Antineutrophil cytoplasm antibodies and vasculitis

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Systemic vasculitis is a rare condition and diagnosis is often difficult. Any organ system can be affected and patients can present with an insidiously progressive illness, a remitting/relapsing pattern, or acute multisystem failure. The early institution of aggressive and potentially toxic immunosuppression may be necessary to minimise permanent organ damage, and, despite the difficulties, rapid and unambiguous diagnosis is sometimes of great importance. The development of assays for antineutrophil cytoplasm antibodies (ANCAs) over the past decade has provided clinicians with a laboratory test for vasculitis. Classification of the vasculitides is confusing and has been the subject of a recent international consensus conference.1 The conditions most associated with the presence of ANCAs are small vessel necrotising vasculitides such as Wegener's granulomatosis, microscopic polyangiitis (microscopic polyarteritis), and pauci-immune crescentic glomerulonephritis. ANCAs were first described in 1982 in eight Australians with segmental necrotising glomerulonephritis,2 and the association with vasculitis was soon recognised.3 In 1985 van der Woude et al reported that ANCAs were specific for Wegener's granulomatosis, and that the titre was associated with the degree of disease activity.4 In 1988 ANCAs were first described in children, in three cases of necrotising glomerulonephritis,5 and the association with vasculitis was soon recognised.1 In 1985 van der Woude et al reported that ANCAs were specific for Wegener's granulomatosis, and that the titre was associated with the degree of disease activity.1 In 1988 ANCAs were first described in children, in three cases of necrotising glomerulonephritis.7 Subsequently ANCAs have been reported, in both adults and children, in numerous conditions characterised by vasculitis and inflammation.4 Recent work has also suggested that they may be pathogenic in vasculitis.

Laboratory assays for ANCA

INDIRECT IMMUNOFLUORESCENCE (IIF)
IIF detects the binding of antibodies to antigens in the cytoplasm of whole ethanol fixed neutrophils. It is subjective, semiquantitative, and requires experience to interpret. However, it is a rapid and standard technique and is widely available.2 Two major staining patterns can be distinguished: granular cytoplasmic staining with central accentuation, known as C-ANCA, and perinuclear staining, known as P-ANCA. A C-ANCA pattern usually corresponds to antibodies against proteinase 3,6 and P-ANCA staining to antibodies against myeloperoxidase,7 although binding to other antigens can give the same patterns. Antinuclear antibody positive sera may give a false positive P-ANCA result on ethanol fixed neutrophils, but can be distinguished using formalin fixed cells. A third pattern is diffuse homogeneous staining of the whole cytoplasm, known as atypical ANCA or x-ANCA. The target antigen is unknown and the staining is often weak. A similar pattern is seen with antibody binding to the plasma membrane.10

ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)
Solid phase methods such as radioimmunoassay and ELISA were developed to provide objective and quantitative assays for ANCA on a large scale. Initially, complex mixtures of antigens from whole neutrophils or neutrophil granules were used.11 ELISAs using these crude preparations give a high incidence of false positive results because of antibody binding to the disparate antigens present. Later, ELISAs that specifically detect antibodies to proteinase 3 and myeloperoxidase were developed. Unfortunately the preparation of both the crude extracts and the specific antigens, as well as the performance of the assays, are poorly standardised, with poor agreement between even expert laboratories.12 An international collaborative study is in progress to investigate these issues. Other quantitative methods using intact unmodified cells are in the early stages of development.13 Because of its ease and rapidity, IIF is still the preferred laboratory method.

