses when sprayed onto skin.

■ CHRONIC POISONING

The main route of occupational poisoning is via the respiratory tract. The main toxic effect of the xylenes is a psycho-organic syndrome with disordered memory concentration and personality and reduced intellectual faculties.

Xylenes have a drying and decreasing contact effect on the skin and cause chronic irritation dermatoses (dry scaling skin).

INDICATIONS FOR MEASUREMENT

Measurement of urinary, methylhippuric acids at the end of the working shift is a specific indicator of exposure on the same day, which correlates well with the extent of exposure. Excretion of the meta isomer predominates compared to the other isomers, regardless of the respected proportion of each isomer in the xylene mixture. Measurement of blood xylenes within 2 hours after the end of exposure is an accurate reflection of recent exposure because of its rapid removal. Measurement is specific, subject of individual variations and has no advantage over measurement of metabolites.

INFORMATION

■ SAMPLE

5 ml of whole, heparinised or EDTA blood.

20 ml of an unacidified urine sample into a plastic bottle.

The sample should preferably be taken at the end of the work shift.

■ SAMPLE STORAGE AND TRANSPORT

Whole blood and urine samples can be stored for several days and transported to the laboratory at between + 2 and + 8°C.

■ INTERFERENCES

Enzyme inducing medicinal products, aspirin, ethylbenzene, toluene and trichloroethane inhibit xylene metabolism. On the other hand, alcohol may either increase or reduce metabolism following high exposure to xylenes or chronic ingestion.

ASSAY METHODS

Gas phase chromatography (GPC).

High performance liquid chromatography with UV detection (HPLC/UV).

REFERENCE VALUES

According to INRS recommendations:

– Blood xylenes:

- General population value < 5 µg/l
- Guideline reference value (Switzerland, Germany): 1.5 mg/l at the end of the work shift.

– Urinary methylhippuric acids:

- General population value < 50 mg/g of creatinine
- Guideline reference value in France: 1500 mg/g of creatinine at the end of the work shift

FOR FURTHER INFORMATION

■ Toxicologie industrielle et intoxications professionnelles, *Xylènes,* 3e édition, Lauwerys R., Masson.

■ Fiche toxicologique des xylènes, INRS, 2005.

YELLOW FEVER

DEFINITION

Yellow fever (YF) is an arboviral infection causing hepatitis and nephritis in a haemorrhagic context. It is due to an arbovirus *(arthropod borne virus)*.

This virus, also called amaril virus or Yellow Fever Virus (YFV), belongs to the Flaviviridae family and to the Flavivirus genus. It has a viral envelope, measures 40 to 60 nm in diameter and possesses a non-segmented single-stranded RNA with a positive-sense polarity.

Synonyms: Yellow Fever Virus, Amaril Virus and YFV.

INTRODUCTION

EPIDEMIOLOGY

Despite the existence of an effective vaccine, YF is still rife in Africa and South America in endemic and epidemic forms. It is clearly recrudescing in Africa. By contrast, it is becoming less common in South America and is not found in Asia or Oceania. Some imported cases are regularly reported.

YF affects around 200,000 people per year, causing 30,000 deaths.

It presents in 3 ways:

– ***Animal YF***, which involves mosquitoes and monkeys and rarely affects man.

– ***Jungle YF***, responsible for rural epidemics, sometimes with increased mortality.

– ***Urban YF***, responsible for devastating epidemics with very high mortality rates.

Man is an accidental host, with the definitive reservoir being animal (predominantly the monkey). The vector is the *Aedes* mosquito.

SYMPTOMS

After a short incubation period (3 to 6 days) the illness develops in 3 phases:

The red phase: Marked by the sudden appearance of high fever with non-specific general features (headaches and myalgia) and facial flushing.

The remission phase: Very short (a few hours).

The yellow phase: Haemorrhagic hepatitis and nephritis, in which haemorrhagic features are associated with hepatic damage (muco-cutaneous jaundice and bloodstained vomiting) and renal involvement (oliguria, anuria, frank albuminuria and high urea).

Progression to death occurs over 5 to 10 days, as a result of hypovolaemic shock, acute renal failure or acute hepatic failure.

In some cases the illness progresses favourably to recovery without sequelae.

Besides these classical forms, there is also a great variety of other clinical pictures, such as asymptomatic, hidden forms with few symptoms, fulminant disease, and forms with neurological or cardiovascular involvement.

SEARCH INDICATIONS

Diagnosis of high fever with suggestive clinical features in someone returning from an endemic area.

Differential diagnosis of hepatitis, malaria, other arboviruses and other haemorrhagic fevers.

Check on immunity after vaccination.

INFORMATION

SAMPLE

Peripheral blood for direct isolation of the virus.

