Anti-neutrophil cytoplasmic antibody (ANCA) levels directed against proteinase-3 and myeloperoxidase are helpful in predicting disease relapse in ANCA-associated small-vessel vasculitis

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Since the discovery of the link between anti-neutrophil cytoplasmic antibodies (ANCA) directed against proteinase-3 (PR3) and myeloperoxidase (MPO) and small-vessel vasculitis, the diagnostic potential of these antibodies has been appreciated [1,2]. High sensitivity and specificity of validated antigen-specific tests for Wegener’s granulomatosis (WG), microscopic polyangiitis (MPA) and the renal limited form of small-vessel vasculitis, pauci-immune necrotizing glomerulonephritis (renal limited vasculitis; RLV), have been demonstrated [3]. It should be stressed that it is essential to use antigen-specific tests such as ELISA with purified antigen in addition to the screening by indirect immunofluorescence (IIF) on ethanol-fixed neutrophils as the finding of a diffuse cytoplasmic (C-ANCA) or perinuclear fluorescence pattern (P-ANCA) is not equivalent to the presence of antibodies directed against PR3 and MPO, respectively (Table 1). Especially the finding of P-ANCA lacks specificity as these can be found in many other conditions.

The finding that ANCA levels fall or become negative once disease remission has been obtained and in vitro data suggesting a pathophysiological role for these antibodies have fuelled the thought that, in addition to their diagnostic value, ANCA are more directly related to disease activity of the vasculitic process [4].

Does ANCA specificity have prognostic value?

Most larger series that describe patients with WG, MPA and RLV, find higher relapse rates for patients with WG (≥ 50% within 5 years) as opposed to MPA and RLV (~ 30% in 5 years) [3–8]. As WG is more frequently associated with antibodies against PR3 (70–80%) than to MPO (10–30%), while the opposite is true for MPA and RLV, the question is whether it is the ANCA specificity or the clinical syndrome that is related to the relapse risk. Many groups reported an increased relapse rate for patients with PR3- or C-ANCA-related, as compared with MPO- or P-ANCA-related, small-vessel vasculitis but did not subdivide these groups according to the clinical syndrome [9–13]. In one study in 75 patients with ANCA-related MPA/RLV who achieved remission after induction treatment, no difference in relapse rate was observed according to C- or P-ANCA specificity [7], although survival was worse in patients with C-ANCA [14]. In WG patients this issue has as yet not been addressed. In a retrospective study of 137 patients with WG, diagnosed and treated at our hospital between 1990 and 2000, we found a difference in relapse rate between WG patients with PR3, as compared with the small group with MPO-ANCA specificity [7], although survival was worse in patients with C-ANCA [14]. In WG patients this issue has as yet not been addressed. In a retrospective study of 137 patients with WG, diagnosed and treated at our hospital between 1990 and 2000, we found a difference in relapse rate between WG patients with PR3, as compared with the small group with MPO-ANCA specificity (Figure 1). Also, in 47 patients diagnosed during the same time period with MPA/RLV we observed a lower relapse-free survival in PR3-ANCA (n = 9; 63% at 5 years) compared with MPO-ANCA-positive patients (n = 38; 84% at 5 years), although the difference was not statistically significant.

Do ANCA levels during initial immunosuppressive treatment predict treatment failure or relapse?

Standard immunosuppressive therapy with cyclophosphamide, corticosteroids and plasmapheresis is highly effective in inducing remission in ANCA-associated vasculitis [1,2]. However, a significant proportion of patients with active disease relapse within the first year after initial remission [3–8]. The results of two previous studies [7,14] and our own findings suggest that these relapses are not entirely due to therapy failure but may be caused by a sustained or recurrent ANCA activity. This is supported by the finding that ANCA levels fall or become negative once treatment-induced remission has been achieved and in vitro data suggesting a pathophysiological role for ANCA [4]. The use of these antibodies as a marker of disease activity is important when planning therapy and assessing treatment response.

