Anca associated vasculitis
Boomsma, Maarten Michiel

IMPORTANT NOTE: You are advised to consult the publisher’s version (publisher’s PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2001

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Prevention of relapses in antineutrophil cytoplasmic antibody associated vasculitis by treatment based on autoantibody levels as detected by solid phase assay: a preliminary report

Maarten M. Boomsma 1, Coen A. Stegeman 2, Jo Hermans 3, Cees G.M. Kallenberg 1, Ronald J. Hené 5, Pieter C. Limburg 4, Jan Willem Cohen Tervaert 6

Department of Internal Medicine, Division of 1 Clinical Immunology, 2 Nephrology, 4 Rheumatology, University Hospital Groningen, Groningen, 3 Division of Medical Statistics, Leiden University Medical Center, Leiden, 5 Division of Nephrology, University Hospital Utrecht, 6 Division of Clinical Immunology, University Hospital Maastricht, Maastricht, The Netherlands.

In preparation
Abstract

Relapses of Wegener’s granulomatosis (WG) can be prevented by pre-emptive treatment based on changes in levels of antineutrophil cytoplasmic antibodies (ANCA). To assess the value of early treatment with azathioprine in combination with prednisolone after a rise in ANCA a randomized multi-center study was conducted in PR3-ANCA positive patients with vasculitis. Hundred PR3-ANCA positive patients attending outpatient clinics in four different hospitals in the Netherlands were studied prospectively from 1998 to 2001. Serum samples were analyzed every 6 weeks for ANCA levels by antigen specific ELISA. Disease activity was prospectively assessed without knowledge of the ANCA levels. Following a rise in ANCA patients were randomized to receive no treatment or to start immunosuppressive therapy. Over 36 months, ANCA rose in 40 patients, 20 patients were randomly assigned to pre-emptive therapy with azathioprine (9 months) and prednisolone (4.5 months); and 20 patients to follow-up without pre-emptive treatment. During follow-up 4 of 20 (20%) patients with pre-emptive treatment and 10 of 20 (50%) patients without pre-emptive treatment relapsed after a median period of 12.3 months (range 0.2 to 29) following randomization. Actuarial disease-free survival with pre-emptive treatment was 100% and 89% whereas without pre-emptive treatment disease-free survival was 80% and 73% at 6 and 12 months, respectively. Early treatment with azathioprine and prednisolone after a rise in PR3-ANCA prevents imminent relapses in patients with PR3-ANCA positive vasculitis. However, since late relapses were observed during long-term follow-up after stopping preventive treatment, a nine month course of azathioprine in combination with a 4.5 months course of prednisolone is likely to be too short for all patients and, hence, cannot be recommended.