Two serum specimens at a 1 week interval for serological diagnosis.

CSF (if encephalitic) for virus isolation or antibodies.

Post-mortem biopsy specimens (especially liver) for confirmation of viral aetiology.

QUESTIONS FOR THE PATIENT

Return from a stay in an endemic area?

Clinical symptoms?

Vaccination?

SAMPLE STORAGE AND TRANSPORT

Serum should be stored and transported at + 4°C.

DIAGNOSTIC METHODS

DIRECT DIAGNOSIS

The virus can be detected in peripheral blood taken during the acute phase, between the 1st and the 6th day after the onset of fever.

It can be isolated by cell culture in mammalian VeroE6 cells or in cultures of mosquito cells. The technique of RT-PCR can be used to expedite diagnosis by detecting the viral genome. There is no satisfactory test for the detection of the antigen in blood.

For all methods, the YF virus should be handled under level 3 bio-safety conditions.

INDIRECT DIAGNOSIS

In general, IgM antibodies appear at about 4 to 5 days after the fever develops, they increase rapidly and persist for 3 to 6 months.

IgG antibodies appear after a period, the length of which depends on whether the infection is primary or secondary. Serological diagnosis of an acute infection is made using EIA (enzyme immunoassay) looking for specific IgM by immunocapture.

Testing for anti-amaril antibodies after vaccination employs a sero-neutralisation test with determination of the titre of neutralising antibodies.

NON-SPECIFIC DIAGNOSIS

There are blood test results which are non-specific but suggestive, such as leukopaenia with neutropaenia and lymphopaenia, high ESR, increased blood transaminases, inceased bilirubin and urea, plus changes in coagulation measurements (increase in prothrombin time and APTT). There may also be albuminuria and haematuria.

INTERPRETATION

The diagnosis of yellow fever is urgent. The request for testing must always be accompanied by clinical and epidemiological information.

Except for simple situations where it has been possible to identify the viral genome or the virus itself, the interpretation of results must be performed carefully, and needs to take account of the date of sampling in relation to that of the first day of fever.

Serological diagnosis always poses the problem of cross-reactivity within the flavivirus group and especially with Dengue Fever and Japanese or West Nile Encephalitis. It is necessary to take another serum sample one week later to be able to interpret results.

TREATMENT

There is no specific treatment for YF. Therapy is, therefore, purely symptomatic, requiring intensive care facilities, which sometimes need to be sophisticated and thus difficult to establish in the affected countries.

There are many possible prophylactic measures (tackling the vector and isolation of patients, for example) but vaccination remains the most effective. This involves a live attenuated vaccine grown in fertilised chicken eggs (Rockefeller 17D strain). A single injection confers immunity for 10 years. It is contraindicated in children aged less than 6 months and in immunodeficient patients. Theoretically, vaccination is mandatory for anybody travelling to or returning from an endemic zone, but this does not always happen. The difficulty in storing heat sensitive vaccines and their high cost, have made it impossible to perform general vaccination in many endemic areas.

FOR FURTHER INFORMATION

RODHAIN F., *Fièvre jaune, dengue et autres arboviroses*, Encycl Med Chir, Maladies infectieuses, 8-062-A-10 ; 2001: p 1 à 18.

YERSINIOSIS

DEFINITION

Yersiniosis is a clinically varied set of infectious diseases caused by bacteria belonging to the *Yersinia* genus and *Enterobacteriaceae* family. They are zoonoses, which are occasionally transmitted to human beings. Three species are indicated in human disease, *Yersinia enterocolitica* and *Y. pseudotuberculosis* responsible for gastro-intestinal disease, and *Yersinia pestis*, the agent responsible for plague. Each species is divided into serotypes or serovars according to their somatic O antigen. The *Yersinia* are Gram-negative bacilli.

INTRODUCTION

EPIDEMIOLOGY

Most infections are sporadic.

– ***Yersinia enterolitica***, the most common species is found widely in the environment, in earth, ground rainwater, plant and animal foods. It is also found in the gastro-intestinal tract of some animals (pigs, cattle, sheep, goats, dogs, cats, rodents and poultry). The main reservoir appears to be butchery pork. It is found throughout the world, with a higher incidence in cold months due to its particular feature of growing at low temperature. Because of this it is found in refrigerated foods. It is transmitted to human beings faeco-orally, usually from eating contaminated food but also from direct contact with an infected or sick animal.

– ***Yersinia pseudotuberculosis*** is found in the earth and in some animals, particularly rodents, and is ubiquitous. The pseudotuberculosis species contains 6 serotypes (I to VI) the most common of which is the type I serotype.

