Titres of anti-neutrophil cytoplasmic antibodies in inflammatory bowel disease are not related to disease activity


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Summary

In the systemic vasculitides, serial measurement of titres of anti-neutrophil cytoplasmic autoantibodies (ANCA) is useful for follow-up of disease activity and prediction of relapses. ANCA have been detected in patients with inflammatory bowel disease, but their relation to disease activity in these diseases is unclear. We analysed the relation between disease activity and ANCA titres as determined by indirect immunofluorescence in paired samples obtained during active disease and at remission from individual patients with ulcerative colitis and Crohn's disease. In contrast to the ANCA-associated systemic vasculitides, serial measurement of ANCA titres is not useful in the monitoring of disease activity in patients with inflammatory bowel disease.

Introduction

Anti-neutrophil cytoplasmic autoantibodies (ANCA), directed against cytoplasmic constituents of neutrophil granulocytes, have originally been detected in sera from patients with systemic vasculitides, in particular Wegener's granulomatosis. Their diagnostic significance in these disorders has been well established. The elucidation of the target antigens of ANCA in the vasculitides, proteinase 3 and myeloperoxidase, has further increased their diagnostic value.

ANCA have also been detected in serum samples from 50–80% of patients with ulcerative colitis and 10–20% of patients with Crohn's disease. ANCA in inflammatory bowel disease are directed against a wide variety of antigens, including lactoferrin, bactericidal/permeability-increasing protein, and the cytosolic antigens catalase and α-enolase.

In contrast to the systemic vasculitides, in which ANCA titres are related to disease activity, conflicting results have been reported on the relation between ANCA and clinical expression of inflammatory bowel disease. In ulcerative colitis, ANCA titres were reported to correlate with disease activity. Furthermore, ANCA-positive patients were found to have a more refractory type of disease than ANCA-negative patients. In Crohn's disease, ANCA have been suggested to be markers for colonic involvement. However, many other studies failed to detect clinical differences between ANCA-positive and ANCA-negative patients in both ulcerative colitis and Crohn's disease. Very few studies have used serial samples to investigate the relation between ANCA and disease activity.
To elucidate the relation between ANCA titre and disease activity, we analysed the presence and titre of ANCA in paired samples from patients with ulcerative colitis and Crohn’s disease during active disease and at remission. In addition, patients with ulcerative colitis and Crohn’s disease were followed prospectively, to study fluctuations of ANCA with time in relation to disease activity.

Methods

Patients

Consecutive patients with ulcerative colitis (n=60) or Crohn’s disease (n=101) from two different centres were included in the study between November 1994 and May 1998 after having given informed consent. The diagnosis of ulcerative colitis or Crohn’s disease was based on accepted clinical, endoscopic, and radiological criteria supported by histopathology. At least two plasma samples from each participating patient were analysed for the presence and titre of ANCA. The first sample was obtained at the time of inclusion in the study. Disease activity of ulcerative colitis or Crohn’s disease was scored at this time, to classify the patients as having either active or quiescent disease (for definitions, see below). If a patient was classified as having active disease at entry, a second plasma sample was analysed that was obtained during a quiescent phase of the disease (group I). If a patient was classified as having quiescent disease at entry, a second plasma sample was analysed obtained during an active phase of the disease (group II). If a patient was classified as having active (group III) or quiescent (group IV) disease during the total observation period, a second plasma sample was analysed that was obtained at a random second time point during the observation period with an interval of at least one month from the first sample.

To analyse more accurately possible fluctuations of ANCA with time in relation to disease activity, at least four serial samples were obtained from 21 unselected patients with ulcerative colitis and 26 with Crohn’s disease, a subset of the total group. All plasma samples were stored at −20°C until ANCA testing.

For ulcerative colitis, disease activity was measured using a clinical activity score. This score is based on the presence and intensity of eight key symptoms of ulcerative colitis, i.e. blood loss, mucoid discharge, frequency of defaecation, consistency of faeces, tenesmus, abdominal pain, rectal pain, and nausea/vomiting. Each symptom is scored from 0 to 2, resulting in a total score ranging from 0 (no symptoms) to 16 (all symptoms present, most severe degree). A score >4 was considered as active disease. For Crohn’s disease, disease activity was measured using the Harvey-Bradshaw index, which measures clinical disease activity based on five clinical items, and using the Van Hees index, which measures inflammatory activity based on several clinical and laboratory variables. A Harvey-Bradshaw index >3 was considered as active disease, as was a Van Hees index >150.