Introduction

Active small-vessel vasculitides that are associated with anti-neutrophil cytoplasmic antibodies (ANCA) are potentially life-threatening diseases with substantial morbidity and mortality. Prolonged immunosuppressive therapy (>1 year) with cyclophosphamide and steroids is effective in inducing disease remission and preventing early relapses in most vasculitic disorders (Balow et al., 1993; Fauci et al., 1978; Gaskin et al., 1992; Hoffman et al., 1992). Continuous use of cyclophosphamide to sustain remission can not be recommended, since this treatment regimen is associated with severe and potentially lethal adverse effects such as the occurrence of opportunistic infections and the development of malignancies (Radis et al., 1995; Stillwell et al., 1988; Westman et al., 1998). Therefore, cyclophosphamide is tapered or stopped once remission is achieved. The majority of patients, however, experience relapses during tapering or when treatment has been stopped (Hoffman et al., 1992; Gordon et al., 1993; Guillevin et al., 1999; Nachman et al., 1998). Since relapses are associated with morbidity and mortality (Exley et al., 1997), effective prevention of relapses is of great potential importance.
We have previously reported that rises in the titer of cytoplasmic/classic ANCA (C-ANCA) as detected by indirect immunofluorescence (IIF) on ethanol fixed neutrophils often precede disease activity, suggesting that serial measurement of ANCA titers is useful in the follow-up of patients with WG (Cohen Tervaert et al., 1989). Recently we demonstrated in a large prospective study in patients with WG that measuring PR3-ANCA by antigen-specific enzyme-linked immunosorbent assay (ELISA) is superior to measuring ANCA by IIF for the prediction of an ensuing relapse (Boomsma et al., 2000). Furthermore, we reported in 1990 in a small prospective clinical trial in which treatment with a 9 month course of cyclophosphamide in combination with a 3 months course of prednisolone instituted after a rise in C-ANCA proved to be successful for the prevention of relapses (Cohen Tervaert et al., 1990a). The use of cyclophosphamide in this trial has been criticized since such a treatment is potentially harmful and it is known that not all rises in C-ANCA as detected by IIF are followed by a relapse. Alternatively, azathioprine may be used (Gaskin et al., 1992). Azathioprine is considered less effective in inducing remission than cyclophosphamide, but its long-term toxicity is much lower and it is successfully used for maintenance therapy in ANCA associated vasculitis (Bouroncle et al., 1967; Norton et al., 1968; Gaskin et al., 1992). Based on the results of our previous study (Boomsma et al., 2000) 82% of the relapses in patients with WG will be preceded by a significant rise in PR3-ANCA as detected by ELISA. We hypothesized that by treatment based on a significant rise in ANCA levels the risk for a subsequent relapse can be reduced by 80%, resulting in a reduction of relapses by approximately two-thirds.

We report the preliminary results of a randomized multi-center study in 100 PR3-ANCA positive patients with vasculitis in order to assess the value of early treatment with 9 and 4.5 months courses of azathioprine and prednisolone based on serial determination of ANCA levels as detected by ELISA for the prevention of relapses.

**Patients and methods**

**Patients**

Patients with ANCA associated vasculitis who had been positive for PR3-ANCA (Cohen Tervaert et al., 1990b) during an active phase of the disease were eligible for this study. Eligibility required 50 mg / day or less of cyclofosfamide and 15 mg / day or less of prednisolone. All patients were classified according to the Chapel Hill Consensus Conference on nomenclature of systemic vasculitides (Jennette et al., 1994). Inclusion started in March 1998 and ended in March 2001. At inclusion, all patients were in complete remission. The institutional review boards of the participating hospitals approved the study and written informed consent was obtained from all participants. The study was carried out in accordance
with the 1997 Declaration of Helsinki of the World Medical Association (World Medical Association, 1997).

Patients were closely monitored at the outpatient clinic of the participating centers according to a strict protocol. Patients were seen at least every 3 months and serum PR3-ANCA levels were determined at least every 6 weeks. At each visit, the same physicians performed the clinical examination and at each visit the urine sediment, and 24-hour urine excretion of protein and creatinine were analyzed. In addition, C-reactive protein concentration, erythrocyte sedimentation rate, and serum creatinine were measured.

Disease activity scoring

At each visit, disease activity was scored using the Birmingham Vasculitis Activity Score (BVAS) (Luqmani et al., 1994). Complete remission was defined as the complete absence of symptoms or signs attributable to active vasculitis (BVAS = 0), in combination with a normal serum C-reactive protein concentration (<10 mg/l) with absence of infection. A relapse was defined as described previously (Cohen Tervaert et al., 1989). Patients had either the recurrence of biopsy-proven granulomatous inflammation of the respiratory tract, glomerulonephritis, or recurrent arthralgias in combination with other signs of vasculitis with or without rising C-reactive protein levels. Signs of vasculitis included diffuse or nodular pulmonary infiltrates with or without cavitation, decreased renal function in combination with (microscopic) glomerular hematuria and proteinuria, necrotizing scleritis, episcleritis, vasculitis of the skin, paresis with loss of sensory function, and serous otitis media. If infection proved to be the cause of symptoms, the patient was not considered to have an exacerbation.