– The reservoir for ***Yersinia pestis*** is wild rodents (rats). Plague still occurs in some geographical areas limited to South East Asia, South America and East and South Africa.

PATHOPHYSIOLOGY

Y. enterocolitica serovars O: 3, O: 5, O: 8 and O: 9 are responsible for most of the human infections. The pathogenic potential of *Y. enterolitica* is due to the secretion of an enterotoxin and to its invasive potential, which itself depends on chromosomal genes and a virulence plasmid pYV (present in all pathogenic strains).

SYMPTOMS

Gastro-intestinal disease:

Y. enterocolitica and *Y. pseudotuberculosis* cause febrile gastro-enteritis, which is more common in young children than in adults. The diarrhoea is watery or bloody. Mesenteric adenitis is common, particularly with *Y. Pseudotuberculosis* and causes right iliac fossa pain mimicking acute appendicitis. Yersiniosis recovers spontaneously in 1 to 2 weeks in healthy adults, although takes longer in children. Rare generalised septicaemia however may occur in specific situations: cirrhosis, diabetes, immunodeficiency and haemochromatosis. Post-transfusion septicaemia may also occur.

Non-invasive non-gastro-intestinal complications (mostly autoimmune):

– Reactive arthritis may develop 1 to 3 weeks after gastro-intestinal infection, particularly in young adults of tissue group HLA B27.

– Erythema nodosum may develop 1 to 2 weeks after gastro-intestinal infection, particularly in young women.

– Other complications described: Fiessinger-Leroy-Reiter syndromes, pericarditis, thyroiditis and glomerulonephritis.

SEARCH INDICATIONS

Diagnosis of Yersiniosis in a setting of febrile childhood gastro-enteritis.

Diagnosis of mesenteric adenitis or pseudo-appendicitis.

Diagnosis of Yersiniosis with autoimmune complications after gastro-intestinal infection.

Differential diagnosis from other causes of bacterial (Salmonellae, *E. coli*), viral or parasitic gastro-enteritis.

Differential diagnosis between *Y. enterocolitica and pseudo-tuberculosis*.

INFORMATION

SAMPLE

Stool sample collected into a sterile container for stool culture.

Mesenteric lymph nodes.

Venous blood for blood cultures.

Serum samples for serological diagnosis.

QUESTIONS FOR THE PATIENT

Clinical signs (diarrhoea and fever)?

History of eating a potentially contaminated food?

Current antibiotic treatment?

SAMPLE STORAGE AND TRANSPORT

Stool: must be transported promptly to the laboratory at room temperature or stored for up to a maximum of 12 hours at + 4 °C. Beyond this time a transport medium must be used.

Blood cultures: transported as quickly as possible at room temperature.

Serum samples: stored at + 4°C for up to a week, then frozen at – 20 °C for up to 1 year.

DIAGNOSTIC METHODS

DIRECT BACTERIOLOGICAL DIAGNOSIS

Isolation of the organism from faeces requires a selective medium: the colonies seen are very small and translucent.

They are identified biochemically from fermentation of sugars, the immobile nature of the mobility of the organism at 37°C and mobility below 30°C and their urease activity.

Antigenic or identification or serotyping is performed via type of somatic O antigen (more than 75 O antigens have been identified in *Y.enterocolitica* and neighbouring species).

INDIRECT SEROLOGICAL DIAGNOSIS

The microtitre plate sero-agglutination method is the most widely adopted and uses somatic antigens obtained from strains of *Yersinia pseudotuberculosis* (serotypes I to V) and the main serotypes of *Yersinia enterolitica* (O3, O9, O5). Other methods are available including complement fixation, indirect immunofluorescence and haemagglutination. Cross-reactions occur particularly between *Y. enterocolitica serotype O9* and the *Brucella* genus and between *Y. pseudotuberculosis* and the Salmonellae. Elisa techniques can be used to test for antibodies against proteins coded for by the virulence plasmid pYV. This method is very sensitive and uses an antigen produced from strains of *Y. enterocolitica O3*, limiting the cross-reactions with *Brucella* genus.

INTERPRETATION OF RESULTS

Stool culture is the diagnosis of choice for *Y. enterocolitica* and *pseudotuberculosis* infections. *Y. enterocolitica* is found in faeces during the episode of diarrhoea but also after the diarrhoea and even following clinical recovery.

Y. pseudotuberculosis can only be isolated from faeces at the time of the diarrhoea.

Sero-diagnosis is useful if difficulties are experienced isolating the bacterium or to make a late or even retrospective diagnosis of the disease. This is positive in all forms of the disease: mesenteric adenitis, reactive arthritis, erythema nodosum and spondyloarthritides.

Serotyping is of epidemiological use.

