A new generation of ANCA tests using capture antibodies

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There are frequently discrepancies in the results of assays for anti-neutrophil cytoplasmic antibodies (ANCA) depending on whether the assay is carried out by the traditional indirect immunofluorescence (IIF) or the more recent ELISA tests. Such discrepancies are thought to be due to differences in the way the antigen is presented in the two test systems. The use of capture antibodies in newly developed MPO and PR3 ELISA test systems ensures that the antigen is presented in its native conformation, with the result that the new tests have higher sensitivity and specificity for vasculitis.

Systemic small vessel vasculitis comprises a heterogeneous group of rare but serious diseases. The first report of these diseases dates back to 1866, and describes a patient with necrotising arteritis referred to as periarteritis nodosa [1]. By the 1950s it was realised that there were a number of clinically distinct forms of arteritis, often manifesting as a component of vasculitis. In 1982, antibodies were first described that stained the cytoplasm of neutrophils in necrotising glomerulonephritis. The antibodies were named anti-neutrophil cytoplasmic antibodies (ANCA). Later it was shown that the presence of specific autoantibodies was related to Wegener’s granulomatosis. ANCA are a family of autoantibodies specific for antigens in neutrophil granules and monocyte lysosomes. Subsequent studies revealed that ANCA are closely associated with three major categories of small-vessel vasculitis: Wegener’s granulomatosis (mainly affecting the respiratory tract and kidneys), microscopic polyangiitis and Churg-Strauss syndrome (allergic granulomatous angiitis).

Small vessel vasculitis is a difficult disease to diagnose and often involves taking biopsy samples, a process which is associated with a certain morbidity. Determination of ANCA has proved to be a specific and sensitive aid in the diagnosis. Traditionally, the detection of ANCA has been carried out by indirect immunofluorescence (IIF) using ethanol-fixed granulocytes as the substrate. The presence of ANCA generates two distinguishable patterns, referred to as perinuclear (P-ANCA) and cytoplasmic (C-ANCA) [Figure 1]. The P-ANCA pattern is generated as an artificial distribution of cationic proteins towards the nucleus as a result of the ethanol treatment. It has been shown that ANCA are specific for a great number of different antigens, although in most cases each antigen generates either a C-ANCA or a P-ANCA pattern. Several antigens have been identified and characterised; these include lactoferrin, elastase, cathepsin G, and bacterial permeability-increasing protein (BPI). However, most of the ANCA activity seen in small vessel vasculitis is related to two particular antigens, namely proteinase 3 (PR3) and myeloperoxidase (MPO). Anti-PR3 gives a C-ANCA pattern and anti-MPO gives a P-ANCA pattern in most, but not all, cases. Thus, antibodies to PR3 are called PR3-ANCA and antibodies to MPO are called MPO-ANCA. It should be noted that although it is generally true that PR3-ANCA is observed as a C-ANCA pattern, a number of ANCA with other antigen specificities also show a C-ANCA pattern. Even more heterogeneous behaviour is seen with the P-ANCA pattern where MPO-ANCA is most prominent, but antibodies to a great number of other proteins also generate this pattern. It is also difficult to distinguish between P-ANCA and the GS-ANA pattern which represents true nuclear staining. Additionally, in some cases MPO-ANCA has been shown to give a C-ANCA pattern which further complicates the interpretation of IIF patterns. Finally, not all autoantibodies to granulocyte proteins generate a detectable staining with IIF. Clinically, the ANCA reactivity is usually associated with small vessel vasculitis but the P-ANCA/MPO-ANCA reactivity in particular is fairly often found in other autoimmune diseases such as SLE, rheumatoid arthritis and Sjögren’s syndrome.

Discrepancy between IIF and ELISA results

IIF is a simple, well-established and fairly cost-effective technique. However, as shown above, IIF analysis is associated with a number of pitfalls. As ANCA antigen specificities have been determined and defined, specific immunoassays such as RIA followed by ELISA have been developed. These tests have proven valuable for the determination of ANCA, in particular PR3-ANCA and MPO-ANCA. Often the immunoassay is used as an adjunct to IIF or as a confirmatory test following IIF screening. In this way it has become clear that there is a discrepancy in results obtained by these two methods of testing. This discrepancy is most likely caused by a difference in antigen presentation. A particular antigen will adopt a certain structural conformation when attached to a plastic surface, which may dramatically differ from the structure it adopts when fixed by ethanol in granulocytes in IIF tests [Figure 2]. Hence the epitopes presented may be very different in the two systems.