Detection of ANCA by direct ELISA

Detection of PR3-ANCA by ELISA was performed as previously described (Boomsma et al., 2000). In short, 96-well microtiter plates (Maxisorp; NUNC NS, Roskilde, Denmark) were coated at 37°C for 1.5 hours with 5 mM phenylmethyl-sulphonyl fluoride (PMSF)-inactivated PR3 (1 µg/ml). After washing, sera were applied at a dilution of 1:100 and 1:300 and incubated for 1 hour at 37°C, washed, and incubated with affinity purified F(ab')2 goat anti-human IgG conjugated with alkaline phosphatase (dilution 1:3000; American Qualex, San Clemente, CA) for 1 hour at 37°C. The plates were developed using P-nitrophenyl-phosphate disodium substrate. The reaction was blocked with 5 M NaOH, and optical densities were measured at 405 nm. A standard curve was made using a reference serum sample included in each test. Values were expressed as arbitrary units/ml. Values of 6 units/ml (mean + 2 SD of 65 normal controls) or more were considered to be positive. Each sample was compared with that in the previously obtained sample in the same assay. The intra- and interassay variations
were <10%. Rises in ANCA had to occur within a period of 6 weeks; a significant rise was defined as an increase in titer of 75% or more with respect to the previous determination. Only rises in ANCA that amounted to at least 10 arbitrary units were taken into account. (Boomsma et al., 2000).

Randomization and treatment

When a significant rise of ANCA was detected, patients were randomized to receive either no treatment or to start pre-emptive immunosuppressive therapy. Randomization was done in one center (Groningen University Hospital). Block randomization (block length 4) was made after stratification for co-trimoxazole use at the time of ANCA rise since we have previously shown that co-trimoxazole maintenance therapy decreases the risk for developing relapses (Stegeman et al., 1996).

When the patient was randomized for pre-emptive treatment, immunosuppressive treatment was started with azathioprine (1 mg/kg per day; maximum 75 mg/day) and prednisolone (30 mg per day). When the patient was using either of these drugs at the time of the ANCA rise, the dose of azathioprine and prednisolone was increased by 1 mg/kg/day (maximum 150 mg/day) and 30 mg/day, respectively. The azathioprine dose was tapered in steps of 25 mg every 3 months. Prednisolone dose was tapered in steps of 5 mg every 3 weeks. For clinical relapses patients were treated with cyclophosphamide and steroids, with or without plasma exchange, according to our standard protocols (Franssen et al., 1998; Boomsma et al., 2000). The moment of a clinical relapse was defined as the time at which immunosuppressive treatment for clinically active disease was started or intensified.

Statistical methods

Data are presented as median value with total range unless stated otherwise. In the study, we focused on the relapse-free (or disease-free) period after a rise in ANCA. We calculated the risk of a first relapse following a rise in ANCA detected between March 1998 and March 2001. It is planned to follow-up randomized patients through March 2002. In the present paper we report the preliminary analysis of the data with a follow-up which was completed through April 2001.

Disease-free survival after randomization was estimated with the Kaplan-Meier method and survival curves were generated using GraphPad Prism™ software. Differences in disease-free survival were analyzed by log-rank test. Hazard ratios (or relative risks) are reported as relative risks with 95% confidence intervals. Differences in baseline characteristics between both randomization limbs were tested with the Fischer’s exact test for categorical quantities, and the Mann-Whitney test (unpaired observations) or Wilcoxon test (paired observations) for continuous quantities when appropriate. A two-sided p-value of < 0.05 was considered to indicate statistical significance.
Chapter 7

Table 1 Patient characteristics according presence or absence of PR3-ANCA rise during follow and treatment allocation after PR3-ANCA rise

