Antineutrophil Cytoplasmic Antibodies Tests: What Should We Know?

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Introduction

Antineutrophil cytoplasmic antibodies (ANCA) are serological markers for supporting the diagnosis of ANCA-associated small-vessel vasculitis (AASV), namely Wegener’s granulomatosis (WG), microscopic polyangiitis (MPA), Churg-Strauss syndrome (CSS) and pauci-immune crescentic glomerulonephritis. ANCA also help in the diagnosis of other non-vasculitic inflammatory disorders such as inflammatory bowel disease (IBD), primary sclerosing cholangitis (PSC) and autoimmune hepatitis (AIH). ANCA tests are currently determined by an indirect immunofluorescence (IIF) assay using polymorphonuclear (PMN) cells as a substrate and an ELISA for detection of antibodies against specific antigens; proteinase3 (PR3) and myeloperoxidase (MPO). Different ANCA patterns are demonstrated by IIF. Some patterns are associated with specific antigens, while some patterns have multiple specificities. Positive C-ANCA or P-ANCA in combination with positive PR3-ANCA or MPO-ANCA, respectively, occurs in AASV. Positive ANCA with antigen specificities other than PR3 and MPO occurs in other inflammatory disorders. The interpretation of ANCA test is somewhat complicated both in laboratory and clinical aspect. Understanding the interpretation of ANCA test leads to appropriate request of ANCA test. In this review, we summarized the general information of ANCA test, including indications, methods and interpretation of the test and proposed the appropriate ANCA test request.

Indications of ANCA tests

The ANCA test is mainly indicated to diagnose AASV, e.g. Wegener’s granulomatosis (WG), microscopic polyangiitis (MPA), Churg-Strauss syndrome (CSS) and pauci-immune crescentic glomerulonephritis. The International Consensus Statement on testing and reporting of ANCA published in 1999 stated the use of ANCA testing in patients suspected of small vessel vasculitis (SVV). Clinical manifestations suggestive of AASV that ANCA test is warranted are as follows:
- Glomerulonephritis, especially rapidly progressive glomerulonephritis
- Pulmonary hemorrhage, especially pulmonary-renal syndrome
- Cutaneous vasculitis, especially with systemic features
- Multiple lung nodules
- Chronic destructive disease of the upper airways
- Long-standing sinusitis or otitis
- Subglottic tracheal stenosis
- Mononeuritis multiplex or peripheral neuropathy
- Retro-orbital mass

Although histopathological findings remain the gold standard for the diagnosis of AASV, in some circumstances tissue biopsy cannot be obtained or do not yield diagnostic findings, ANCA testing constitute an important adjunct to the diagnosis.

Determination of ANCA is also useful for diagnosing other inflammatory disorders, i.e. IBD, PSC and AIH. ANCA have been reported 50-80% of patients with ulcerative colitis (UC) and 5-30% of patients with Crohn disease (CD). The combination of ANCA test by IIF and anti-Saccharomyces cerevisiae antibody (ASCA) test by ELISA often helps differentiate between UC and CD. ANCA occurs in 60-90% of patients with PSC and 50-90% of patients with AIH.

The role of ANCA test for monitoring patients to detect disease relapse is still under debate. The correlation of ANCA titers and disease activity varied in different studies. Nevertheless, a negative result during a follow-up indicates that the disease is still in remission. Reappearance or increase of ANCA titer indicates that the disease likely relapses.

Methods for ANCA detection

Indirect immunofluorescence (IIF) is the standard method for ANCA test at present. This method is mainly used for ANCA pattern screening. The use of ethanol-fixed PMN as a substrate for IIF was recommended by the first ANCA workshop in 1989. Four patterns of ANCA demonstrated on ethanol-fixed PMN have been described. First, cytoplasmic pattern (C-ANCA) is defined as diffuse granular cytoplasmic staining with central interlobular accentuation. Second, perinuclear pattern (P-ANCA) is defined as perinuclear staining with nuclear extension. Third, C-ANCA (atypical) pattern is defined as cytoplasmic staining without central interlobular accentuation or cytoplasmic granularity. The forth pattern called atypical ANCA pattern is defined as homogeneous cytoplasmic staining in combination with nuclear staining.

The use of formalin-fixed PMN helps differentiate ANCA pattern, in particular distinguish between P-
ANCA specific to formalin-resistant antigens (mostly PR3 and MPO) and P-ANCA specific to formalin-sensitive antigens (antigens other than PR3 and MPO). P-ANCA specific to formalin-resistant antigens shows cytoplasmic staining on formalin-fixed PMN (positive), while P-ANCA specific to formalin-sensitive antigens shows no fluorescence staining on formalin-fixed PMN (negative).

The employment of other substrates such as Hep-2 cells is helpful for better interpretation of ANCA IIF test. Hep-2 cells substrate, a substrate of antinuclear antibodies (ANA) test, helps suggest the presence of ANA which can mimic both C-ANCA and P-ANCA patterns.

ELISA is another method widely used for ANCA detection. This method has an advantage in characterization of ANCA specificity. The detection of ANCA with PR3 and MPO specificity provides adjunctive information for the diagnosis of AASV. ELISA for PR3- and MPO-ANCA are widespread commercially available. Moreover, ACNA specific to antigens other than PR3 and MPO can be detected by some commercial ELISA kits.