TREATMENT

CURATIVE

Diarrhoea is treated symptomatically. Antibiotic therapy is reserved for patients with persistent enterocolitis, septicaemia or generalised disease or in immunodeficiency: fluoro-quinolones, 3rd generation cephalosporins, co-trimoxazole or oral aminoglycosides. Strains of *Yersinia pseudotuberculosis* are generally sensitive to the β-lactams, *Y. enterocolitica* strains may produce chromosomal β-lactamases (a constitutional penicillinase and inducible cephalosporinase).

PREVENTIVE

Observing hygiene measures during food transport, adequate cooking of pork.

FOR FURTHER INFORMATION

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■ Benoit C., Guiyoule A., Carniel E., *Sérodiagnostic des infections humaines à Yersinia pathogènes*, Presse Méd., 1996 ; 25/34 : 1627-1630.

ZINC

DEFINITION

Zinc (Zn) is an essential trace element involved in many physiological functions. Zinc requirements are in the region of 10 to 15 mg/day in children and adults and are increased in pregnancy (approximately 20 mg/d) and breast-feeding (25 mg/d).

The main sources of zinc in the diet are red meats, fish and seafood (particularly oysters). Deficiency is common in human beings, even in countries with high living standards. Poisoning also occurs in exposed workers by inhalation or skin contact with metal vapours. The main sources of exposure are zinc mines, zinc metallurgy, galvanisation processes, alloy manufacture (brass: zinc + copper) and use of zinc containing pigments and salts.

METABOLISM

Zinc is the most abundant trace element after iron in the body, which contains approximately 2.5 g (38 mmoles), 30% of which are in bone and 60% in muscle. The richest zinc containing tissues are prostate, hair and eye.

Gastro-intestinal absorption takes place mostly in the jejunum. The exact mechanism is not entirely understood, although it appears that zinc is bound by the brush border, as a complex and that prostaglandins play a role in absorption. The route of occupational absorption, is principally by the lungs as smoke or dust (zinc oxide). After gastro-intestinal absorption, zinc is distributed throughout the intestinal cell, some is used *in-situ* by binding to metalloenzymes or membrane proteins and some is excreted from the cell by the basolateral membrane and passes into the systemic circulation. A final component is stored as metallothioneines (low molecular weight proteins which play a regulatory role in release of zinc, copper and cadmium). In the blood, zinc is concentrated in red blood cells (80%), 10% is found in leucocytes and platelets and 10% in plasma. In plasma, zinc is 60% bound to albumin, 30-40% bound tightly to alpha-2-macroglobulin and 2 to 3% in an ultrafilterable form. The plasma peak is found 2 to 4 hours after oral administration. Plasma zinc has a half-life of 12.5 days.

Zinc is mostly removed in faeces (70 to 80%) but also in urine and sweat (15 to 25%). Urine zinc excretion is constant in healthy human beings, in the region of 0.5 mg per day. Excretion depends on the ultrafilterable plasma fraction, dietary intake and level of exposure in occupationally exposed workers. Urine zinc concentrations are subject to diurnal variations and are increased in some diseases (cirrhosis and nephrotic syndrome).

MECHANISM OF ACTION

Zinc is involved in the major metabolic pathways through its role in enzyme systems either as an integral part of the active site of many enzymes or as a co-factor regulating the activity of so-called "zinc-dependent enzymes" or with its own structural role.

As such it influences the activity of more than 70 enzymes including carbonic anhydrase, superoxide dismutase, alkaline phosphatase, glutamate and lactate dehydrogenase, RNA and DNA polymerases, amino-peptidases etc. It also is involved in the activity of some hormones: prostaglandins, insulin, growth hormone, prolactin, testosterone, thymuline and gustine. Zinc's involvement in these enzymes and hormones explains its importance in all metabolism (protein, lipid and carbohydrate) and all body functions (immunity, growth, fertility, healing, taste and vision). The prostate is the gland, which concentrates and contains most zinc in body.

INDICATIONS

Measurement in occupationally exposed workers.

Measurements in anti-oxidant assessments.

Suspected Pick's disease (hereditary familial hyperzincaemia).

Suspected acrodermatitis enteropathica (deficient zinc absorption): alopecia, diarrhoea, skin lesions and growth retardation.

Zinc in semen is a marker of prostate function.

INFORMATION

■ SAMPLE

Erythrocyte zinc: 5 ml of whole heparinised blood (do not use a glass tube).

Plasma zinc: 2 ml of plasma collected into sodium heparinate (special trace elements tube) or failing this into a dry plastic tube with no gel or coagulation activator (be aware of dry vacuum tubes as most contain these), preferably at the end of the work shift at end of week in exposed workers. Avoid any haemolysis in plasma samples as red cell zinc concentrations are 10 times greater than plasma concentrations.

