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, 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

**ANCA: vasculitis, chronic inflammatory bowel diseases**

- 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, II10 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>
<thead>
<tr>
<th>Wegener’s granulomatosis</th>
<th>Microscopic polyangiitis</th>
<th>Churg Strauss syndrome</th>
<th>Polyarteritis nodosa</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANCA aspect by IIF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75 % c-ANCA</td>
<td>35 % c-ANCA</td>
<td>10 % c-ANCA</td>
<td>10 % c-ANCA</td>
</tr>
<tr>
<td>10-15 % p-ANCA</td>
<td>50 % p-ANCA</td>
<td>60 % p-ANCA</td>
<td>20 % p-ANCA</td>
</tr>
<tr>
<td>ELISA specificities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR3 (85 %)</td>
<td>PR3 (25 %)</td>
<td>PR3 (10 %)</td>
<td>PR3 (85 %)</td>
</tr>
<tr>
<td>MPO (10 %)</td>
<td>MPO (60 %)</td>
<td>MPO (60 %)</td>
<td>MPO (10 %)</td>
</tr>
<tr>
<td>Eosinophilia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rarely</td>
<td>Never</td>
<td>Always &gt; 10 %</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: ANCA positivity and specificities during vasculitis
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.

**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 2: IIF screening of human polynuclear neutrophils [PNN]**

<table>
<thead>
<tr>
<th>PNN fixed in ethanol</th>
<th>PNN fixed in formalin</th>
<th>PNN fixed in methanol</th>
<th>HEp-2 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>c-ANCA aspect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granular cytoplasmic fluorescence</td>
<td></td>
<td></td>
<td>Negative</td>
</tr>
<tr>
<td>p-ANCA aspect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perinuclear fluorescence</td>
<td></td>
<td>Granular cytoplasmic fluorescence</td>
<td>Negative</td>
</tr>
<tr>
<td>x-ANCA aspect</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Granular cytoplasmic fluorescence</td>
<td></td>
<td>Granular cytoplasmic fluorescence</td>
<td>Negative</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>Positive</td>
<td>Positive</td>
</tr>
</tbody>
</table>

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.

**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 protocollitis (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 protocollitis and Crohn’s disease**

<table>
<thead>
<tr>
<th>Haemorrhagic protocollitis</th>
<th>Crohn’s disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>SENSITIVITY</td>
<td>SPECIFICITY</td>
</tr>
<tr>
<td>x-ANCA+</td>
<td>65</td>
</tr>
<tr>
<td>ASCA+</td>
<td>57</td>
</tr>
<tr>
<td>ASCA-</td>
<td>57</td>
</tr>
</tbody>
</table>

**Table 5: epidemiology - clinical**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Incidence in France</th>
<th>Gender ratio F/M</th>
<th>Average age at diagnosis</th>
<th>NOD2/CARD15</th>
<th>Role of tobacco</th>
<th>Topography</th>
<th>Abdominal pain</th>
<th>Diarrhoea</th>
<th>Anal fistulas</th>
<th>Malabsorption syndrome</th>
<th>Extra-intestinal manifestations:</th>
<th>Dysplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemorrhagic protocollitis</td>
<td>2,8/100 000 inhabitants</td>
<td>0.5</td>
<td>36 years old</td>
<td>x 20 % have 1 mutation</td>
<td>“protector”</td>
<td>left colon, rectum, transmural intermittent interference</td>
<td>++ left</td>
<td>++</td>
<td>&lt; 10 %</td>
<td>0</td>
<td><strong>Arthritis</strong></td>
<td>++</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>3,6/100 000 inhabitants</td>
<td>0.9</td>
<td>27 years old</td>
<td>50 % have 1-2 mutations</td>
<td>favoured</td>
<td>Jejunum, ileum, right colon, rectum, anus and anastomosis</td>
<td>++ right</td>
<td>++</td>
<td>20 – 30 %</td>
<td>+</td>
<td><strong>-Cholangitis</strong></td>
<td>++</td>
</tr>
</tbody>
</table>

IBD treatment includes: 5- aminosalicylic acid (5-ASA), corticoids and topical or oral antibiotics; 2nd line, immunesuppressors (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.

Carole Emile, following a communication from Georges Chyderiotis, Biomnis Lyon.