ANCA testing by indirect immunofluorescence

Detection of ANCA by indirect immunofluorescence was performed on ethanol-fixed granulocytes from one single donor as previously described, with minor modifications. Longitudinal samples from one patient were always analysed in one single experiment. Samples were diluted 1:40 in phosphate-buffered saline and tested at two-fold dilutions up to 1:640. A fluorescein-isothiocyanate-conjugated rabbit anti-human IgG antibody (F315, Dakopatts, dilution 1:100) was used for detection of bound IgG autoantibodies. All slides were read by the same observer, who was not aware of the clinical diagnosis or the state of disease activity. A titre ≥1:40 was considered positive.

Statistical analysis

Statistical analysis of the data was performed using Fisher’s exact test, the Mann-Whitney U test, or the Spearman rank correlation test. For analysis of serial samples, correlations between disease activity and ANCA titre were determined using the Spearman rank correlation test in each patient. The mean correlation coefficient was calculated after Z-transformation. A p value <0.05 was considered significant for all tests.

Results

Ulcerative colitis

Sixty patients with ulcerative colitis were included in this study. Thirty-eight (63%) patients had at least one sample included that was positive for ANCA. All positive samples showed perinuclear (pANCA) staining, with the exception of one sample with cytoplasmic staining. The presence of ANCA was not related to sex, age, duration of the disease, localization of the disease, or use of immunosuppressive medication (corticosteroids, azathioprine, or both). Previous bowel resection did not influence the presence of ANCA: eight patients had undergone bowel resection, of whom four (50%) were positive for ANCA, whereas 30/52 (58%) patients without
previous bowel resection were positive for ANCA ($p=0.72$; Fisher’s exact test).

**Paired samples from patients with ulcerative colitis**

To determine any correlation between ANCA titre and disease activity in individual patients, pairs of samples from the 60 patients were analysed. Patients were divided into four different groups based on the course of disease activity during the study period, as stated in the methods section. Thirteen patients were in group I (active-quiescent), 14 were in group II (quiescent-active), six were in group III (active-active), and 27 were in group IV (quiescent-quiescent). Clinical characteristics of the four groups of patients are given in Table 1.

ANCA prevalence was not different between the four groups (Table 1). Figure 1 shows the individual paired ANCA titres in the four groups. Changes in ANCA titre between the first and the second sample were not related to changes in ANCA titre. Changes in ANCA titre were also observed in group IV, in which the disease was persistently quiescent during the total observation period. The use of immunosuppressive medication did not influence the presence or titre of ANCA.

**Serial samples from patients with ulcerative colitis**

To study the fluctuation of ANCA titres with time in relation to disease activity, we analysed ANCA titre and disease activity in serial samples from 21 ulcerative colitis patients who had at least four (median 6, range 4–11) samples included in the study. Mean (median) follow-up period was 24.6 (24) months (range 12–42 months).

Fifteen of these 21 patients were at least once positive for ANCA during the observation period.

### Table 1  Clinical characteristics of patients with ulcerative colitis (for group definitions, see text)

<table>
<thead>
<tr>
<th></th>
<th>Group I ($n=13$)</th>
<th>Group II ($n=14$)</th>
<th>Group III ($n=6$)</th>
<th>Group IV ($n=27$)</th>
<th>All groups ($n=60$)</th>
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</thead>
<tbody>
<tr>
<td><strong>Male/female</strong></td>
<td>3/10</td>
<td>8/6</td>
<td>4/2</td>
<td>15/12</td>
<td>30/30</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td>28 (23–42)</td>
<td>42 (17–71)</td>
<td>50 (31–75)</td>
<td>36 (18–83)</td>
<td>35 (17–83)</td>
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<td>8 (1–27)</td>
<td>2 (0–11)</td>
<td>2 (0–23)</td>
<td>3 (0–27)</td>
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<td>4</td>
<td>11</td>
<td>30</td>
</tr>
<tr>
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<td>1</td>
<td>14</td>
<td>26</td>
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<td>1</td>
<td>2</td>
<td>4</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
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<td>13</td>
<td>4</td>
<td>23</td>
<td>52</td>
</tr>
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<td><strong>Interval between samples (months)</strong></td>
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<td>14 (1–35)</td>
<td>2 (1–19)</td>
<td>13 (2–41)</td>
<td>12 (1–41)</td>
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<td><strong>ANCA-positive</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At least one of two samples</td>
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<td>9</td>
<td>4</td>
<td>16</td>
<td>38</td>
</tr>
<tr>
<td>Neither of two samples</td>
<td>4</td>
<td>5</td>
<td>2</td>
<td>11</td>
<td>22</td>
</tr>
</tbody>
</table>