Urine zinc: 20 ml of a urine sample (or 24 hour urine collection), unacidified: urine should preferably be collected at the end of the shift at end of week.

Zinc in semen: 2 ml of semen collected after absence from sex for 5 days. Collect into a fluorinated tube. Record the ejaculate volume, centrifuge the sample and freeze the supernatant within an hour of sampling.

■ SAMPLE STORAGE AND TRANSPORT

Whole blood, plasma or urine samples can be stored and transported to the laboratory at between + 2 and + 8°C.

Semen must be frozen at – 20°C.

ASSAY METHODS

Atomic absorption flame spectrophotometry.

REFERENCE VALUES

Plasma zinc: 0.50 to 1.50 mg/l.

Erythrocyte zinc: 10 to 15 mg/l, i.e. 152 to 229 µmol/l.

Urine zinc: 350 to 600 µg/24h, i.e. 5.3 to 9.1 µmol/24h, or < 0.70 mg/g of creatinine.

Semen zinc: 80 to 200 mg/l, i.e. 1.2 to 3.0 mmol/l.

PATHOLOGICAL VARIATIONS

■ ZINC DEFICIENCIES

■ **Symptoms**

– Diarrhoea
– Growth retardation
– Skin lesions
– Poor healing
– Alopecia
– Loss of taste and smell
– Ocular disturbances
– Reduced immune response.

■ **Causes of deficiency**

Apart from dietary deficiencies, many pathological situations may cause zinc deficiency:

Malabsorption
– Pancreatic insufficiency
– Biliary obstruction
– Gastrectomy
– Jejuno-ileostomy
– Intestinal diverticuli
– Tropical sprue
– Coeliac disease
– Cystic fibrosis.

Inflammatory bowel disease
– Protein-losing enteropathies
– Inflammatory colitis.

Liver diseases
– Cirrhosis and hepatitis.

Renal disorders
– Chronic renal insufficiency
- Nephrotic syndrome.

Neuro-psychiatric disorders
– Anorexia nervosa
– Endogenous depression
– Alcoholism.

Genetic diseases
– Acrodermatitis enteropathica: an autosomal disease due to partial deficiency of intestinal zinc absorption. This develops usually during the first months of life and is characterised by skin rash with secondary infections and erythematous and then vesicular pustules and hyperkeratotic skin lesions. The lesions only develop in breast-fed children after weaning. Zinc sulphate treatment must be taken for life.
– Thalassaemia, sickle cell anaemia
– Diabetes
– Trisomy 21
– Phenylketonuria.

Parasitic diseases
– Ankylostomiasis
– Schistosomiasis
– Malaria
– Giardiasis.

Medical drugs
– Diuretics
– Penicillamine.

■ HYPERZINCAEMIA

Acute poisoning

By industrial exposure to zinc through inhalation of metal vapours causing pulmonary features and fever.

Chronic poisoning

Causes allergic dermatoses, conjunctivitis and bronchitis.

Pick's disease

Familial hereditary hyperzincaemia.

FOR FURTHER INFORMATION

■ Toxicologie industrielle et intoxications professionnelles, *Zinc*, Lauwerys. R., 3e édition, MASSON : p. 246-248.

■ Fiche du zinc plasmatique et urinaire : guide BIOTOX 2002 – INRS.

■ Chappuis P., Les oligoéléments en médecine et biologie : *le zinc,* Ed. Lavoisier-Tec & Doc, 1991 : p. 347-397

ZINC PROTOPORPHYRIN

DEFINITION

Porphyrins are pigments, and are cyclical tetrapyrrole derivatives, grouped into uro-, copro- and protoporphyrins (2, 7, 12, 18-tetramethyl-3, 8-divinyl-13 and 18-dipropanoic-porphins). Erythrocyte protoporphyrins are either free or incorporate zinc (zinc protoporphyrin or ZPP) under the action of ferrochelatase. ZPP regulates heme catabolism by inhibiting heme oxygenase and its half-life is 2 to 3 weeks.

INTRODUCTION

– In subjects suffering from lead poisoning (sometimes referred to as saturnism), lead modifies the properties of numerous cytosol and membrane proteins by irreversibly bonding to thiol groups. As a result of this, the lead inhibits enzymes, particularly those involved in the heme biosynthesis pathway, such as aminolevulinic acid dehydratase (ALAD) and ferrochelatase. ALAD inhibition results in increased urinary secretion of ALA. Ferrochelatase inhibition causes an accumulation of free erythrocyte protoporphyrin (not bonded to iron) and results in heme deficiency, which affects haemoglobin synthesis and also cellular processes, such as mitochondrial respiratory activity and oxidative metabolism.

