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ANCA Tests (IFA and ELISA)

Professor Burçin Şener, MD
ORCID: 000-0002-0724-3166

Anti-neutrophil cytoplasm antibodies (ANCA) are autoantibodies that target the antigens present in the granules of neutrophils and monocytes. ANCA are directly involved in small blood vessel damage and are usually detected in patients with small vessel vasculitis. ANCA tests have an important value particularly in the diagnosis and follow-up of patients with ANCA-associated vasculitis which is a subtype of small vessel vasculitis. ANCA tests are important in the differential diagnosis and follow-up of the inflammatory activity in small vessel vasculitis. Besides small vessel vasculitis ANCA tests are also used in the diagnosis of drug induced vasculitis, active romatoid arthritis, ulcerative colitis, Crohn's disease, primary sclerosing cholangitis, primary biliary cirhosis and type 1 autoimmune hepatitis.

ANCA tests are performed by two methods: indirect immunofluorescence (IIF) and ELISA. IIF method which uses the substrate prepared from peripheral blood granulocytes, helps to determine the binding pattern of ANCA to granulocytes. For that purpose ethanol and formalin fixed granulocytes are used as ANCA substrates. To rule out a false positive result due to the concomitant presence of anti-nuclear antibody (ANA), the use of an additional HEp-2 substrate is recommended. ANA positivity which may mask the presence of p-ANCA appears as a problem in ANCA IIF method. ANCA are defined as p-ANCA, c-ANCA and atypical ANCA according to the immunofluorescence staining pattern. Usually the first step is to screen the presence of ANCA in the serum by IIF and then to determine the specific ANCA (PR-3, MPO and the other antibodies) by ELISA.

Soluble granulocyte contents such as MPO, lactoferrin, elastase, cathepsin G and basic proteins move towards the granulocyte nucleus in ethanol-fixed subtrates and this leads to a perinuclear fluorescence staining which is defined as p-ANCA (perinuclear ANCA). Diffuse cytoplasmic fluorescence usually with intralobular nuclear intensification in ethanol-fixed slides is defined as c-ANCA (cytoplasmic ANCA). Since formalin fixes the granules in the granulocyte cytoplasm, in formalin-fixed slides the fluorescence pattern is seen as if cytoplasmic if the ANCA is formalin-resistant. However, some of the ANCA types (Bactericidal Permeability Increasing Protein (BPI), lactoferrin and cathepsin G) which are sensitive to formalin, can not be detected in formalin-fixed slides and such ANCA are defined as formalin sensitive ANCA. The examination of both ethanol and formalin fixed substrates help to differentiate perinuclear and cytoplasmic staining (p-ANCA, c-ANCA) together with prediction of the related specific antibody based on formalin sensitivity.

There is a correlation between the ANCA IIF pattern and the related monospecific antibody. c-ANCA staining is due to the presence of PR3-ANCA in 90% of the cases and rarely the other related antibodies are BPI and MPO. p-ANCA positivity is due to MPO in 10% of the cases and the remaining antibodies are known as lactoferrin, elastase, cathepsin G, lysozyme, alpha-enolase, BPI and beta-glucuronidase.

c-ANCA positivity is strongly associated with Wegener's granulomatosis, whereas p-ANCA with primary vasculitis (microscopic polyangiitis, Churg-Strauss syndrome), collagen tissue disease (SLE, romatoid arthritis), chronic inflammatory bowel disease and chronic liver diseases.

In addition to p-ANCA and c-ANCA patterns, a-ANCA defined as "atypical ANCA" can also be observed in IIF ANCA tests. a-ANCA pattern is characterized by the presence of both cytoplasmic and perinuclear staining, the target antigen is usually BPI. A-ANCA is frequently associated with drug induced vasculitis, chronic diseases, inflamatory bowel disease or rheumatoid arthritis.

IIF testing provides an ANCA positive and negative result, only predicting the possible presence of a p-ANCA, c-ANCA or an a-ANCA pattern. ANCA IIF results should always be confirmed by monospecific ELISA test. A positive PR3-ANCA or an MPO-ANCA result is highly suspicious for the diagnosis of ANCA-associated vasculitis. The use of antigen specific ANCA ELISA tests is not recommended unless the clinical correlation of specific antigens are confirmed. Patient follow-up with PR3 ANCA titers is recommended to asses the disease activity. Persistent ANCA positivity or a rise in ANCA titer is reported prior to disease relapses. Increases in ANCA titer have a predictive value especially in vasculitis with renal involvement.

Hacettepe University Faculty of Medicine, Department of Medical Microbiology, Ankara

As in all laboratory test requests, following a test strategy based on clinical findings, evaluating ANCA IIF and ANCA monospecific/panel ELISA tests together will support the correct diagnosis and effective patient follow-up.

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