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group I&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Group II&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Group III&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No treatment (n = 20)</td>
<td>Treatment (n = 20)</td>
<td>no rise in ANCA (n = 60)</td>
</tr>
<tr>
<td>Age, y</td>
<td>52 (18 - 74)</td>
<td>65 (38 - 84)</td>
<td>56 (22 - 80)</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>10 / 10</td>
<td>9 / 11</td>
<td>38 / 22</td>
</tr>
<tr>
<td>Ever used Cyclophosphamide, yes / no</td>
<td>20 / 0</td>
<td>18 / 2</td>
<td>52 / 8</td>
</tr>
<tr>
<td>Ever used Azathioprine, yes / no</td>
<td>13 / 7</td>
<td>7 / 13</td>
<td>25 / 35</td>
</tr>
<tr>
<td>Ever used Prednisolone</td>
<td>20 / 0</td>
<td>20 / 0</td>
<td>54 / 6</td>
</tr>
<tr>
<td>Previous relapse</td>
<td>0 (0 - 6)</td>
<td>0 (0 - 5)</td>
<td>0 (0 - 5)</td>
</tr>
<tr>
<td>Disease duration, y</td>
<td>2.6 (0.6 - 12.2)</td>
<td>2.8 (0.6 - 21.1)</td>
<td>2.8 (0.4 - 28.0)</td>
</tr>
<tr>
<td>Immunosuppression at inclusion, yes / no</td>
<td>14 / 6</td>
<td>15 / 5</td>
<td>31 / 29</td>
</tr>
<tr>
<td>Co-trimoxazole maintenance at inclusion, yes / no</td>
<td>11 / 9</td>
<td>9 / 11</td>
<td>30 / 30</td>
</tr>
<tr>
<td>Creatinine clearance, ≤ 60 / &gt; 60 ml / min</td>
<td>10 / 10</td>
<td>11 / 9</td>
<td>19 / 41</td>
</tr>
</tbody>
</table>

<sup>a</sup> At time of entry: Group I = anti-neutrophil cytoplasmic antibody (ANCA) rise (no treatment); Group II = ANCA rise (pre-emptive treatment); Group III = no ANCA rise. Values with brackets are median values with ranges.

Results

Patient characteristics during follow-up

Hundred patients with PR3-ANCA associated vasculitis (57 males, 43 female) with a median age (range) of 56 years (18 - 84) were included in this study at 4 participating centers. The time between diagnosis of disease and inclusion was 2.7 years (median, range: 0.4 - 28.1). Thirty-seven percent of the patients (n=37) were included in 1998, while the remaining patients were included afterwards. Patient characteristics are listed in Table 1. Ninety-four patients were classified as WG, and 6 as microscopic polyangiitis. At inclusion, 60 patients were receiving decreasing doses of immunosuppressive drugs (34 being treated after their initial presentation, 26 after a relapse) and 40 patients were free of immunosuppressive medication (27 after initial treatment, 13 after treatment of a relapse). Patients were followed up for a median of 694 days (range 8 – 946 days) until March 2001 or until death (2 patients died with no apparent relation to active disease 483 and 753 days following inclusion) or until exclusion (one patient developed an interstitial nephritis 539 days following inclusion; one patient was excluded 8 days following inclusion because of a malignant B-cell lymphoma; one patient stopped visiting the outpatient clinic 273 days following inclusion).

From the 100 included patients a total of 1,536 serum samples were prospectively analyzed for the presence of PR3-ANCA between March 1998 until March 2001. A median of 17
samples per patient (range 2-23) were analyzed. One or more significant rises in PR3-ANCA were seen in 40 (40%) patients, whereas in 60 patients (60%) no rise in ANCA was detected during the study (Group III). The time between inclusion and randomization was 7 months (median, range: 1 - 28).