– When an iron deficiency is present, ferrochelatase uses the zinc as an alternative substrate and we see an increase in intraerythrocyte ZPP concentrations. This rise in ZPP is among the first biochemical responses to iron depletion.

INDICATIONS FOR MEASUREMENT

■ DIAGNOSIS OF LEAD POISONING

Certain tests reveal exposure and these include plumbaemia, spontaneous or provoked plumburia and osseous lead. Other tests reveal the repercussions on the body, such as urinary δ-aminolevulinic acid (δ-ALA), intraerythrocyte protoporphyrins, particularly in the form of zinc protoporphyrin (ZPP), and also free protoporphyrins. Erythrocyte protoporphyrins, or their fraction bonded to zinc (ZPP), indicate exposure in the preceding months.

ZPP measurement is simple, rapid and inexpensive. It is also more sensitive than urinary ALA measurement and independent of any contamination by lead. ZPP measurement is strongly correlated with plumbaemia at between 350 and 800 µg/l. In cases of stable and prolonged exposure, ZPP is a good indicator of the biologically-active pool of lead. ZPP rises at a later stage than urinary ALA. ZPP measurement, however, is not highly specific: iron deficiency, haemolytic anaemia and disorders of haemoglobin and porphyrin metabolism can cause a rise in ZPP.

INFORMATION

■ SAMPLE

Heparinated or EDTA whole blood (5 ml).

■ SAMPLE STORAGE AND TRANSPORT

Samples can be stored and transported for several days at +4°C, protected from light.

ASSAY METHODS

Haematofluorometry: Micromethod measurement is possible. Detection limit: 0.2 µg/g haemoglobin, accuracy: coefficient of variation < 10 %.

High performance liquid chromatography followed by fluorometric detection.

EXPECTED VALUES

Intraerythrocyte ZPP concentration: < 715 nmol/l i.e. < 2.5 µg/g haemoglobin (< 400 µg /l).

Workers exposed to lead: < 20 µg/g Hb

Obvious poisoning: > 20 µg/g Hb

PATHOLOGICAL VALUES

– Lead poisoning: Elevated intraerythrocyte ZPP concentration becomes detectable when plumbaemia exceeds 200-250 µg/l. If plumbaemia reached 800 µg/l, ZPP concentrations do not rise any further. Iron deficiency, haemolytic anaemia or erythropoietic protoporphyria interfere with the results. An elevated carboxyhaemoglobin result causes an underestimation of ZPP if the assay is performed using haematofluorometry.

– Iron deficiency: Early increase in intraerythrocyte ZPP

– Ferrochelatase enzyme deficiency (erythropoietic protoporphyria): Free erythrocyte protoporphyrin ↗↗↗, and ZPP ↗↗ (NOTE: ZPP and erythrocyte protoporphyrin are two distinct biological markers but are too often confused).

FOR FURTHER INFORMATION

■ *Plomb et composé minéraux.* Fiche toxicologique N° 59 de l'INRS (Institut National de Recherche et de Sécurité). www.inrs.fr.

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HAEMOSTASIS PREANALYTICS

BLOOD SAMPLING FOR HAEMOSTASIS

Certain clinical details about the patient (severe acute phase reaction, pregnancy), circumstances (bleeding, thrombosis, pre-operative assessment), the request and treatments (particularly anti-platelet treatments, anticoagulants, fibrinolytics and hormone treatments) which are being taken and which may influence the results are essential in order for the results to be correctly interpreted.

The blood group must be given for measurement of Von Willebrand factor and factor VIII, as reference levels are lower in blood group O people.

The sample should preferably be taken in the morning from a patient who has been resting for more than 5 minutes in the seated position: a light low fat breakfast is permitted. Smoking and caffeine (coffee, cola) should be avoided in the morning of the sample. Patients must also avoid all intense physical activity for at least 24 hours before the sample and any "rushing" on the morning itself.

The quality of the blood sample is extremely important. The sample should be taken using a tourniquet which is not too tight and which must not be left in place for more than a minute. The puncture site must be distant to any infusion.

