RHEUMATOLOGY

Antineutrophil cytoplasm antibodies and vasculitis

M C Nash, M J Dillon

This is the fifth article in a series on rheumatology.

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 (ANCA) 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 ANCA are small vessel necrotising vasculitides such as Wegener's granulomatosis, microscopic polyangiitis (microscopic polyarteritis), and pauci-immune crescentic glomerulonephritis. ANCA 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 ANCA were specific for Wegener's granulomatosis, and that the titre was associated with the degree of disease activity.⁴ In 1988 ANCA were first described in children, in three cases of necrotising glomerulonephritis.⁵ Subsequently ANCA have been reported, in both adults and children, in numerous conditions characterised by vasculitis and inflammation.⁶ 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.⁷ 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 3,⁸ and P-ANCA staining to antibodies against myeloperoxidase,⁹ 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 vasculitis.^{4 6 9 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

Nephrology Unit,
Institute of Child
Health, 30 Guilford
Street, London
WC1N 1EH
M C Nash
M J Dillon

Correspondence to:
Dr Dillon.

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.^{6 15 16} 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).¹⁷ 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.^{4 11} 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.¹⁸ 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.¹⁹

IN OTHER DISEASES

ANCAs are detected in a proportion of patients with systemic lupus erythematosus (SLE), rheumatoid arthritis, inflammatory bowel disease, autoimmune hepatic disease, and other chronic inflammatory disorders. C-ANCA patterns are uncommon. Most are P-ANCA or atypical on IIF, and, although some bind to myeloperoxidase on specific ELISAs, others bind to elastase, lactoferrin, cathepsin G, or β -glucuronidase, or are of undefined specificity.^{6 20-25} The prevalence and specificity of ANCAs vary in different reports and the clinical significance is uncertain. Reports for IgA nephropathy are conflicting but generally negative.^{26 27} ANCAs have also been described

in patients with infections such as amoebiasis, HIV, and cystic fibrosis,^{6 17 28} and in haemodialysis, without vasculitis.²⁹

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.³⁰⁻³² Although numbers are small, clinical experience suggests that findings in adults with these conditions can be extrapolated to children. ANCAs have also been reported in chronic inflammatory disorders in children. In inflammatory bowel disease, ANCAs are more common in ulcerative colitis than in Crohn's disease, a phenomenon seen with other autoantibodies.³³ In one study, 16 of 23 children with active SLE (70%) were positive for ANCAs. However, the antibodies were directed against a variety of antigens, and there was no correlation with organ involvement or disease activity.³⁴ As in the literature on adults, the significance of ANCAs in chronic inflammatory conditions in childhood is unclear. ANCAs are not present in haemolytic uraemic syndrome.³⁵ IgG and IgM ANCAs are not found in children with Henoch-Schönlein purpura, a relatively common systemic vasculitis. The prevalence and significance of IgA isotype ANCA in Henoch-Schönlein purpura is a matter of debate.²⁶

Early reports suggested that positive ANCA tests were found in acute Kawasaki disease, with an elevated titre and atypical pattern on IIF.³⁶ Later studies using only samples taken before administration of gammaglobulin therapy did not confirm this, and the same IIF pattern was seen in children with fever resulting from acute infection as in those with Kawasaki disease.^{37 38} This is consistent with the experience in adults in which an atypical pattern can be seen in a range of acute and chronic inflammatory conditions, and suggests that this pattern may be due to non-specific binding of antibody secondary to generalised immune activation and polyclonal B cell activation.

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 ANCAs are redistributed to the cell surface and therefore are available to interact with circulating antibodies.³⁹ Exposure to ANCAs can cause activated neutrophils to degranulate, undergo an oxidative burst, and damage endothelial cells in culture.⁴⁰⁻⁴² Interaction of ANCAs with cytokine activated endothelial cells, which express the major C-ANCA antigen proteinase 3 on their surface, can increase neutrophil adhesion to the cells.⁴³⁻⁴⁴ 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.⁴² Other evidence suggests that binding of C-ANCAs to circulating proteinase 3 may inhibit inactivation of this enzyme by α_1 -antitrypsin.⁴⁵ Continuing proteolytic activity may contribute to inflammation and tissue damage. Support for this theory comes from work showing a link between α_1 -antitrypsin phenotype and ANCA positive vasculitis.⁴⁶

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 ANCAs 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.