**Interpretation of ANCA tests in laboratory aspect**

The C-ANCA pattern usually corresponds to PR3 antigen. C-ANCA with MPO specificity can be found in less percentage. The P-ANCA pattern corresponds to a number of antigens, i.e., MPO, which is the major antigen, bactericidal/permeability-increasing protein, lactoferrin, lysozyme, elastase and cathepsin G. P-ANCA with PR3 specificity can be rarely found. C-ANCA (atypical) and atypical ANCA patterns have been shown to target multiple antigens.

To correctly interpret the ANCA tests, it is necessary to understand the relationship of ANCA results from IIF and ELISA as shown in Table 1. The clinician should consult laboratory specialist for correct interpretation.

**Interpretation of ANCA tests in clinical aspect**

The ANCA tests will be helpful when used in

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**TABLE 1. Interpretation of ANCA results.**

<table>
<thead>
<tr>
<th>IIF (EOH)</th>
<th>IIF (HCHO)</th>
<th>ANA</th>
<th>ELISA (PR3&amp;MPO)</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>ANCA specific to PR3, MPO</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>+/-weakly</td>
<td>ANCA specific to other Ag † (rarely PR3, MPO)</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>ANCA specific to PR3, MPO with ANA</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+/-weakly</td>
<td>+</td>
<td>ANA induced ANCA pattern or</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>ANCA specific to other Ag † (rarely PR3, MPO) with ANA</td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>ANCA specific to PR3, MPO (5%)</td>
</tr>
</tbody>
</table>

EOH = ethanol fixed PMN, HCHO = formalin-fixed PMN, + = positive, - = negative, Ag = antigens
† Not associated with AASV

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Fig 1. Immunofluorescence patterns. C-ANCA with PR3 specificity on (a) ethanol-fixed PMN and (b) formalin-fixed PMN. P-ANCA with MPO specificity on (c) ethanol-fixed PMN and (d) formalin-fixed PMN.

Fig 2. Immunofluorescence patterns. P-ANCA with lactoferrin specificity on (a) ethanol-fixed PMN and (b) formalin-fixed PMN.

Fig 3. Immunofluorescence patterns. P-ANCA specific to MPO with positive ANA. (a) ethanol-fixed PMN. (b) formalin-fixed PMN. (c) ANA

Fig 4. Immunofluorescence patterns. ANA-induced ANCA pattern. (a-c) P-ANCA pattern induced by coarse speckled ANA. (d-f) C-ANCA pattern induced by anticentoplasmic antibodies.
proper clinical setting. The results of ANCA test should be interpreted with caution. ANCA results from IIF alone give some suggestion according to different ANCA pattern. C-ANCA and P-ANCA is suggestive, but not diagnostic, of AASV. C-ANCA (atypical) and atypical ANCA is not typically associated with AAVS. ANCA results from both IIF and ELISA give more information to support both diagnosis. Positive C-ANCA with positive PR3-ANCA or positive P-ANCA with positive MPO-ANCA strongly supports the diagnosis of AAVS. Positive PR3- or MPO-ANCA alone can be found in AAVS. Positive any of ANCA patterns with negative PR3- and MPO-ANCA occurs in infections, malignancies and other inflammatory disorders. The negative ANCA result does not exclude AAVS because 10-20% of patients with AAVS have been found ANCA negative. The positive ANCA result in the absence of suggestive clinical manifestation does not give any diagnosis.

ANCA patterns found in patients with non-vasculitic ANCA-associated inflammatory disorders; UC, CD, PSC and AIH are usually described as P-ANCA or atypical ANCA. These antibodies recognized many different antigens, but occasionally PR3 and MPO.6,7 Adherence to the International Consensus Statement, the demonstration of distinctive peripheral rim (or cytoplasmic or atypical) fluorescence pattern on ethanol fixed PMN and the absence of PR3- and MPO-ANCA by ELISA helps in the diagnosis of non-vasculitic ANCA-associated inflammatory disorders.

**Appropriate ANCA test request**

For patients with clinical manifestations highly suggestive of AAVS, as described in the topic of indications of ANCA tests, IIF assays including ethanol-fixed PMN, formalin-fixed PMN and ANA should be requested simultaneously. Positive ANCA on both ethanol-fixed PMN and formalin-fixed PMN strongly suggest PR3 or MPO specificity, depending on ANCA pattern, thus ELISA for PR3- and MPO-ANCA is required in some cases for confirmation. Positive ANCA on ethanol-fixed PMN but negative on both formalin-fixed PMN and ANA should be subsequently tested by ELISA for ANCA specific to PR3, MPO and other antigens. Negative ANCA by IIF should also be tested by ELISA for PR3- and MPO-ANCA because 5% of patients with AAVS have positive PR3- or MPO-ANCA alone.

For patients with clinical manifestations minimally suggestive of AAVS and patients with suspected other ANCA-associated inflammatory disorders, ANCA test by IIF on ethanol-fixed PMN should be firstly requested. Positive ANCA on ethanol-fixed PMN regardless of patterns should be subsequently tested by IIF assays including formalin-fixed PMN and ANA. ELISA for ANCA specific to PR3, MPO or other antigens should be requested, according to ANCA results from IIF as mention above. Negative ANCA on ethanol-fixed PMN has no further ANCA test required (Fig 5).

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**REFERENCES**


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Fig 5. Appropriate ANCA test request.

EOH = ethanol fixed PMN, HCHO = formalin-fixed PMN