*Values are given as median (range).*

**Figure 1.** Reciprocal ANCA titres in paired samples from patients with ulcerative colitis. Patients were divided into four groups based on the course of clinical disease activity (for details, see text).
Two patients had persistently active disease during the total observation period (one ANCA-positive and one ANCA-negative). Three patients had persistently quiescent disease during the total observation period (two ANCA-positive and one ANCA-negative). The remaining 16 patients had intermittently active disease (twelve ANCA-positive and four ANCA-negative).

There was no correlation between disease activity and ANCA titre in the 15 patients who were at least at one occasion positive for ANCA (mean \( r = -0.073 \)). Clinical activity scores and ANCA titres during follow-up in six representative ANCA-positive ulcerative colitis patients are shown in Figure 2.

**Crohn’s disease**

In this study, 101 patients with Crohn’s disease were included. Twenty-one (21%) patients had at least one sample included that was positive for ANCA. Samples from 17 patients showed perinuclear (pANCA) staining, whereas samples from the other four patients showed cytoplasmic staining. The presence of ANCA was not related to sex, age, duration of the disease, localization of the disease, or use of immunosuppressive medication. Previous bowel resection did not influence the presence of ANCA: 54 patients had undergone bowel resection, of whom seven (13%) were positive for ANCA, whereas 10/47 (21%) patients without previous bowel resection were positive for ANCA (\( p = 0.30 \); Fisher’s exact test).

Clinical disease activity was measured with the Harvey-Bradshaw index. In addition, inflammatory activity was measured with the Van Hees index. Since the correlation between the two indices was low in this study (\( r = 0.38 \); \( p < 0.001 \); Spearman rank correlation test), we separately analysed the relation between clinical disease activity (Harvey-Bradshaw index) and ANCA titres and that between inflammatory activity (Van Hees index) and ANCA titres.

**Paired samples from patients with Crohn’s disease**

To investigate correlations between ANCA titre and clinical disease activity in individual patients, pairs of samples from the 101 patients were analysed. Patients were divided into four groups (I–IV) based on the course of clinical disease activity during the study period, as assessed by the Harvey-Bradshaw index. Twenty patients were in group I (active-quiescent), 18 were in group II (quiescent-active), six were in group III (active-active), and 57 were in group IV (quiescent-quiescent). Clinical characteristics of the four groups are given in Table 2.

ANCA prevalence was not different between the four groups. Figure 3 shows the individual paired ANCA titres in the four groups. Changes in disease activity status in groups I and II were not related to changes in ANCA titre. Changes in ANCA titre were also observed in groups III and IV, in which the disease was either persistently active or persistently quiescent during the total observation period. The use of immunosuppressive medication did not influence the presence or titre of ANCA. Likewise, ANCA prevalence and titre were not related to inflammatory disease activity as assessed by the Van Hees index (data not shown).

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**Figure 2.** Reciprocal ANCA titres and clinical disease activity scores in serial samples from six representative ANCA-positive patients with ulcerative colitis.
Table 2  Clinical characteristics of patients with Crohn’s disease (for group definitions, see text)