Twenty patients were randomized to no treatment (Group I), and 20 to pre-emptive immunosuppressive treatment (Group II). At the moment of randomization, 10 of 20 patients randomized for pre-emptive treatment and 11 patients randomized for no treatment still used (decreasing doses of) immunosuppressives (not significant; NS). Sex, age, previous treatment, treatment at time of inclusion, the relapse rate, creatinine clearance (Table 1) and cumulative organ involvement of the patients (Table 2) were not significantly different between the two groups. The median level of ANCA (79 [range 24 - 1210] vs 66 [21 - 409] AU, $P = 0.99$), serum C-reactive protein levels (5 [range <3 - 34] vs 5 [<3 - 27] mg/liter, $P = 0.76$), serum creatinine (114 [range 74 - 539] vs 119 [74 - 359] µmol/liter, $P = 0.93$) at randomization did not differ between group I and II. However, analysis of the magnitude of rises in ANCA revealed significantly lower rises in group I compared to group II (99% in 6 weeks [range 75 - 200] or 2.0% / day [range 1.5 - 5.0] vs 161% in six weeks [range 77 - 669] or 3.5% / day [range 1.4 - 14.6], $P = 0.02$).

Table 2 Cumulative organ system involvement according presence or absence of PR3-ANCA rise during follow and treatment allocation after PR3-ANCA rise

<table>
<thead>
<tr>
<th></th>
<th>Group I¹</th>
<th>Group II¹</th>
<th>Group III¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No treatment (n = 20)</td>
<td>Treatment (n = 20)</td>
<td>No rise in ANCA (n = 60)</td>
</tr>
<tr>
<td>Ear, nose and / or throat</td>
<td>18 (90)</td>
<td>15 (75)</td>
<td>55 (92)</td>
</tr>
<tr>
<td>Lungs</td>
<td>12 (60)</td>
<td>12 (60)</td>
<td>27 (45)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>17 (85)</td>
<td>17 (85)</td>
<td>38 (63)</td>
</tr>
<tr>
<td>Eyes</td>
<td>6 (30)</td>
<td>9 (45)</td>
<td>20 (33)</td>
</tr>
<tr>
<td>Skin</td>
<td>5 (25)</td>
<td>6 (30)</td>
<td>18 (30)</td>
</tr>
<tr>
<td>Joints</td>
<td>17 (85)</td>
<td>16 (80)</td>
<td>41 (69)</td>
</tr>
<tr>
<td>Nervous system</td>
<td>11 (55)</td>
<td>8 (40)</td>
<td>22 (37)</td>
</tr>
</tbody>
</table>

¹ At time of entry: Group I = anti-neutrophil cytoplasmic antibody (ANCA) rise (no treatment); Group II = ANCA rise (pre-emptive treatment); Group III = no ANCA rise.

Relapses

In our ongoing study, 4 of 20 patients (20%) assigned to pre-emptive treatment relapsed 13.6 months after randomization (median, range 10.1 to 14.8) and 10 of 20 (50%) patients assigned to no treatment relapsed 10.8 months after randomization (median, range 0.2 to 29.1).
Although comparison of the two disease-free survival curves revealed a significant difference at 9 months after randomization (P = 0.05), analysis of long-term disease-free survival revealed no significant difference (relative risk 1.4; confidence interval 0.4 to 4.4, P = 0.56) (Figure 1). Estimated disease-free survival without pre-emptive treatment (group I) was 90% (95% confidence interval, 77 to 100%) at 3 months, 80% (95% confidence interval, 62 to 98%) at 6, and 73% (95% confidence interval, 53 to 94%) at 12 months as compared to 100% at 3 and 6 months and 89% (95% confidence interval, 68 to 100%) at 12 months with pre-emptive treatment (group II) (Figure 1).