ANCA assays in adult medicine
IN SYSTEMIC VASCULITIS AND CRESCENTIC GLOMERULONEPHRITIS
Numerous reports have confirmed that a positive ANCA assay result in the appropriate clinical setting is useful in the diagnosis of systemic vasculitides.6,7,14 The type of ANCA staining can also be of some assistance in diagnosis, although there is substantial overlap between different conditions. C-ANCA staining occurs most commonly in Wegener’s granulomatosis (but also in microscopic polyangiitis), and P-ANCA staining in microscopic polyangiitis (but also in Wegener's granulomatosis, classic polyarteritis nodosa, and Churg-Strauss syndrome). P-ANCA staining is also
seen in pauci-immune crescentic glomerulonephritis, which may be a renal-limited form of microscopic polyangiitis. A positive ANCA assay in such a case would support a plan to treat with immunosuppressive drugs such as steroids and cyclophosphamide, and possibly even plasma exchange. In other vasculitides including polyarteritis nodosa, Takayasu’s disease, giant cell arteritis, Behçet’s disease, and familial Mediterranean fever, ANCAs are rare or absent. Although the sensitivity and specificity of C-ANCA staining for Wegener’s granulomatosis was initially thought to be 90% or better, more recent studies have been less optimistic. Of 738 consecutive patients tested in one reference laboratory, 48 of 68 patients with Wegener’s granulomatosis were positive (38 C-ANCA and 10 P-ANCA) and 22 of 43 patients with microscopic polyangiitis (12 C-ANCA and 10 P-ANCA). This gives a sensitivity and specificity for Wegener’s granulomatosis of 71 and 80% respectively and for microscopic polyangiitis of 51 and 80% respectively. The predictive value of a test depends on the population in which it is applied. Even in this high risk population, with a prevalence of systemic vasculitis of 17%, the positive predictive value of the presence of ANCAs for any form of systemic vasculitis was only 40%. It would be lower if applied to a population with a lower a priori risk of vasculitis. A positive ANCA test is not sufficient to make a diagnosis of vasculitis, but must be interpreted in the clinical context. A link between ANCA titre and the degree of clinical activity in Wegener’s granulomatosis has been suggested by both cross sectional and longitudinal studies. In a prospective randomised study of 58 patients with Wegener’s granulomatosis there were fewer clinical relapses and lower cumulative doses of prednisolone and cyclophosphamide in the group in which therapy was intensified on the basis of an increase in C-ANCA titre. These results are encouraging but it would be premature to conclude that a rise in ANCA titre in a clinically stable patient should prompt an increase in immunosuppression, as this was a small study with only nine patients randomised to treatment, and a later report found an increase in ANCA titre to be neither a sensitive nor a specific marker of impending relapse.

**Does ANCA detection have a role in paediatrics?**

In comparison with the extensive literature on ANCA testing in adults, work in children has been limited. A number of reports have described typical C-ANCA and P-ANCA patterns in children with vasculitis, predominantly Wegener’s granulomatosis, microscopic polyangiitis and crescentic glomerulonephritis.

ANCAs and the pathogenesis of vasculitis

In systemic vasculitis there is leucocyte accumulation and vessel wall infiltration, cytokine release and activation of the inflammatory cascade, endothelial damage, and ultimately vessel wall destruction, thrombosis, and distal tissue infarction. The precipitant of this series of events is unknown but many workers believe it may be an infection. The prominent endothelial damage may be partly mediated by release from neutrophils of lytic enzymes and reactive oxygen species. There are several lines of experimental evidence that suggest that ANCAs may play a role in disease pathogenesis.
Circulating neutrophils in Wegener’s granulomatosis appear to be activated, probably by proinflammatory cytokines. In activated neutrophils, antigens recognised by ANCs are redistributed to the cell surface and therefore are available to interact with circulating antibodies. Exposure to ANCs can cause activated neutrophils to degranulate, undergo an oxidative burst, and damage endothelial cells in culture. Interaction of ANCs with cytoxin activated endothelial cells, which express the major C-ANCA antigen proteinase 3 on their surface, can increase neutrophil adhesion to the cells. This may potentiate neutrophil mediated endothelial injury, with neutrophil derived agents released into the relatively sheltered microenvironment formed when the cells bind. Similarly, activated endothelial cells are damaged when incubated with primed neutrophils and antibodies to the main P-ANCA antigen, myeloperoxidase. Other evidence suggests that binding of C-ANCs to circulating proteinase 3 may inhibit inactivation of this enzyme by α-antitrypsin. Continuing proteolytic activity may contribute to inflammation and tissue damage. Support for this theory comes from work showing a link between α-antitrypsin phenotype and ANCA positive vasculitis.

Conclusion

In both children and adults a positive IIF ANCA result in an appropriate clinical setting suggests the presence of active vasculitis, usually Wegener’s granulomatosis, microscopic polyangiitis, or pauci-immune crescentic glomerulonephritis. Recent evidence suggests that ANCs may be pathogenically important, although this is not yet proved. In Wegener’s granulomatosis the ANCA titre may be helpful in monitoring disease activity and in choosing appropriate therapy. In other inflammatory disorders such as systemic lupus erythematosus, inflammatory bowel disease, and rheumatoid arthritis the specificity of a positive ANCA test is lower and the antigens recognised are less well defined. Results are conflicting and, in these diseases, ANCA detection should still be considered a research tool, as the meaning of a positive result is not established, and may in fact be misleading. The antigenic specificity and clinical significance, if any, of the atypical pattern of staining seen in Kawasaki disease, inflammatory diseases, and infection have not been determined.