biomnis
SPECIALISED MEDICAL PATHOLOGY
Focus
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Autoimmune hepatitis (AH) and primary biliary cirrhosis (PBC)

Autoimmune hepatitis: definition

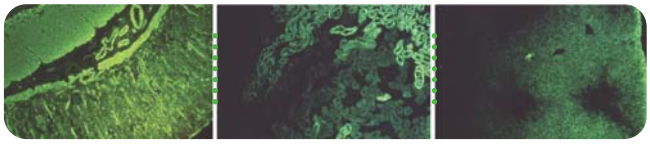
Autoimmune hepatitis (AH) is a chronic, necro-inflammatory liver disease. Its etiology is unknown and it is associated with auto-antibodies and hypergammaglobulinemia. This is a rare condition and its frequency is 0.1 - 1.2 cases per 100,000 inhabitants, representing for example less than 6% of chronic hepatitis cases in France. AH can start at any age and is predominately found in women (F/M ratio: 4/1). It is often associated with other autoimmune diseases.

Diagnosis is essential

- Diagnosis is essential in order to initiate treatment (corticosteroid therapy and/or immunosuppressive treatment). Untreated autoimmune hepatitis can quickly develop into cirrhosis.
- Establishing a differential diagnosis between a possible viral origin or autoimmune hepatitis is essential as interferon treatment for autoimmune hepatitis could be fatal if the patient in fact has viral hepatitis.
- To distinguish autoimmune hepatitis from other autoimmune liver disorders: cholestatic forms (PBC, primitive sclerosing cholangitis (PSC), autoimmune cholangitis, overlap syndrome) and cytolytic forms (hepatitis C with autoimmune symptoms). Finally, the diagnosis of autoimmune hepatitis is a diagnosis of exclusion.

Autoimmune hepatitis - which antibody tests?

The antibodies to be screened for are: Anti-nuclear antibodies (ANA), anti-smooth muscle actin antibodies, anti-LKM1 antibodies (anti liver-kidney microsome antibodies), anti-cytosol antibodies (LC1), anti-soluble liver antibodies (SLA) and anti mitochondrial antibodies (AMA) in order to exclude PBC.
In the first instance, it is recommended to request screening for anti-tissue antibodies via indirect immunofluorescence using slides with 3 cuts of rat tissue (liver, kidney, stomach: triple substrate). This is then followed by anti SLA antibodies (which is not detected by triple substrate but the immunodot technique among others). Following anti-tissue antibody screening (identification according to the fluorescent pattern, see photos) and titration by indirect immunofluorescence, the specificity must be confirmed: if the anti-smooth muscle antibodies titre is › 1/60 then the anti-actin specificity must be confirmed using HEp-2 colchicine treated cells or on VSM47 cell lines. Following titration, anti-LKM1 antibodies, anti-LC1 and AMA specificity confirmation can be performed via the immunodot technique.



Typical anti-smooth muscle expression on the stomach | *Typical anti-LKM expression on the kidney* | *Typical anti-cytosol expression on the liver*

Autoimmune hepatitis classification into 3 classes according to the identified antibodies:

- **Type I (ANA, ASMA):** 80% of cases
- **Type II (LKM1 and/or LC1):** 10%
- **Type III (SLA):** 10% (according to certain writers this is related to type I autoimmune hepatitis).

Autoimmune hepatitis type I

Onset is seen between the ages of 10 and 25 (80% females) or between 40 and 65 (65% females). The onset is acute or insidious in 20% of cases (diagnosis can not be made simply from the stage of cirrhosis). The suspicion of possible autoimmune hepatitis is raised following an alteration of the general state, amenorrhoea, arthralgia, myalgia and diarrhoea etc. Hepatomegaly often occurs (70%) and can be the only clinical sign. This disease is often associated with other autoimmune diseases: auto immune thyroiditis, Biermer, Sjögren syndrome, rheumatoid polyarthritis etc. In terms of the clinical pathology, transaminase levels are raised (10 - 20), GGT and ALP are moderately raised, significant hypergammaglobulinemia is present (IgG › 30 g/L) and actin type ASMA is detected.

Autoimmune hepatitis type II

The disease is essentially a paediatric disease with onset occurring before the age of 15 in 50% of cases (90% in female cases). Autoimmune hepatitis is associated with the presence of anti-LKM1 antibodies in 85% of cases and anti-LC1 antibodies

in 30% of cases. Anti-LC1 antibodies have been detected in 10% of cases.
The onset is acute and suggestive of viral hepatitis with rapid progression towards cirrhosis. The associated autoimmune diseases are similar to those linked to autoimmune hepatitis except for type 1 diabetes and vitiligo which are more frequently encountered and rheumatoid poly arthritis which is more rare.

Autoimmune hepatitis type III

Type III autoimmune hepatitis is characterised by the presence of anti-SLA antibodies but does not differ from type I autoimmune hepatitis. The individuality of type III autoimmune hepatitis remains controversial. ANA and actin type ASMA can be present.