- **Tube sampling order:** it is generally recommended that the haemostasis tube be taken second, after a dry tube without coagulation activator or a blood culture bottle, and before tubes containing other anti-coagulants such as citrate or coagulation activators. If only haemostasis studies are requested the citrate tube can be taken first if the phlebotomist is experienced, if there are no visible sampling difficulties and if the tourniquet is not too close to the puncture site, not too tight or kept in place for too long. Otherwise the first 2 millilitres of blood should be discarded (except when monitoring oral anticoagulation with the PT/INR, when this precaution is not required).
- **Tube identification:** tubes must be very carefully identified when the sample is taken, in front of the patient, stating as a minimum the patient's surname and forename and the sampling date and time.
- **Choice of tubes and needles:** Blood should be collected into sodium citrate anticoagulant, 0.129 M (3.8%) or 0.109 M (3.2 %) (1 vol/9 vol) and mixed by turning the tube over for 4 to 6 times in succession. Many publications recommend that 0.109 M (3.2%) citrate be used. Tubes containing a CTAD mixture (citrate, theophylline, adenosine, dipyridamole) can be used for measurement of unfractionated heparin and to study platelet membrane glycoproteins by flow cytometry. These must be stored away from light.

  Vacuum tubes should preferably be used, made of siliconised glass or polyethylene terephthalate (PET). The needle used to take the sample should ideally be between 19 and 21 gauge diameter for adults (≈ 1 mm); 23 gauge is acceptable for children. "Epicranial" butterfly needles can be used if the tubing is short (< 6 cm and dead space < 150 µl).
- **Anticoagulant/blood ratio and tube filling:** The volume of blood to be taken should be observed in order to maintain the ratio of 1 volume of anticoagulant/9 volumes of blood. Tubes must be at least 80% filled (ideally > 90%).

  The anticoagulant/blood ratio is no longer valid and must be taken into account in the results if the patient's haematocrit is > 55% or < 30%.
- **Sample, storage and transport temperature:** it is recommended that samples be transported and stored vertically before analysis, at between 18 and 22°C. Temperatures of between 2 and 4°C are strongly advised against. If the test is to be performed later, the samples must be aliquoted and frozen promptly *(cf below)*. Storage of frozen samples: 2 weeks at - 20°C and 6 months at – 70°C. The samples must be thawed promptly (5-10 min) in a water bath at 37°C and the test must be performed as soon as possible after the sample has been thawed and mixed by turning the tube over for 6 to 8 times in succession. If samples are to be tested later they should be stored at + 4°C, for no more than 2 hours. They cannot be refrozen for any function tests.
- **Sample *pH*** must be kept between 7.3 and 7.45. To do this the storage and transport conditions must be observed (transporting the tubes vertically before centrifuging and separating and avoiding any unintentional shaking, store closed tubes at room temperature). **N.B:** factors V and VIII deteriorate at pH > 8.
- **Dual centrifugation:** samples should be centrifuged twice (to obtain plasma which is very platelet and phospholipid particle depleted) to test for lupus anticoagulants. The activated protein C resistance test using the original method on fresh plasma and for any plasma must be frozen. Centrifuge as soon as possible and always within 3 hours of sampling at 2,500 g for 15 minutes at temperatures of between 18 and 22°C; remove the plasma carefully taking care to avoid removing cells from the centrifugation pellet and transfer the plasma into another PET tube and centrifuge again for 15 minutes at 2,500 g to 18-22°C; freeze promptly at – 20°C or below. The residual platelet count must be less than 10 G/L.
- **Time before sampling and the examination:** ideally this should be 1 to 2 h and must not be more than 4 h (6 h at room temperature for the Quick time). If this time cannot be met, centrifuge and separate the serum promptly and then freeze at -20 °C. Aliquots must be of small volume (500 to 1200 µl) in tubes with screw closures made of a non-wettable material and of a capacity suitable for the sample (lowest air volume possible). **Centrifugation:** for usual haemostasis tests on fresh plasma centrifuged for 15 min at 2000 – 2500 g.

Radius of centrifuge from the axis to the base of the centrifugation tube	Speed (rotations per minute) to obtain acceleration of 2,500 g
9 cm	5 000 rpm
10 cm	4 500 rpm
12 cm	4 300 rpm
15 cm	3 800 rpm
17 cm	3 600 rpm
20 cm	3 400 rpm
22 cm	3 200 rpm

The least trace of coagulation occurring during or after sampling may completely invalidate the test results. In particular, artefactual factor V rises have been seen in early coagulation. It is occasionally necessary to filter the blood through gauze to ensure that no microclots are present.

Sampling time for heparin, danaparoid or fondaparinux treatment

Table: Sampling time to monitor treatment with heparins (unfractionated heparin and a low molecular weight heparin) and danaparoid or fondaparinux.

