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The ANCA test: its clinical relevance

In theory, the diagnosis of vasculitis depends on the presence of certain clinical features and the demonstration of vascular disease by either biopsy or angiography.¹ In practice, there have been many difficulties in formulating a satisfactory classification of the vasculitides. In Wegener's granulomatosis, for example, where there are prominent pulmonary changes, infections, other granulomatous conditions, and neoplastic diseases may produce a similar clinical picture, and biopsy specimens are often non-diagnostic.² The demonstration that antineutrophil cytoplasmic antibodies (ANCA) are present in most cases of Wegener's granulomatosis has provided a useful diagnostic tool and may shed light on the pathogenesis of vasculitis.³

Ten years ago, ANCAs were found in a small number of patients with glomerulonephritis.⁴⁻⁵ In 1985 van der Woude *et al* demonstrated the high sensitivity of ANCA in the diagnosis of Wegener's granulomatosis and a correlation with disease activity.³ These findings were corroborated by other groups.⁶⁻⁷ Indirect immunofluorescence is the most widely accepted method for detecting ANCAs, and a standardised methodology has been recently adopted.⁸ So far, two different patterns have been identified: classical ANCA, which produces a coarsely granular, centrally accentuated immunofluorescence and perinuclear ANCA, which gives a perinuclear staining pattern. The significance of other patterns, such as a diffuse cytoplasmic immunofluorescence, is unclear at present.

Antineutrophil cytoplasmic antibody reacts against cytoplasmic antigens of neutrophils and monocytes of human origin but not those from other species. Leucocyte alkaline phosphatase was initially thought to be the target antigen.⁹ Recent investigations, however, have shown that classical ANCA is directed against a soluble, single chain 29 kD protein contained in the primary or azurophilic granules of the neutrophils and in the lysosomal granules of monocytes.¹⁰⁻¹¹ This is most likely to be proteinase 3, a serine protease.¹² Perinuclear ANCA reacts mainly with myeloperoxidase but also with elastase, both of which are contained in the primary granules of the neutrophil.¹³⁻¹⁴ The different staining pattern is due to an artefactual staining of alcohol fixed neutrophils, but in formalin fixed neutrophils the same antibodies produce a cytoplasmic staining similar to classical ANCA.¹³ The previous observations suggest that ANCAs represent a novel class of autoantibodies directed against proteinase 3, myeloperoxidase, elastase, and, probably, other enzymes present in the lysosomal granules of neutrophils and monocytes.

Enzyme linked immunosorbent assays (ELISAs) using purified primary granules of neutrophils or myeloperoxidase as antigens have been developed.¹³⁻¹⁵ They permit a more objective quantitative determination of classical and perinuclear ANCA respectively. The diagnostic sensitivity of the

ELISA seems to be as high as that of indirect immunofluorescence. An ELISA may also detect ANCA in serum samples from patients with Wegener's granulomatosis and negative indirect immunofluorescence titres, and is useful in cases where there are difficulties in reading immunofluorescence patterns because of simultaneous antinuclear antibodies, which may obscure the cytoplasmic staining. The use of purified antigens also results in greater specificity than previous ELISAs and radioimmunoassays which used crude antigen preparations.⁶ As the indirect immunofluorescence recognises the different types of ANCA it is quite likely that this will remain the preferred screening test.

What is the clinical significance of ANCA? There is no doubt that determination of ANCAs is a valuable test in the diagnosis of Wegener's granulomatosis, as they are present in 50 to 96% of all patients with this diagnosis.⁶⁻⁷ Its sensitivity is 78 to 100% in active and disseminated Wegener's granulomatosis, 60 to 70% in cases with disease limited to the respiratory tract, and roughly 30% in patients with Wegener's granulomatosis in remission. The specificity of the test in the diagnosis of Wegener's granulomatosis has been consistently high (90 to 100%).⁷⁻¹⁶ The presence of ANCA does not absolve the doctor from actively pursuing a histological diagnosis, however. Most proved cases of Wegener's granulomatosis show a classical ANCA pattern. Titres of ANCA may fluctuate with the clinical activity of the disease and decrease or disappear with treatment.⁷⁻¹⁶ Classical ANCA has also been found in microscopic polyarteritis nodosa⁶ and in Kawasaki disease,¹⁷ though in the latter the immunofluorescence pattern is not quite typical. Therefore ANCA determination is not only a good marker for the presence and activity of the disease, but may also be helpful in distinguishing between exacerbations of the disease and infection, a common complication in Wegener's granulomatosis.

