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ANCA: vasculitis, chronic inflammatory bowel diseases

Screening for anti-neutrophil cytoplasmic antibodies (ANCA) is recommended in 2 very different contexts:
• systemic or localised vasculitis in the kidney (glomerulonephritis); and
• inflammatory bowel diseases (IBD).

Clinical diagnosis of vasculitis

Vasculitis causes inflammatory lesions in the vessel walls with infiltration, necrosis and even thrombosis. The clinical symptomology of vasculitis is varied because numerous organs can be affected; this is especially the case for highly vascularised organs such as the kidneys and the lungs. The diagnosis of vasculitis relies on serology testing (ANCA) and radiology and is confirmed by histology.

The clinical profile tests usually performed include:
- Kidney profile, lung profile, radiology, tomography, ECG, MRI, skin biopsy and kidney biopsy;
- FBC, CRP, anti nuclear abs (DNA, ENA), rheumatoid factors, C3, C4, ANCA, anti-glomerular basement membrane antibodies (anti-GBM), cryoglobulins, ASLO, anti-cardiolipin antibodies, anti-HCV antibodies, Hbs antigen, (HIV).

Vasculitis classification

Secondary vasculitis

- Infectious vasculitis: tuberculosis, hepatitis C and B, HIV and parvovirus
- Mixed cryoglobulinemia associated with hepatitis C
- Cystic fibrosis complications
- Collagen vascular disease (lupus, rheumatoid arthritis, scleroderma, Gougerot Sjögren syndrome)
- Drug-induced vasculitis: penicillamine, propylthiouracil, hydralazine and minocycline etc.
- Drug abuse: cocaine (+++ caution: false positive ANCA result)
- Vasculitis and malignant disease: lymphoma, solid tumour

Essential vasculitis

In 1994, a new classification system for systemic vasculitis was established during the Chapel Hill Consensus Conference. This new classification takes into account the size of the vessels:

Large vessel vasculitis (giant cells)
- Horton disease
- Takayasu disease

Medium vessel vasculitis
- Polyarteritis nodosa
- Kawasaki disease

Small vessel vasculitis
- Henoch Schonlein purpura
- Hypersensitivity vasculitis
- Deposition of cryoglobulinemia immune complexes
- Microscopic polyangiitis
- Wegener's granulomatosis
- Churg Strauss syndrome.

ANCA antibodies are associated with these last three diseases (see table 1).

Epidemiology

Both genetic and environmental factors can trigger vasculitis.

Genetic factors

- Family history, linked to class I HLA
- Allergy, alpha 1-antitrypsin
- Fc gamma polymorphisms, CD18, C3 and C4, Il10 and CTLA4 etc.
- South › north gradient for microscopic polyangiitis
- South ‹ north gradient for Wegener's granulomatosis

Facteurs environnementaux

- Nasal carriage of *Staphylococcus aureus*
- Repeated inhalation of silica, solvents, pesticides, asbestos, cocaine etc.
- Contact with cattle
- Vaccination, using adjuvants

The treatment of vasculitis relies on prednisolone combined with methotrexate or cyclophosphamide and then a prednisolone/azathioprine combination for maintenance therapy. Plasma exchange or rituximab is used in severe cases.

Differential criteria for small vessel vasculitis

The vasculitis is usually distinguished as ANCA positive or ANCA negative. ANCA testing is therefore a significant diagnostic tool: however, the circumstances can be very different (i.e. ANCA-negative Wegener's granulomatosis or even anti-MPO ANCA etc.).

Table 1: ANCA positivity and specificities during vasculitis

Wegener's granulomatosis	Microscopic polyangiitis	Churg Strauss syndrome	Polyarteritis nodosa
ANCA aspect by IIF			
75 % c-ANCA 10-15 % p-ANCA	35 % c-ANCA 50 % p-ANCA	10 % c-ANCA 60 % p-ANCA	10 % c-ANCA 20 % p-ANCA
ELISA specificities			
PR3 (85 %) MPO (10 %)	PR3 (25 %) MPO (60 %)	PR3 (10 %) MPO (60 %)	PR3 (85 %) MPO (10 %)
Eosinophilia			
Rarely	Never	Always › 10 %	

The presence of anti-PR3 antibodies is associated with a greater risk of developing granulomas, extra renal manifestations, inflammatory lesions, carriage of Staphylococcus aureus, a rapid degradation of renal function and more frequent relapses.
No correlation between the ANCA level and the clinical activity of the disease has ever been established; neither has a correlation been established for their persistence after 2 years of treatment and the risk of a relapse.

Recommendations for ANCA screening

Screening

- By IF: magnification of x 400
- Dilution screening: 1/20 If positive: describe the cytoplasmic (c-ANCA), perinuclear (p-ANCA) or atypical, perinuclear (x-ANCA) aspect. Perform identification testing.

