

Predictive Value of Antineutrophil Cytoplasmic Antibodies in Small-Vessel Vasculitis: Is the Glass Half Full or Half Empty?



Since the discovery of antineutrophil cytoplasmic antibodies (ANCA) directed against proteinase-3 (PR3) and myeloperoxidase (MPO), the diagnostic potential of these antibodies in the investigation of patients suspected for small-vessel vasculitis has been appreciated^{1,2}. High sensitivity and specificity of validated antigen-specific tests have been demonstrated for Wegener's granulomatosis, microscopic polyangiitis, and the renal-limited form of small-vessel vasculitis, pauciimmune necrotizing glomerulonephritis³. More controversial is the possible association of ANCA levels with vasculitic disease activity. ANCA levels tend to fall or become negative in many patients once disease remission is induced with immunosuppressive treatment^{1,4-6}. In addition, data suggest a pathophysiological role for these antibodies⁷.

In this issue of *The Journal*, Lurati and Spertini report their data on a cohort of 36 patients with PR3- and MPO-ANCA associated vasculitis, of which 23 were classified as Wegener's granulomatosis and 13 as microscopic polyangiitis⁸. They retrospectively analyzed the presence of ANCA at diagnosis and the predictive value during followup, once remission was achieved, of persistently elevated or rising levels of PR3- and MPO-ANCA as determined by a commercially available direct antigen-specific ELISA.

PRESENCE OF ANCA AT DIAGNOSIS

At diagnosis, 22 of 23 patients (96%) classified by Lurati and Spertini as Wegener's granulomatosis were positive by indirect immunofluorescence (IIF) on ethanol-fixed neutrophils for cytoplasmic (c) ANCA, while antigen-specific ELISA was positive for PR3 antibodies in 18 (78%) patients and MPO antibodies in one (4%). Similarly, in 13 patients with microscopic polyangiitis, IIF revealed perinuclear (p) ANCA in 11 (85%) and cANCA in 2 (15%) patients at diagnosis. Again, ELISA appeared somewhat less sensitive, with MPO antibodies in only 8 (61%) and PR3 antibodies in one (8%). In both patient groups disease activity was biopsy-

proven in the great majority (32 of 36)⁸. Although not new and from a small retrospective study, these data again highlight some important points in the clinical use of ANCA testing in diagnosing small-vessel vasculitis. First, it is essential to use validated antigen-specific tests such as ELISA with purified antigen in addition to the screening by IIF on ethanol-fixed neutrophils, as the finding of a diffuse cytoplasmic (cANCA) or perinuclear fluorescence pattern (pANCA) is not equivalent to the presence of antibodies directed against PR3 and MPO, respectively⁶. In particular, the finding of pANCA lacks specificity, as these can be found in many other conditions and are not directed against MPO. Second, even in histologically verified disease direct PR3- and MPO-ELISA systems seem to have a somewhat reduced sensitivity as compared to IIF. Previous studies have suggested that antigen-specific capture ELISA systems are more sensitive without jeopardizing specificity³. As many different IIF and ELISA systems are (commercially) available, every clinician interpreting results of ANCA testing in the diagnostic investigation of a patient suspected of small-vessel vasculitis should be aware of the different characteristics these tests may have.

RELAPSE AND ANCA SPECIFICITY: IS THERE A DIFFERENCE BETWEEN PR3- AND MPO-ANCA?

Lurati and Spertini provide data in 28 patients with a mean followup of 5 years. More patients who were classified as Wegener's granulomatosis relapsed (14 of 19; 74%) as compared to patients with microscopic polyangiitis (3 of 9; 33%). In addition, time to first relapse was shorter in those with Wegener's granulomatosis⁸. As PR3- and MPO-ANCA specificity to a significant extent segregates with Wegener's granulomatosis and microscopic polyangiitis, respectively, it is obvious that PR3-ANCA specificity is associated with higher risk of relapse. Although the number of patients is small and duration of followup is not given for

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both disease groups separately, these data are in accord with previously reported findings. Even within cohorts of patients classified as having either Wegener's granulomatosis or microscopic polyangiitis, PR3- as compared to MPO-ANCA is associated with increased risk of relapse^{6,9}.