- Jennette JC, Falk RJ, Andrassy K, et al. Nomenclature of systemic vasculitides. Proposal of an international consensus conference. *Arthritis Rheum* 1994;37:187-92.
- Davies DJ, Moran JE, Niall JF, Ryan GB. Segmental necrotising glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology? *BMJ* 1982;285:606.
- Hall JB, Wadham BM, Wood CJ, Ashton V, Adam WR. Vasculitis and glomerulonephritis: a subgroup with an antineutrophil cytoplasmic antibody. *Aust N Z J Med* 1984;14:277-8.
- van der Woude FJ, Rasmussen N, Lobatto S, et al. Autoantibodies against neutrophils and monocytes: tool for diagnosis and marker of disease activity in Wegener's granulomatosis. *Lancet* 1985;i:425-9.
- Walters MD, Savage CO, Dillon MJ, Lockwood CM, Barratt TM. Antineutrophil cytoplasm antibody in crescentic glomerulonephritis. *Arch Dis Child* 1988;63:814-17.
- Kallenberg CG, Brouwer E, Weening JJ, Tervaert JW. Antineutrophil cytoplasmic antibodies: current diagnostic and pathophysiological potential. *Kidney Int* 1994;46:1-15.
- Wiik A. Delineation of a standard procedure for indirect immunofluorescence detection of ANCA. *APMIS Suppl* 1989;6:12-13.
- Goldschmeding R, van der Schoot CE, ten Bokkel Huinink D, et al. Wegener's granulomatosis autoantibodies identify a novel diisopropylfluorophosphate-binding protein in the lysosomes of normal human neutrophils. *J Clin Invest* 1989;84:1577-87.
- Falk RJ, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies with specificity for myeloperoxidase in patients with systemic vasculitis and idiopathic necrotizing and crescentic glomerulonephritis. *N Engl J Med* 1988;318:1651-7.
- Stroncek DF, Egging MS, Eiber GA, Clay ME. Neutrophil alloantibodies react with cytoplasmic antigens: a possible cause of false-positive indirect immunofluorescence assays for antibodies to neutrophil cytoplasmic antigens. *Am J Kidney Dis* 1993;21:368-73.
- Savage CO, Winearls CG, Jones S, Marshall PD, Lockwood CM. Prospective study of radioimmunoassay for antibodies against neutrophil cytoplasm in diagnosis of systemic vasculitis. *Lancet* 1987;i:1389-93.
- Hagen EC. Standardisation of ANCA assays using purified proteinase-3 (PR3) and myeloperoxidase (MPO) [abst]. *Clin Exp Immunol* 1995;101:41.
- Yang YH, Hutchinson P, Littlejohn GO, Boyce N. Flow cytometric detection of anti-neutrophil cytoplasmic autoantibodies. *J Immunol Methods* 1994;172:77-84.
- Hagen EC, Ballieux BE, van Es LA, Daha MR, van der Woude FJ. Antineutrophil cytoplasmic autoantibodies: a review of the antigens involved, the assays, and the clinical and possible pathogenetic consequences. *Blood* 1993;81:1996-2002.
- Vaipopoulos G, Hatzinikolaou P, Tsiroyanni A, et al. Anti-neutrophil cytoplasmic antibodies in Adamantidis-Behçet's disease. *Br J Rheumatol* 1994;33:406-7.
- Rozenbaum M, Rosner I, Naschitz Y, Golan TD. Absence of antineutrophil cytoplasmic autoantibodies in Familial Mediterranean Fever. *J Rheumatol* 1995;22:376-7.
- Davenport A, Lock RJ, Wallington TB, Feest TG. Clinical significance of anti-neutrophil cytoplasm antibodies detected by a standardized indirect immunofluorescence assay. *Q J Med* 1994;87:291-9.
- Cohen Tervaert JW, Huitema MG, Hene RJ, et al. Prevention of relapses in Wegener's granulomatosis by treatment based on antineutrophil cytoplasmic antibody titre. *Lancet* 1990;336:709-11.
- Kerr GS, Fleisher TA, Hallahan CW, Leavitt RY, Fauci AS, Hoffman GS. Limited prognostic value of changes in antineutrophil cytoplasmic antibody titer in patients with Wegener's granulomatosis. *Arthritis Rheum* 1993;36:365-71.
- Schnabel A, Csernok E, Isenberg DA, Mrowka C, Gross WL. Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. Prevalence, specificities, and clinical significance. *Arthritis Rheum* 1995;38:633-7.
- Coremans IE, Hagen EC, Daha MR, et al. Antilactoferrin antibodies in patients with rheumatoid arthritis are associated with vasculitis. *Arthritis Rheum* 1992;35:1466-75.
- Cambridge G, Rampton DS, Stevens TRJ, McCarthy DA, Kamm M, Leaker B. Anti-neutrophil antibodies in inflammatory bowel disease: prevalence and diagnostic role. *Gut* 1992;33:668-74.
- Mulder AH, Horst G, Haagsma EB, Limburg PC, Kleibeuker JH, Kallenberg CG. Prevalence and characterization of neutrophil cytoplasmic antibodies in autoimmune liver diseases. *Hepatology* 1993;17:411-7.
- Helsloot J, Virgo S, McGuigan L, Sturgess A. Antineutrophil cytoplasmic antibodies in inflammatory arthritis: potential for misdiagnosis? *Br J Rheumatol* 1995;34:820-4.
- Nassberger L, Ljungh A. β -Glucuronidase antibodies in ulcerative colitis. *Lancet* 1992;340:734-5.
- O'Donoghue DJ, Nusbaum P, Noel L, Halbwachs-Mecarelli L, Lesavre P. Antineutrophil cytoplasmic antibodies in IgA nephropathy and Henoch-Schönlein purpura. *Nephrol Dial Transplant* 1992;7:534-8.
- Sinico RA, Tadros M, Radice A, et al. Lack of IgA antineutrophil cytoplasmic antibodies in Henoch-Schönlein purpura and IgA nephropathy. *Clin Immunol Immunopathol* 1994;73:19-26.
- Eftimiou J, Spickett G, Lane D, Thompson A. Antineutrophil cytoplasmic antibodies, cystic fibrosis, and infection. *Lancet* 1991;337:1037-8.
- Weidemann S, Andrassy K, Ritz E. ANCA in haemodialysis patients. *Nephrol Dial Transplant* 1993;8:839-45.
- Wong S, Shah V, Dillon MJ. The spectrum of antineutrophil cytoplasmic antibodies (ANCA) in childhood systemic vasculitis [abst]. *Clin Exp Immunol* 1993;93:30.
- Ellis EN, Wood EG, Berry P. Spectrum of disease associated with anti-neutrophil cytoplasmic autoantibodies in pediatric patients. *J Pediatr* 1995;126:40-3.
- Vogt BA, Kim Y, Jennette JC, Falk RJ, Burke BA, Sinaiko A. Antineutrophil cytoplasmic autoantibody-positive crescentic glomerulonephritis as a complication of treatment with propylthiouracil in children. *J Pediatr* 1994;124:986-8.
- Winter HS, Landers CJ, Winkelstein A, Vidrich A, Targan SR. Anti-neutrophil cytoplasmic antibodies in children with ulcerative colitis. *J Pediatr* 1994;125:707-11.
- Wong SN, Shah V, Dillon MJ. Anti-neutrophil cytoplasmic antibodies in childhood systemic lupus erythematosus. *Eur J Pediatr* 1995;154:43-5.