Identification

- Simultaneously screen for anti-MPO and anti-PR3 antibodies
- Favour ELISA and Luminex Western Blot techniques (false negatives): 5% of IF negatives are positive following ELISA testing (some pathologists combine IF and ELISA at the outset of testing). In the majority of cases, any discrepancies are caused by ANAs causing interference or ANCAs against target antigens other than PR3/MPO (cathepsin, BPI, elastase, lactoferrin etc. These specificities are not screened for unless specific associated clinical details are supplied).

Table 2: IIF screening of human polynuclear neutrophils (PNN)

PNN fixed in ethanol	PNN fixed in formalin	PNN fixed in methanol	HEp-2 cells
c-ANCA aspect			
Granular cytoplasmic fluorescence	Granular cytoplasmic fluorescence	Granular cytoplasmic fluorescence	Negative
p-ANCA aspect			
Perinuclear fluorescence	Granular cytoplasmic fluorescence	Negative	Negative
x-ANCA aspect			
Granular cytoplasmic fluorescence	Negative	Granular cytoplasmic fluorescence	Negative
Aspect: Anti nuclear antibodies			
Nuclear or perinuclear	Negative	Positive	Positive

The diagnostic sensitivity and specificity of ANCA depends on the clinical presentation: sensitivity › 90% and specificity › 95% in cases of suspected rapidly progressive glomerulonephritis. Caution: a negative ANCA result does not exclude vasculitis.

Rapidly progressive glomerulonephritis (GN)

In this context, ANCA screening is combined with anti glomerular basement membrane antibody (GBM) screening as the clinical symptoms of GN and vasculitis are similar.

Table 3: Acute glomerulonephritis: histology classification (by direct immunofluorescence)

Type I	Anti-GBM antibodies	*Linear fluorescence*
	■ with alveolar haemorrhage: Goodpasture's syndrome ■ without alveolar haemorrhage: GN with anti-GBM	
Type II	Immune complexes	*Granular fluorescence*
	■ post-infectious ■ secondary to lupus, cryoglobulinemia, Henoch Schonlein purpura ■ moderately progressive GN complications ■ idiopathic	
Type III	Pauci-immune (ANCA +)	*Negative fluorescence (or very weak)*
	■ Wegener's granulomatosis ■ microscopic polyangiitis ■ limited renal vasculitis ■ Churg Strauss syndrome	

IBD: Inflammatory Bowel Disease

xANCA or atypical ANCA have been observed during IBD. Nowadays, it is usual to combine ANCA screening with ASCA screening (anti-*Saccharomyces cerevisiae* antibodies: IgG and IgA) for the differential diagnosis of haemorrhagic proctocolitis (associated with xANCA) and Crohn's disease (associated with ASCA). These pathologies should also be differentiated from irritable bowel syndrome, infectious colitis, ischaemic colitis, drug induced colitis and coeliac disease.

Table 4: differential diagnosis of haemorrhagic proctocolitis and Crohn's disease

	Haemorrhagic proctocolitis			Crohn's disease		
	SENSITIVITY	SPECIFICITY	PPV*	SENSITIVITY	SPECIFICITY	PPV*
x-ANCA+	65	85	74			
ASCA+				61	88	89
x-ANCA+ ASCA-	57	97	92			
x-ANCA- ASCA-+				49	97	96

*PPpositive predictive value : positive predictive value

Table 5: epidemiology - clinical

	Haemorrhagic proctocolitis	Crohn's disease
Incidence in France	2,8/100 000 inhabitants	3,6/100 000 inhabitants
Gender ratio F/M	0.5	0.9
Average age at diagnosis	36 years old	27 years old
NOD2/CARD15	‹ 20 % have 1 mutation	50 % have 1- 2 mutations
Role of tobacco	"protector"	favoured
Topography	left colon, rectum Continual superficial interference	Jejunum, ileal, right colon, rectum and anus. Transmural intermittent interference
Abdominal pain	++ left	+++ right
Diarrhoea	++	++
Anal fistulas	‹ 10 %	20 – 30 %
Malabsorption syndrome	0	+
Extra-intestinal manifestations:		
- Arthritis	+++	+++
- Cholangitis	++	+/-
Dysplasia	Possible	Rare

IBD treatment includes: 5- aminosalicylic acid (5-ASA), corticoids and topical or oral antibiotics; 2nd line, immunosuppressors (azathioprine, 6 mercaptopurine, methotrexate), biotherapies (anti TNF alpha) and surgery (80% of patients are operated on during the disease development).

Calprotectin

Calprotectin is present in a large quantity in the granulocytes. Its presence in stool indicates intestinal inflammation. Quantification can be used for diagnosis and for monitoring intestinal inflammatory diseases, bacterial and parasitic infections and colorectal cancer.
In adults, the quantification sensitivity for the diagnosis of IBD is 0.93 (0.85 - 0.97) and its specificity is 0.96 (0.79 - 0.99). Its good negative predictive value limits the need for coloscopies.

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