The interpretation of perinuclear ANCA is somewhat less clear. It has been found associated mainly with necrotising and crescentic glomerulonephritis.¹³⁻¹⁸ As up to two thirds of patients with crescentic glomerulonephritis have extra-renal vasculitic disease, and some of them have clinical disease also characteristic of Wegener's granulomatosis, the same authors have proposed that all patients with ANCA fall within a continuum of pathological features ranging from limited renal disease to varying degrees of systemic involvement.

Antineutrophil cytoplasmic antibody has been occasionally found in Churg-Strauss disease, Takayasu's disease, systemic lupus erythematosus, relapsing polychondritis, and Behçet's disease.¹⁹ Most of these studies have detected ANCA of the IgG class. Lockwood, however, identified a small group of patients with exclusively IgM ANCA, who developed pulmonary haemorrhage and severe glomerulonephritis.²⁰

Recently, IgA ANCA has been reported in patients with Henoch-Schönlein purpura and IgA nephropathy.²¹

The question remains whether ANCA is simply a disease marker or has a pathogenic role. It has been proposed that immune complexes play a part in the pathogenesis of Wegener's granulomatosis. Circulating immune complexes have been detected in some patients, but their role is unclear as serum complement concentrations are not depressed and immunofluorescence studies have indicated deposits of immunoglobulins and complement in only few cases of Wegener's granulomatosis.²² Recent studies support the hypothesis that ANCAs are more than a useful serum abnormality. By electron microscopy, intravascular lysis of neutrophils has been found as an early event in the inflammatory process.²³ A chemotactic abnormality has also been reported,²⁴ as well as evidence of neutrophil activation *in vitro*²⁵ and *in vivo*.²⁶ IgG from patients with antibody to myeloperoxidase inhibits the enzymatic activity of myeloperoxidase in a chemiluminescence system, implying a disturbance in neutrophil function.²⁷ Furthermore, it has been shown that ANCA might enter live neutrophils,³ and ANCA antigen may be expressed in the cellular membrane or be released from neutrophils stimulated with cytochalasin B *in vitro*.¹¹ For many years it has been thought that Wegener's granulomatosis is an immunological reaction triggered by a specific antigen. The lung, an organ exposed to the outer environment and commonly affected in Wegener's granulomatosis, has unique anatomical and physiological features with a large marginated pool of leucocytes.²⁸ Conceivably, a triggering event such as infection might lead to activation of leucocytes, and the presence of ANCA might tilt the balance toward frank neutrophil activation, release of lysosomal enzymes, production of oxygen metabolites, and tissue injury. Reports of the beneficial effects of sulphamethoxazole-trimethoprim on the course of the disease lend additional support to this theory.²⁹ Theoretically and practically it is interesting that high dose intravenous immunoglobulin contains inhibitory antibodies reactive with ANCA.³⁰ Studies of ANCA in the interaction of neutrophils with endothelial vascular cells may help to clarify its importance in the pathogenesis of vasculitis.

Using a monoclonal antibody against the neutrophil cytoplasmic antigen, Abbott *et al* detected antigenic determinants on glomerular endothelial and epithelial cells, suggesting that such cross reactivity might initiate the development of vasculitis in patients with ANCA.³¹

Cellular immune mechanisms should not be ruled out in the pathogenesis of the disease. The presence of granulomas is characteristic of cellular immune disturbances. Immunohistochemical studies of biopsy specimens have shown that monocytes and T lymphocytes are the dominant cell types in the cellular infiltrates.³² Recent studies emphasise the complex ways in which the T lymphocyte may affect neutrophil function.³³

Although too much importance may have been given to the discovery of ANCA and other tests providing evidence of active blood vessel injury, these are, nonetheless, welcome advances in the understanding of the pathogenesis of vasculitis.³⁴

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