![Figure 1](image-url)

**Figure 1** Percentage of patients with ANCA associated vasculitis who did not experience disease relapses in the indicated time period (vertical axis) after a rise in antineutrophil cytoplasmic antibodies (horizontal axis) as measured by antigen-specific enzyme-linked immunosorbent assay (ELISA; n = 40). The numbers above the horizontal axis indicate the number of patients still at risk who were randomized for pre-emptive treatment (n = 20, lower numbers) or no treatment (n = 20, upper numbers). The numbers between brackets are the actuarial disease-free survival percentages.

At the moment of relapse, ANCA were detectable by ELISA in all 14 patients. Three relapses in Group II were preceded by a second rise in ANCA. Remarkably, none of the relapses in group I were preceded by more than one rise in ANCA. Eight of 14 patients (57%) with a relapse had no previous history of relapses. The remaining 6 patients (55%) had a relapse rate of 0.3 - 0.5 relapses/year (median 0.4). Ten of 14 patients (71%) had relapses involving more than one organ system. Seven patients (50%) had renal involvement, whereas the remaining 7 patients (50%) had non-renal relapses characterized by granulomatous inflammation of the respiratory tract with or without other symptoms and signs of vasculitis (Table 3). The median disease activity score was 14 (range 3 - 25) and the median C-reactive protein level was 36 mg/liter (range <3 -152) at the moment of clinical diagnosis of relapse.
No differences were found in clinical manifestations, BVAS score, C-reactive protein levels, erythrocyte sedimentation rate, and duration of hospitalization in patients who relapsed with (n=4) or without (n=10) pre-emptive treatment based on a rise in ANCA.

**Table 3** Clinical characteristics at the moment of relapse of randomized patients who relapsed

<table>
<thead>
<tr>
<th>Patient/age/sex</th>
<th>Organ involvement during a relapse</th>
<th>Days Hospitalized</th>
<th>Biopsy proven</th>
<th>CRP (mg/liter)</th>
<th>ESR (mm/hour)</th>
<th>BVAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/56/F</td>
<td>Renal, ENT, Syst, Muc, Chest</td>
<td>17</td>
<td>Kidney</td>
<td>37</td>
<td>66</td>
<td>24</td>
</tr>
<tr>
<td>2/51/M</td>
<td>Renal, Syst, Muc, Chest, Cardiac</td>
<td>23</td>
<td>-</td>
<td>152</td>
<td>105</td>
<td>25</td>
</tr>
<tr>
<td>3/74/M</td>
<td>Renal, Syst</td>
<td>29</td>
<td>Kidney</td>
<td>49</td>
<td>56</td>
<td>14</td>
</tr>
<tr>
<td>4/66/F</td>
<td>Renal, Syst</td>
<td>23</td>
<td>Kidney</td>
<td>86</td>
<td>99</td>
<td>15</td>
</tr>
<tr>
<td>5/52/M</td>
<td>Renal</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>109</td>
<td>12</td>
</tr>
<tr>
<td>6/18/F</td>
<td>ENT, Syst, Muc, Nerv</td>
<td>-</td>
<td>-</td>
<td>19</td>
<td>25</td>
<td>17</td>
</tr>
<tr>
<td>7/52/F</td>
<td>ENT, Syst, Cut, Muc</td>
<td>-</td>
<td>-</td>
<td>26</td>
<td>47</td>
<td>10</td>
</tr>
<tr>
<td>8/60/M</td>
<td>ENT, Syst</td>
<td>-</td>
<td>Nose</td>
<td>&lt;3</td>
<td>37</td>
<td>7</td>
</tr>
<tr>
<td>9/60/F</td>
<td>ENT</td>
<td>-</td>
<td>Nose</td>
<td>22</td>
<td>70</td>
<td>6</td>
</tr>
<tr>
<td>10/28/M</td>
<td>ENT</td>
<td>-</td>
<td>Nose</td>
<td>8</td>
<td>12</td>
<td>6</td>
</tr>
</tbody>
</table>