Medicinal product	Treatment regimen	Sampling time
Standard heparin		
Unfractionated heparin (UFH)	Continuous IV injection	Any time after treatment for 4 hours
	Discontinuous injection	Mid-time between both injections
Low molecular weight heparin (LMWH)		
LMWH administered as 2 injections/24 h	SC	3 to 4 hours after injection
LMWH administered as 1 injection/24 h	SC	4 to 6 hours after injection
Danaparoid sodium	Continuous IV regimen	Any time after initial bolus
	SC regimen	Mid-time between two injections
Fondaparinux - Pentasaccharide	SC regimen	3 to 4 hours after injection

INVESTIGATION OF CAUSE OF THROMBOSIS ("THROMBOPHILIA SCREEN")

This should ideally be performed in patients who have been preselected, distant (4 to 8 weeks) to the episode of thrombosis and any acute inflammatory state and preferably off any anticoagulant. If the patient is receiving anticoagulation (heparin or AVK), this must be considered in the results *(cf. table on the next page)*.

INFLUENCE OF TREATMENTS

PROTEINS C AND S

Vitamin K dependent proteins are reduced during AVK treatment which makes their measurement difficult to interpret. The measurement should be performed 3 to 4 weeks after stopping the AVK (end of treatment or treatment window: possibly temporary switch to heparin). It should be noted that protein S falls during oestrogen-progestogen treatment (contraception, hormone replacement therapy), physiologically in pregnancy and in some inflammatory states. A period of 2 months after childbirth should be left or after stopping hormone treatments before measurements are performed.

Activated PS and PC can be over-estimated in patients treated with heparin or direct thrombin inhibitors (dabigatran, hirudins). Measurements can be invalidated in patients being treated with rivaroxaban.

ANTITHROMBIN

Heparin can produce a usually minor fall in anti-thrombin, which is less with the LMWH. Anti-thrombin can be measured in patients treated with these drugs. Deficiency is excluded if the anti-thrombin is normal: if the result is moderately low a repeat should be requested when the patient is not receiving any treatment. A large fall suggests deficiency which is independent of heparin treatment. In emergency situations, family studies can provide a presumptive diagnosis.

TESTING FOR LUPUS ANTICOAGULANT

– Testing for LA in patients receiving unfractionated heparin: some commercial reagents for dRVVT or APTT contain a heparin inhibitor (polybren or heparinase) up to a concentration of 0.6 to 0.8 U/ml (check that the thrombin time is normal).

– On low molecular weight heparins (LMWH): LA testing is recommended at least 12 hours after the last dose (the interference depends on the anti-IIa/anti-Xa ratio of the LMWH).

– In patients taking AVK, the test should ideally be performed 1 to 2 weeks after stopping the AVK. It is only recommended if the INR is < 1.5. At INR values of between 1.5 and 3, the LA test is acceptable provided that a mixing test is performed.

– There is currently no answer for patients on anti-IIa (dabigatran). Testing for LA is not recommended in patients receiving Rivaroxaban (risk of false positive results).

ACTIVATED PROTEIN C RESISTANCE (PCAR)

Heparin therapy can interfere with the test. The modified Dahlbäck method which is currently used is not influenced by anti-vitamin K treatments. PCaR testing can be invalidated by treatment with dabigatran or rivaroxaban. Hormone therapies (oral contraception and hormone replacement therapy) can also interfere with the test and must be stopped for at least 2 months before the test.

MOLECULAR BIOLOGY TESTING

Molecular biology testing for the Q506 mutation which is an arginine R to glutamine Q mutation (Factor V Leiden) causing PCaR, the prothrombin FII G>A 20210 mutation and other mutations (MTHFR methylene tetrahydrofolate reductase, etc.) are not influenced by any treatments.

TESTS WHICH CAN BE INTERPRETED IN PATIENTS RECEIVING ANTICOAGULATION

	Protein C	Protein S	AT	Lupus CAC	RPCa (modified coagulation test)	Molecular biology
Heparin	yes	yes	yes*	no**	no***	yes
AVK	no	no	yes	yes	yes	yes

** If the result is low it should be checked off treatment or by family studies.*
*** For tests using the APTT, tests measuring diluted Russell Viper venom can be used in the presence of heparin (anti-Xa activity < 1 IU/ml).*
**** Some tests contain a heparin inhibitor allowing the test to be performed if anti-xa activity is < 1 IU/ml.*

yes = sample can be taken, no = avoid taking the sample

On dabigatran or rivaroxaban treatment: activated PC and PS measurements (measured using the coagulation technique) and PCaR tests can be invalidated. Progressive AT (or anti-factor-Xa) measurements cannot be interpreted in patients taking rivaroxaban. Molecular biology mutation tests are not influenced by these treatments.

Heparin-induced thrombocytopaenia

The Health Authorities recommend monitoring platelet counts in patients treated with heparin anticoagulation: before treatment and twice per week throughout treatment. Monitoring is no longer indicated after the third week of treatment however and samples can for example be taken a month apart (empirical approach).

Fondaparinux is not liable to induce thrombocytopaenia and systematic monitoring is not therefore required.

FOR FURTHER INFORMATION

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