Overlap syndrome autoimmune hepatitis/PBC: Overlap syndrome

Relatively frequent (5 - 10% of PBC), it meets at least two of the criteria of PBC and 2 of autoimmune hepatitis, which can follow on from or overlap one another.

Autoimmune hepatitis and PBC diagnostic criteria (International Autoimmune Hepatitis Group)

PBC criteria	ALP › 2N and/or GGT › 5N for over 6 months
	Type M2 anti-mitochondrial antibodies ›1/40
	Biliary cirrhosis
Autoimmune hepatitis criteria	Hepatic cytolysis: AST or ALAT › 5N
	IgG › 2N
	Actin specific anti-smooth muscle antibodies › 1/80
	Significant periportal and lobular inflammatory lesions

Autoimmune hepatitis and viral hepatitis C auto-antibodies (HCV)

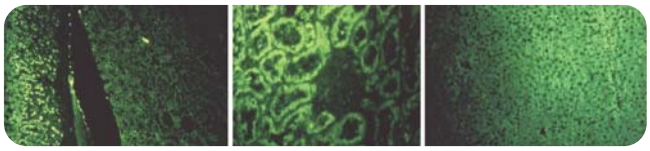
During viral hepatitis C, ANAs are found in 40-70% of cases, anti-LKM1 antibodies in 3-5% of cases, ASMAs in 15-65% of cases, anti-SLA antibodies in 0 - 10% of cases anti-LC1 and AMA antibodies in 0 - 2% of cases. Taking into account the significant prevalence of HCV in France in comparison to autoimmune hepatitis, it is no wonder that when autoimmune hepatitis auto antibodies are detected it is often associated with HCV.

Primary biliary cirrhosis (PBC)

Among biliary canal disease, one can distinguish between primary biliary cirrhosis and primitive sclerosing cholangitis. PBC is a chronic cholestatic disease of an unknown cause associated with the presence of anti-mitochondrial antibodies (AMAs) and poorly specific histological lesions (destruction of biliary canals, inflammation, necrosis and terminal phase cirrhosis). Indeed, AMAs are a great help in the diagnosis of a patient as they are found in 95% of cases of PBC.
The prevalence of PBC is 10-15/100,000 inhabitants. This disease mainly affects women (7/10 cases) aged 50 - 65.
On a clinical level this disease is characterised by chronic cholestatis, which evolves in 3 phases. The first, preclinical stage is asymptomatic. The second phase is associated with pruritus and asthenia, without jaundice, and possible skin xanthomas caused by secondary hypercholesterolemia from prolonged bile duct obstruction. Progressive weight loss is frequently encountered. The disease evolves towards jaundice, hepatosplenomegaly and in the terminal phase significant bile duct obstruction and cirrhosis.
In terms of pathology, the liver profile is atypical with a significant increase in GGT, ALP, hyperbilirubinemia and moderately raised transaminase levels. The gammaglobulins are raised to a lesser level than in autoimmune hepatitis but the IgM increase is net. ANAs are positive in 30-50% of cases (membrane fluorescence, nuclear dots).
PBC is often associated with sclerodermia (CREST), Gougerot-Sjögren syndrome and Hashimoto thyroiditis.

When to screen for AMA?

Following the request from a prescribing physician or following ANA screening showing typical AMA in the cytoplasm of HEp-2 cells. Screening and quantification of AMA via indirect immunofluorescence on triple substrate (rat kidney-liver-stomach):



STOMACH: fluorescence of gastric parietal cells

KIDNEY: fluorescent tubules, no fluorescence of the glomerulus

LIVER: matt and granular fluorescence of hepatocyte cytoplasms

If the titre is ›1/40, the AMA must be identified by immunodot, ELISA or Western Blot. Type 2 or M2 AMAS are the only types associated with PBC. M2 AMAs have a diagnostic value for PBC at a titre of ›1/80. This antibody permits the differential diagnosis between PBC and other intra hepatic cholestasis. It can be prematurely detected, even before the onset of symptoms. AMA titres (especially IgG) are not linked to the severity of the disease nor its prognosis. Following liver transplantation, M2 type AMAs seen through typing investigations can persists yet remain negative via immunofluorescence. AMAs detected by immunofluorescence yet negative in M2 typing often corresponds to viral hepatitis C.

Other PBC antibodies

ANAs are positive in 30-50% of PBC cases. Anti-GP210 antibodies (membrane fluorescence on HEp-2 cells) are very specific, however they are only found in 30% of PBC cases and are linked to a poor prognosis. Anti-SP100 antibodies (nuclear dot fluorescence in HEp-2 cells) are found in 10-40% of PBC cases.
Other ANAs are also sometimes detected:
Non-specific: Anti-SSA/Ro 60 antibodies, anti-SSA/Ro 52 antibodies, anti- SSB/La antibodies, anti-centromere antibodies and anti-Scl70 antibodies.

Fairly specific: Type 10 anti-mitochondrial antibodies are found in 2% of PBC cases.
These antibodies are detected via indirect immunofluorescence on triple substrate, showing fluorescence only on the distal tubules of the kidney (negative parietal cells on the stomach).

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