Table 1. Results of antigen specific testing by capture ELISA according to the fluorescence pattern in 243 samples positive on indirect immunofluorescence on ethanol-fixed neutrophils of 1434 samples (16%) sent for ANCA screening to the Laboratory for Clinical Immunology, University Hospital Groningen between January 1 and July 1, 1997

<table>
<thead>
<tr>
<th>Fluorescence pattern on IIF</th>
<th>PR3-ANCA</th>
<th>MPO-ANCA</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-ANCA (n = 19)</td>
<td>19</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>P-ANCA (n = 106)</td>
<td>2</td>
<td>16</td>
<td>90</td>
</tr>
<tr>
<td>Atypical-ANCA (n = 109)</td>
<td>3</td>
<td>11</td>
<td>97</td>
</tr>
</tbody>
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successful in inducing remission in ANCA-related small-vessel vasculitis, with a response rate of $\geq 80\%$. During induction treatment, ANCA levels fall or become negative in 30 to $>80\%$ of the patients within the first few months. Although persistent disease activity during treatment is associated with persistent ANCA positivity, most patients with persistent ANCA achieve remission [1,9,15]. Persistent or reappearing C-ANCA during the first year, however, is significantly related to subsequent relapse in both WG and other ANCA-related vasculitides [9,11–13,16,17]. For P-ANCA this is less clear [8,10,11,18]. In addition, the relapse risk for patients persistently negative for C- or P-ANCA is very low. As many relapses of ANCA-related vasculitis occur during tapering or within a short period after stopping immunosuppressive therapy [5,6,9,19], the question whether the response of ANCA levels during treatment should guide dose and duration of treatment is logical. We recently found that in patients with PR3-ANCA-associated vasculitis who were switched from cyclophosphamide to azathioprine after 3 months of complete remission, ANCA positivity at the moment of treatment switch was strongly associated with risk for relapse [20]. No prospective trials in this respect have been performed, but they are clearly needed to answer this question.

Do changes in ANCA level during follow up adequately predict relapse?

Relapses of ANCA-related small-vessel vasculitis after previous remission in patients who have been ANCA positive at diagnosis are associated in 80–100% of cases with a persistently or renewed positive ANCA, either measured by IIF or antigen-specific ELISA [8,9,11,13,16–18,21,22]. This has been reported both for PR3- and MPO-ANCA. 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 and should either be histologically proven or other diagnoses have to be ruled out. Whether persistently positive or increasing ANCA levels are good indicators of ensuing vasculitic disease activity is more controversial. A prognostic test should have both high sensitivity and specificity to result in high positive and negative predictive values for future clinical events. In addition, as relapses of small-vessel vasculitis are associated with significant morbidity and mortality due both to the disease and its treatment, an intervention should be possible that results in prolonged prevention of the event at minimal toxic costs. Studies have been published concerning the relation between rises in ANCA levels as measured by IIF or ELISA and disease activity (Tables 2 and 3). Many of these studies, however, are retrospective, involve small numbers of patients and relapses, and do not standardize the

| Table 2. Relation between rises in ANCA as determined by IIF and relapse of ANCA-related small-vessel vasculitis as reported by different studies |
|---|---|---|---|
| Number of patients | ANCA pattern on IIF | ANCA rise prior or at moment of relapse | ANCA rise followed by relapse | Reference |
| 35 | C-ANCA | 100% | 77% | [21] |
| 10 | C-ANCA | 100% | 75% | [30] |
| 58 | C-ANCA | 90% | 82% | [23] |
| 10 | N.R. | 82% | 65% | [31] |
| 68 | C-ANCA | 24% | 56% | [22] |
| 37 | C-P-ANCA | 43% | 23% | [32] |
| 19 | C-ANCA | N.R. | 57% | [11] |
| 85 | C-ANCA | 52% | 57% | [19] |
| 18 | C-P-ANCA | 37% | N.R. | [24] |

N.R., not reported.