It is well known that antibodies from individual patients react with different epitopes on a particular antigen and thus they will give a low or high reaction when tested depending on which and how their epitope(s) is presented. In the extreme case a positive reaction in one test may be negative in the other. For this reason, there is frequently no correlation between IIF titre and ELISA. Another obvious reason for discrepancy between IIF and ELISA is the fact that the IIF detects a number of ANCA specificities. This is in contrast to ELISA, which only detects ANCA reactivity to a single defined antigen. Discrepancies are not only seen between different techniques such as IIF and ELISA, but also between tests using the same technique. Again, such discrepancy is probably caused by different epitope presentation [Figure 2]. Different brands of ELISA kits use various antigen preparations, which are coated to the plastic surfaces in different ways, all adding to the heterogeneous capture antibodies detected at an early stage. ANCA determination

Capture ANCA ELISA: design and performance

In order to preserve the three-dimensional structure of the antigen and to minimise differences in antigen presentation, capture ANCA immunoassays have been developed [2]. The capture assay is based on a monoclonal capture antibody specific for the antigen. The characteristics of the capture antibody which is coated on the plate are very important. It must have a certain avidity/affinity and be specific for an epitope that does not interfere with the binding of the patient’s antibody. With this in mind, the Wieslander capture ANCA assays were developed using carefully characterised monoclonal capture antibodies. For the capture PR3-ANCA, a number of clones were screened for their specificity. One clone was found to present the antigen such that 98% of antibodies against PR3 were detected. A capture monoclonal antibody specific for MPO was selected in a similar way.

The capture PR3-ANCA ELISA has been extensively validated regarding its diagnostic performance [2-8]. The assay is characterised by a high clinical sensitivity for vasculitis combined with a high clinical specificity. The capture format allows the presentation of the PR3 antigen in a native form, preserving important conformational epitopes. This characteristic seems to have significant implications, suggesting that the detected ANCA specificities are diagnostically the most relevant ones, and may even be disease-related. It has been found that there is a correlation between renal survival and the level of PR3-ANCA measured by the capture assay; a higher level gives a lower five-year renal survival [3]. No such correlation was found with conventional PR3-ANCA ELISAs. Preliminary data suggest that the capture PR3-ANCA level also correlates with patient survival [6].

A most important and common diagnostic objective of ANCA analysis is monitoring known vasculitis patients. Wegener’s granulomatosis is a life-long condition that can be treated with extensive drug regimens so that patients are in remission. Eventually relapse will occur and it is essential for the patient that this relapse is detected at an early stage. ANCA determination...
employing the capture format has been shown to detect relapses earlier and with a higher sensitivity compared with conventional PR3-ANCA ELISA [2-4, 7]. Most importantly, a decreasing titre or negative capture PR3-ANCA during remission excludes relapse. In a recent multi-centre study, six different capture PR3-ANCA assays were evaluated. The data obtained by the different centres were closely correlated, and one of the key conclusions was that the overall diagnostic performance of capture PR3-ANCA ELISAs is superior to IIF and direct ELISA, respectively [8]. The good correlation in results from the participating centres also suggests that with a well characterised and carefully selected capture antibody, a robust assay with high inter-assay performance can be designed which should prove valuable for future international standardisation.

The new capture MPO-ANCA assay is an alternative to the direct MPO-ANCA assay. Usually, MPO-ANCA will give a P-ANCA pattern on IIF, but in a few positive cases a C-ANCA pattern is shown [9]. The explanation may be that MPO artificially allocated to the nucleus presents epitopes generating the normal P-ANCA pattern, and granular MPO presents an epitope giving a C-ANCA pattern. Moreover, MPO-ANCA showing a C-ANCA pattern is often not associated with vasculitis but with other conditions. The capture MPO-ANCA assay is based on a monoclonal capture antibody specific for an epitope on MPO giving a C-ANCA pattern with IIF. Hence, the rationale for the assay is to acquire a higher sensitivity and specificity for vasculitis, in particular microscopic polyangiitis. Preliminary data suggest that capture MPO-ANCA assays have a sensitivity and specificity at least as high as the conventional assay but with a higher specificity for vasculitis [10]. An obvious advantage with the capture MPO-ANCA ELISA is the fact that the antigen is always presented in the same way and in a native form. More extensive studies are needed to fully evaluate its diagnostic performance and benefits.

**Conclusions**

The high morbidity and mortality associated with small-vessel vasculitis requires optimal diagnostic efficacy. Diagnostic tools are needed that have a high specificity for the disease as well as a sensitivity not generating false positives. The new generation of ANCA ELISA tests using the capture format has a diagnostic performance superior to the conventional direct assays.

**References**


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