PR3 antibodies: not always an immunological emergency

Sirs,

Antineutrophil cytoplasmic antibodies (ANCA) are autoantibodies directed against antigens in the cytoplasm of neutrophils. A number of antigens have been identified, (*e.g.* elastase, bactericidal/permeability-increasing protein), but myeloperoxidase (MPO) and proteinase-3 (PR3) remain the most important antigens in vasculitis (1, 2). ANCA testing is essential in the work-up of systemic vasculitides. Initial screening with indirect immunofluorescence, looking for perinuclear (pANCA) or cytoplasmic (cANCA) staining patterns, is followed by antigen-specific assays for MPO and PR3, as outlined in the International Consensus Statement (2). The classical pairings of cANCA with PR3 and pANCA with MPO and their respective associations with specific vasculitides are well established (1, 2). The finding of either pairing constitutes an "immunological emergency", and our laboratory policy is to telephone out all ANCA results which are PR3 / MPO positive.

However, when ANCA staining patterns and subsequent antigen-specific assays do not follow convention, their clinical significance remains uncertain. We reviewed our PR3-ANCA and MPO-ANCA results to help establish the clinical significance of discrepant results (*i.e.* PR3 positive with pANCA immunofluorescence or MPO positive with cANCA immunofluorescence). All discordant ANCA results were identified from patients undergoing routine ANCA testing at Partnership Pathology Services (now Surrey Pathology Services) between January 2008 and December 2009. All samples were tested by indirect immunofluorescence followed if positive by MPO and PR3 antibody testing, using the Phadia ImmunoCAP assay. Immunofluorescence results were classified as cANCA, pANCA, or negative. We did not identify other described patterns such as atypical ANCA, or atypical cANCA. Discrepancy was confirmed on repeat ANCA/MPO/PR3 testing to exclude laboratory error. The case notes of patients with discrepant results at the 2 parent hospitals (Frimley Park and Royal Surrey County Hospitals) were then reviewed for underlying clinical diagnosis. We identified 19 patients with discrepant ANCA results. Fourteen patients demonstrated pANCA pattern with positive PR3, with the cANCA-MPO pairing found in the remaining 5 patients. Anti-nuclear antibody (ANA) testing was performed in 11 out of 14 patients with the p-ANCA-PR3 antibody combination. Four patients demonstrated weakly positive ANA with a further patient showing strongly positive ANA staining (centromere pattern).

Within this group of 19 discrepant ANCA patients, only one case of vasculitis was identified. Furthermore we observed a predominance of gastrointestinal/hepatic conditions in these patients. This was particularly prominent within the pANCA-PR3 cohort where 71% of patients had gastrointestinal/hepatic disorders, principally inflammatory bowel disease and autoimmune liver disease (Table I). Within the cANCA-MPO group, 4 out of 5 patients had non-vasculitic conditions though the gastrointestinal/hepatic preponderance was less striking. The only case of vasculitis found in either group was a patient with eosinophilic granulomatosis with polyangiitis (EGPA) who was cANCA positive with strong MPO antibodies (513 U/ml) and low positive PR3 (7 U/ml), (Table I).

Follow-up data was available for a limited number of these patients. In the pANCA-PR3 group 4 out of 14 patients had further testing. In 2 cases the same pANCA-PR3 combination was noted. From the c-ANCA-MPO group 3 out of 5 patients had follow-up testing but none showed the same c-ANCA-MPO pattern. This included the patient with eGPA, who became negative for MPO and PR3 after immunosuppression. His most recent sample, 7 years after follow-up, showed pANCA 1:20, negative MPO and equivocal PR3. We have been unable to verify the role of disease activity and treatment interventions in the other patients who were negative on repeat ANCA testing.

The clinical significance of discrepant ANCA results is as yet uncertain and remains largely unobserved in the literature. Most of our patients with discrepant ANCA immunofluorescence and MPO/PR3 antibodies did not have an underlying vasculitis. Furthermore, gastrointestinal and hepatic conditions were seen frequently, especially in those where pANCA was found together with PR3 antibodies. The association of ANCA, and in particular pANCA, with non-vasculitic conditions such as ulcerative colitis, primary sclerosing cholangitis (PSC) and autoimmune hepatitis has been well documented (3-6). There are more recent studies describing the presence of PR3 antibodies in PSC and ulcerative colitis (7, 8). In our own clinical practice the text accompanying authorised ANCA-MPO-PR3 results has been changed to take into account the above findings. Although we still telephone out all new PR3 and MPO antibody results, we advise the clinicians, particularly for PR3 antibodies with pANCA, to also be aware of the association with gastrointestinal and hepatic disease.

We acknowledge several limitations of our study. Firstly, it involved only small numbers of patients, despite the collection period totalling 2 years. We did not distinguish other described patterns such as atypical ANCA, or atypical cANCA. It is also possible that local patterns of ANCA requesting in our hospitals may have influenced these results, as we do not serve a Renal Unit. This limitation is important to acknowledge, in case we are underestimating the prevalence of discrepant ANCA serology in systemic vasculitis. Further study of a larger patient population and the inclusion of other hospitals with Renal Units in any future studies will be important to substantiate these findings.

Table I. Clinical diagnoses for patients with discordant ANCA results (either pANCA-PR3 or cANCA-MPO positive).

Number of patients	
pANCA-PR3	
Gastrointestinal/Hepatic	
2	Primary sclerosing cholangitis
5	Ulcerative colitis
2	Liver abscess / cyst
1	Colonic angiodysplasia
Other	
1	Psoriatic arthropathy
1	Raynaud's disease
1	Monoclonal gammopathy
1	Diagnosis unspecified
cANCA-MPO	
Gastrointestinal/Hepatic	
1	Drug-induced hepatitis
1	Duodenal ulcer
Renal	
1	Renal calculi
1	Chronic kidney disease
Vasculitis	
1	Eosinophilic granulomatosis with polyangiitis

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