PREDICTIVE VALUE OF ANCA LEVELS DURING FOLLOWUP

Like the data provided by Lurati and Spertini, relapses in patients with Wegener's granulomatosis occur in $\geq 50\%$ within 5 years after diagnosis and initial therapy. In patients with microscopic polyangiitis and renal-limited vasculitis, percentages of $\cong 30\%$ within 5 years are reported⁶. As relapses of these diseases are associated with chronic organ damage (renal failure), increased cumulative immunosuppressive therapy toxicity, and mortality, relapses constitute both a numerically important and a clinically relevant problem^{10,11}. At the moment of relapse of active vasculitis following previous remission, in patients who have been ANCA-positive at diagnosis, ANCA measured by IIF or PR3- and MPO-ANCA ELISA is (again) positive in 80% to 100%¹². Therefore, a diagnosis of relapse in a patient with ANCA-associated small-vessel vasculitis with a persistently negative ANCA at the moment of presumed relapse should be seriously questioned, necessitating either histological proof of disease activity or rigorous exclusion of other diagnoses.

Whether persistently positive or increasing ANCA levels can predict ensuing vasculitic disease activity is more controversial. Lurati and Spertini, using a commercially available direct PR3- and MPO-ANCA ELISA, found no association of persistent positive ANCA titers for ≥ 6 months and relapse. As well, elevations analyzed over a range of 1.2- to 4-fold in PR3- or MPO-ANCA titers were, although statistically significant, only weakly associated with occurrence of relapse. Low level elevations showed moderate sensitivity and very poor specificity, while 3- to 4-fold elevations were rather specific, but at the cost of a sensitivity of only around 20%, making clinical use impossible or futile. Other studies have been published concerning the relation between elevations in ANCA levels as measured by IIF or ELISA and disease activity (see reviews^{6,13}). Unfortunately, many of these studies, like the one reported here by Lurati and Spertini, are retrospective, involve small numbers of patients and relapses, and do not standardize or even state the interval between sequential ANCA measurements. In addition, relapses of vasculitis can be histologically proven, clinically diagnosed, derived from changes in disease activity scores that have not been validated for that purpose, or any combination of these. Also with respect to measurement of ANCA levels, differences exist, not only in the type of assay used, but also whether sequential samples were tested in the same or different assays at the same or different times and whether during the whole study period the same type of assay has been

used. These methodological differences between, and probably within, the studies may be responsible for the widely different results for sensitivity and positive predictive value reported for rises in ANCA levels, ranging from 20% to (nearly) 100% for both. Using predefined criteria for relapse, a standardized interval for sequential ANCA measurements, and eliminating interassay variation by measuring 2 sequential samples in the same assay, we have found predictive values of $\geq 50\%$ for a relapse within 6 or 12 months for 4-fold elevations in cANCA titer by IIF or $> 75\%$ increase in PR3-ANCA level as determined by direct ELISA in 3 prospective studies with a reasonable number of patients and followup¹⁴⁻¹⁶. As in these studies relapse rates were around or below 10% per 12 months in patients without a cANCA or PR3-ANCA rise of this magnitude, prediction of clinically relevant increases in absolute risk for relapse may be feasible. For MPO-ANCA this situation is less well studied. Sparse data suggest that in MPO-ANCA related vasculitis as well, elevations in ANCA are associated with relapse of disease activity^{16,17}. However, probably due to the lower risk of relapse associated with MPO-ANCA associated vasculitis, this relationship has not been well established.

PREDICTIVE VALUE OF SERIAL ANCA DETERMINATIONS: NOT ONLY A DIAGNOSTIC PROBLEM

As relapses of small-vessel vasculitis cause significant morbidity and mortality due to both the disease and its treatment, an ideal intervention should result in prolonged prevention with minimal toxicity. The only prospective, randomized controlled study that has used preemptive immunosuppressive therapy with cyclophosphamide and prednisolone for 9 months after a 4-fold rise in cANCA titer showed that clinical relapses of vasculitis can be prevented, and cumulative exposure to immunosuppressive drugs can be reduced¹⁵. Similarly, an uncontrolled study in which immunosuppressive therapy was increased upon elevations in cANCA titer showed a large reduction in relapses compared to patients in whom therapy was not modified according to ANCA titers¹⁸. Both studies also showed a tendency to reduced treatment related toxicity and side effects in the preemptive treated patients. However, it was also illustrated clearly that not all relapses can be prevented in this way, as not all relapses are preceded by an elevation of ANCA. More worrisome, not all ANCA elevations are followed by clinical relapse, leading to (increased) immunosuppressive treatment with all the associated risks in patients who will not benefit. As currently the positive predictive value of serial ANCA determination for relapses is not close enough to 100% concomitant with useful sensitivity, we might try less aggressive therapies with, for example, azathioprine, although preliminary data from such a trial suggest that this approach may not be effective.

In the end we still face the problem that prediction and

prevention of relapses of ANCA associated vasculitis are not optimal. We should continue our clinical research efforts until we can substantially fill the glass and show that it is not empty.

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