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=20)</th>
<th>Group II (n=18)</th>
<th>Group III (n=6)</th>
<th>Group IV (n=57)</th>
<th>All groups (n=101)</th>
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</thead>
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<td>8/10</td>
<td>1/5</td>
<td>24/33</td>
<td>43/58</td>
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<tr>
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<td>31 (18–62)</td>
<td>34 (21–68)</td>
<td>35 (19–68)</td>
<td>39 (18–73)</td>
<td>35 (18–73)</td>
</tr>
<tr>
<td>Disease duration (years)*</td>
<td>3 (0–30)</td>
<td>8 (0–35)</td>
<td>6 (1–14)</td>
<td>7 (0–34)</td>
<td>6 (0–35)</td>
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<tr>
<td>Localization</td>
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<td></td>
</tr>
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<td>5</td>
<td>1</td>
<td>16</td>
<td>28</td>
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<tr>
<td>Ileum + colon</td>
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<td>3</td>
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<td>46</td>
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<td>2</td>
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<td>37</td>
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<td>3</td>
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<tr>
<td>Interval between samples (months)*</td>
<td>12 (1–35)</td>
<td>14 (1–31)</td>
<td>7 (1–14)</td>
<td>19 (2–42)</td>
<td>14 (1–42)</td>
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<td>ANCA-positive</td>
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<tr>
<td>At least one of two samples</td>
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<td>6</td>
<td>1</td>
<td>8</td>
<td>19</td>
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<tr>
<td>Neither of two samples</td>
<td>16</td>
<td>12</td>
<td>5</td>
<td>49</td>
<td>82</td>
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</tbody>
</table>

*Values are given as median (range).

Figure 3. Reciprocal ANCA titres in paired samples from patients with Crohn’s disease. Patients were divided into four groups based on the course of clinical disease activity (for details, see text).

Serial samples from patients with Crohn’s disease

To study the fluctuation of ANCA titres over time in relation to clinical and inflammatory disease activity, we analysed ANCA and disease activity in serial samples from 26 Crohn’s disease patients who had at least 4 (median 6, range 4–11) samples included in the study. Mean (median) follow-up period was 29.1 (29) months (range 7–43 months).

Six of these 26 patients were at least at one occasion positive for ANCA during the observation period. Nine patients had clinically quiescent disease during the total observation period (one ANCA-positive and eight ANCA-negative). The remaining 17 patients had intermittently active disease (five ANCA-positive and 12 ANCA-negative). There was no correlation between clinical disease activity or inflammatory disease activity and ANCA titre in the six ANCA-positive patients (Harvey-Bradshaw index: mean r = 0.01; Van Hees index: mean r = −0.114). Clinical and inflammatory disease activity and ANCA titres during follow-up in these six patients are shown in Figure 4.

Discussion

In this study, we demonstrated that in patients with inflammatory bowel disease, ANCA titres do not correlate with disease activity. In the ANCA-associated vasculitides, several studies have shown a relation between disease activity and ANCA titre (reviewed in reference 10). In these diseases, ANCA become often undetectable after starting immunosuppressive treatment. Relapses frequently occur in patients being persistently positive for ANCA or in whom ANCA reappear.\(^{23–25}\) Rises in ANCA titre may occur during clinical relapse,\(^{26}\) but may also precede a clinical relapse.\(^{27}\) Cohen Tervaert et al.\(^{28}\) showed
Figure 4. Reciprocal ANCA titres, clinical disease activity as assessed by the Harvey-Bradshaw index, and inflammatory disease activity as assessed by the Van Hees index in serial samples from six ANCA-positive patients with Crohn’s disease.

that treatment with immunosuppressive medication based on rises in ANCA titre prevented the occurrence of relapses in patients with Wegener’s granulomatosis. Thus, serial measurement of ANCA titres may be useful for follow-up and treatment of patients with ANCA-associated vasculitides.

However, in inflammatory bowel disease, the relation between disease activity and ANCA titre has been less clear. In ulcerative colitis, several studies reported that high titres of ANCA were particularly found in patients with active disease, but other studies failed to detect a relation between disease activity and ANCA titre. However, these were all cross-sectional studies. No large studies investigating a possible relation between disease activity and ANCA titres in individual patients have been performed thus far. The single long-term observation of ANCA in ulcerative colitis published until now only reported the presence or absence of ANCA and did not study whether titres varied with disease activity.

The data reported here clearly show that disease activity of both ulcerative colitis and Crohn’s disease are not related to ANCA titres. For measurement of disease activity of ulcerative colitis, we have used an index that has been developed to evaluate disease activity in clinical trials and is widely used in The Netherlands. For Crohn’s disease, two different indices were used to measure disease activity: the Harvey-Bradshaw index which measures clinical disease activity, scoring of which is, to some extent, subject to clinical interpretation; and the Van Hees index, mainly based on inflammatory parameters as measured in the laboratory. Although these indices were not concordant, which might be explained by their different approaches to disease activity, neither index correlated with ANCA titres.