- 35 Fitzpatrick MM, Shah V, Trompeter RS, Dillon MJ, Barratt TM. Interleukin-8 and polymorphonuclear leucocyte activation in hemolytic uremic syndrome of childhood. *Kidney Int* 1992;**42**:951-6.
- 36 Savage CO, Tizard J, Jayne D, Lockwood CM, Dillon MJ. Antineutrophil cytoplasm antibodies in Kawasaki disease. *Arch Dis Child* 1989;**64**:360-3.
- 37 Guzman J, Fung M, Petty RE. Diagnostic value of anti-neutrophil cytoplasmic and anti-endothelial cell antibodies in early Kawasaki disease. *J Pediatr* 1994;**124**:917-20.
- 38 Nash MC, Shah V, Reader JA, Dillon MJ. Anti-neutrophil cytoplasmic antibodies and anti-endothelial cell antibodies are not increased in Kawasaki disease. *Br J Rheumatol* 1995;**34**:882-7.
- 39 Csernok E, Ernst M, Schmitt W, Bainton DF, Gross WL. Activated neutrophils express proteinase 3 on their plasma membrane in vitro and in vivo. *Clin Exp Immunol* 1994;**95**: 244-50.
- 40 Falk RJ, Terrell RS, Charles LA, Jennette JC. Anti-neutrophil cytoplasmic autoantibodies induce neutrophils to degranulate and produce oxygen radicals in vitro. *Proc Natl Acad Sci USA* 1990;**87**:4115-9.
- 41 Savage CO, Pottinger BE, Gaskin G, Pusey CD, Pearson JD. Autoantibodies developing to myeloperoxidase and proteinase 3 in systemic vasculitis stimulate neutrophil cytotoxicity toward cultured endothelial cells. *Am J Pathol* 1992;**141**:335-42.
- 42 Ewert BH, Jennette JC, Falk RJ. Anti-myeloperoxidase antibodies stimulate neutrophils to damage human endothelial cells. *Kidney Int* 1992;**41**:375-83.
- 43 Mayet WJ, Csernok E, Szymkowiak C, Gross WL, Meyer zum Buschenfelde KH. Human endothelial cells express proteinase 3, the target antigen of anticytoplasmic antibodies in Wegener's granulomatosis. *Blood* 1993;**82**: 1221-9.
- 44 Mayet WJ, Meyer zum Buschenfelde KH. Antibodies to proteinase 3 increase adhesion of neutrophils to human endothelial cells. *Clin Exp Immunol* 1993;**94**: 440-6.
- 45 van de Wiele BA, Dolman KM, van der Meer Gerritsen CH, Hack CE, von dem Borne AE, Goldschmeding R. Interference of Wegener's granulomatosis autoantibodies with neutrophil Proteinase 3 activity. *Clin Exp Immunol* 1992;**90**: 409-14.
- 46 Savage JA, Chang L, Cook L, Burdon J, Daskalakis M, Doery J. α 1-antitrypsin deficiency and anti-proteinase 3 antibodies in anti-neutrophil cytoplasmic antibody (ANCA)-associated systemic vasculitis. *Clin Exp Immunol* 1995;**100**:194-7.