| Table 3. Relation between rises in ANCA as measured by ELISA and relapse of ANCA-related small-vessel vasculitis as reported by different studies |
|---|---|---|---|
| Number of patients | ANCA antigenic specificity | ANCA rise prior or at moment of relapse | ANCA rise followed by relapse | Reference |
| 56 | Extract | 41% | 62% | [12] |
| 60 | N.R. | 74% | 79% | [9] |
| 17 | PR3 | 33% | 59% | [11] |
| 19 | MPO | 73% | 79% | [11] |
| 25 | MPO | 100% | 80% | [18] |
| 85 | PR3 | 81% | 71% | [19] |
| 15 | MPO | 75% | 100% | [19] |
| 14 | PR3 (capture) | 43% | N.R. | [24] |
| 18 | MPO/PR3 (direct) | 32% | N.R. | [24] |
| 10 | PR3 (direct) | 79% | 92% | [33] |
| 10 | PR3 (capture) | 100% | 83% | [33] |

N.R., not reported.
Predicting disease relapse in ANCA-associated small-vessel vasculitis

Can the relation between ANCA levels and vasculitic disease activity be improved?

Improvements in ANCA assays by further standardization of antigen sources and preparations used, and international standards for PR3- and MPO-ANCA levels may lead to more reproducible and comparable results within and between centres. Future studies will have to be performed to assess whether further characterization of PR3- and MPO-ANCA with respect to IgG subclass distribution or levels of certain IgG subclasses have a better correlation with disease activity. As especially IgG3 antibodies directed against PR3 were able to activate primed neutrophils in vitro and correlated with the in vitro capacity of activations, it was hoped that IgG3-PR3-ANCA levels were better predictors of disease activity. So far this has proven illusive as IgG3-ANCA levels are low and difficult to quantify with current assays [19,24]. Other in vitro characteristics of ANCA have been correlated with disease activity such as the interference of PR3-ANCA with enzyme activity of PR3 and the formation of a complex of PR3 with its physiological inhibitor x1-antitrypsin [25,26]. However, these tests are difficult to perform and have so far only been tested cross-sectionally. The observations that human PR3- and MPO-ANCA are directed against a limited number of epitopes on the respective target antigens and that epitope specificity changes during follow up may give other possibilities for refining the relation between ANCA and active small-vessel vasculitis [27–29].

Conclusions

A relationship between the presence of PR3-ANCA and to a lesser degree MPO-ANCA, and active small-vessel vasculitis has been reasonably well established. This relationship is far from perfect and, as is often the case, it can be better described at a group level than on an individual patient level. In individual patient care ANCA levels may guide treatment, but to what extent and in which circumstances has to be further elucidated.

References


10. Pettersson E, Heigl Z.


17. Girard T, Mahr A, Noel LH.


22. Kerr GS, Fleisher TA, Hallahan CW et al.


25. Kallenberg CG, Brouwer E, Weening JJ, Tervaert JW.

26. Savage CO, Harper L, Holland M.

27. Chang L, Binos S, Savige J.


29. van der Geld YM, Simpelaar A, Van Der ZR et al.

30. Egner W, Chapel HM.


32. Davenport A, Lock RJ, Wallington T.


34. Kerr GS, Fleisher TA, Hallahan CW et al.


38. Davenport A, Lock RJ, Wallington T.


43. ANCA titers, even of IgG subclasses, and soluble CD14 fail to predict relapses in patients with ANCA-associated vasculitis. Nephrol Dial Transplant 2001; 16: 1631–1637

44. Savage CO, Harper L, Holland M.


48. Anti-neutrophil cytoplasmic antibodies (ANCA) from patients with systemic vasculitis recognize restricted epitopes of proteinase 3 involving the catalytic site. Kidney Int 2001; 59: 147–159


62. Savage CO, Harper L, Holland M.

63. Anti-neutrophil cytoplasmic antibodies (ANCA) from patients with systemic vasculitis recognize restricted epitopes of proteinase 3 involving the catalytic site. Kidney Int 2001; 59: 147–159

64. Egner W, Chapel HM.


67. Davenport A, Lock RJ, Wallington T.