We used two different approaches to analyse the relation between disease activity and ANCA titre. First, we determined ANCA titres in paired samples from groups of patients who were discordant in their course of disease activity. Changes in ANCA titre were found in a significant number of paired samples, with no correlation with changes in disease activity. Changes in ANCA titre were also observed in patients with either persistently active or persistently quiescent disease. Conversions from ANCA-negative to ANCA-positive and vice versa were detected without any relation to disease activity. Secondly, since paired samples may not precisely reflect the course of ANCA titre changes in time, we determined ANCA titres in individual patients have been performed thus far. The single long-term observation of ANCA in ulcerative colitis published until now only reported the presence or absence of ANCA and did not study whether titres varied with disease activity.

The use of immunosuppressive medication had no influence on the presence or titre of ANCA activity in the paired and serial samples from patients with ulcerative colitis or Crohn’s disease. For measurement of disease activity of ulcerative colitis and Crohn’s disease, we have used an index that has been developed to evaluate disease activity in clinical trials and is widely used in The Netherlands. For Crohn’s disease, two different indices were used to measure disease activity: the Harvey-Bradshaw index which measures clinical disease activity, scoring of which is, to some extent, subject to clinical interpretation; and the Van Hees index, mainly based on inflammatory parameters as measured in the laboratory. Although these indices were not concordant, which might be explained by their different approaches to disease activity, neither index correlated with ANCA titres.

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An important difference between the findings in the vasculitides and in the inflammatory bowel diseases is that ANCA in the vasculitides, in contrast to ANCA in inflammatory bowel disease, are usually directed against one single antigen. Antibodies to proteinase 3 are highly specific for Wegener’s granulomatosis, whereas antibodies to myeloperoxidase are associated with microscopic polyangiitis, the Churg-Strauss Syndrome, or idiopathic necrotizing crescentic glomerulonephritis. ANCA in inflammatory
bowel disease are directed against a wide variety of antigens, including lactoferrin, bactericidal/permeability-increasing protein, several nuclear antigens, and several cytosolic antigens. In addition, antibodies to various antigenic specificities may be present in one serum sample. We have shown that ANCA detection by indirect immunofluorescence may fail to detect antibodies to some of these antigens. On the other hand, a substantial number of patients with ANCA as detected by indirect immunofluorescence do not test positive for antibodies to the aforementioned antigens. Furthermore, multiple antigenic targets may be recognized by one serum sample. These findings, together with the lack of standardized assays other than for anti-proteinase 3 and anti-myeloperoxidase, do not permit us to analyse the relation between disease activity and titres of antigen-specific ANCA in the total population of patients with inflammatory bowel disease. In the future, quantitative antigen-specific tests may reveal whether disease activity correlates with ANCA of any defined specificities.

ANCA have been suggested to play a role in the pathophysiology of the systemic vasculitides. Several observations lead to the assumption that ANCA are not involved in the pathogenesis of inflammatory bowel disease. First, this study, like most other studies, has failed to detect any association between ANCA and clinical parameters of inflammatory bowel disease. Furthermore, ANCA in ulcerative colitis and Crohn’s disease bind a variety of antigens, which are not exclusively recognized by ANCA from patients with these disorders but also by ANCA from patients with autoimmune liver diseases and rheumatic diseases. The persistence of ANCA after colectomy in ulcerative colitis, which was also confirmed in this study, demonstrates that an inflamed colon is not a necessary requirement for the presence of ANCA. Finally, a pathogenic role for ANCA from patients with systemic vasculitis has been suggested by their in vitro ability to induce oxygen radical production in cytokine-primed neutrophils. However, ANCA from patients with inflammatory bowel disease are not able to induce oxygen radical formation. We have observed that ANCA directed against the main target antigens in inflammatory bowel disease, that is, lactoferrin, bactericidal/permeability-increasing protein, catalase, and z-enolase, were not able to induce a respiratory burst in primed neutrophils (Roozendaal et al., manuscript in preparation). Thus, whereas ANCA may be a useful marker for subclassification of ulcerative colitis or Crohn’s disease, any pathogenic role in these diseases seems unlikely.

From the data presented in this study we conclude that, in contrast to the ANCA-associated vasculitides, serial measurement of ANCA titres in patients with inflammatory bowel disease does not contribute to the monitoring of the disease process and thus to a better treatment.

Acknowledgements

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References