**Group I - ANCA rise (no treatment)**

**Group II - ANCA rise (pre-emptive treatment)**

<table>
<thead>
<tr>
<th>Patient/age/sex</th>
<th>Organ involvement during a relapse</th>
<th>Days Hospitalized</th>
<th>Biopsy proven</th>
<th>CRP (mg/liter)</th>
<th>ESR (mm/hour)</th>
<th>BVAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/67/M</td>
<td>Renal, ENT, Syst, Chest</td>
<td>9</td>
<td>Kidney</td>
<td>34</td>
<td>81</td>
<td>21</td>
</tr>
<tr>
<td>2/63/F</td>
<td>Renal</td>
<td>2</td>
<td>Kidney</td>
<td>41</td>
<td>48</td>
<td>14</td>
</tr>
<tr>
<td>3/82/F</td>
<td>ENT, Syst, Muc</td>
<td>-</td>
<td>-</td>
<td>37</td>
<td>26</td>
<td>10</td>
</tr>
<tr>
<td>4/38/M</td>
<td>Syst</td>
<td>-</td>
<td>&lt;5</td>
<td>8</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

CRP = C-reactive protein; ESR = erythrocyte sedimentation rate; BVAS = Birmingham vasculitis activity score; Group I = ANCA rise (no treatment); Group II = ANCA rise (treatment); Renal = positive biopsy and/or decreased creatinine clearance in combination with proteinuria and erythrocyturia; ENT = ear, nose and throat; Syst = systemic (e.g. fever, arthralgia, myalgia, malaise); Muc = genital/mouth ulcers or red eyes/proptosis; Cut = cutaneous; Nerv = nervous system.

Finally, in 10 of the 60 patients (17%) with no randomization (group III) at least one relapse occurred during follow-up (Table 4). The median disease activity score was 17 (range 5 - 34) and the median C-reactive protein level was 31 mg/liter (range 10 - 121) at the moment of clinical diagnosis of relapse (Table 4). Three of these 10 patients (Table 4; nr. 3,7,9) had a significant rise in ANCA ranging 3.0 to 39.5 months prior to relapse. In these cases ANCA levels rose prior to inclusion so these patients were not randomized. In 7 patients this relapse was not preceded by a rise in ANCA of 75% or more. However, 6 of these patients had a rise in ANCA ranging from 50% to 75% which occurred 0.0 to 41.3 months prior to relapse (Table 4; nr. 1,2,4-6,10). At the moment of the first relapse during the study period, ANCA were detectable by ELISA in all but one patient (Table 4; nr. 8). Despite the fact that this patient was PR3-ANCA positive by capture ELISA at diagnosis, and a positive C-ANCA
staining pattern by IIF was seen at the moment of relapse, no PR3-ANCA were detectable by
direct ELISA during the study.

Table 4 Clinical characteristics of patients without randomization who underwent a relapse

<table>
<thead>
<tr>
<th>Patient/age/sex</th>
<th>Organ involvement during a relapse</th>
<th>Days Hospitalized</th>
<th>Biopsy proven</th>
<th>CRP (mg/liter)</th>
<th>ESR (mm/hour)</th>
<th>BVAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group III - no randomization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/65/M Renal, ENT, Syst, Chest, Nerv</td>
<td>23</td>
<td>-</td>
<td>204</td>
<td>121</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>2/33/F Renal, ENT, Syst, Muc</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>30</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>3/58/M Renal, ENT, Syst, Muc</td>
<td>-</td>
<td>-</td>
<td>34</td>
<td>18</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>4/56/M Renal, ENT, Syst</td>
<td>41</td>
<td>Kidney</td>
<td>50</td>
<td>100</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>5/53/M Renal, Syst, Chest</td>
<td>37</td>
<td>Kidney</td>
<td>62</td>
<td>ND</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>6/62/M ENT, Syst, Cut, Chest</td>
<td>52</td>
<td>-</td>
<td>86</td>
<td>ND</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>7/55/F ENT, Syst, Muc</td>
<td>-</td>
<td>-</td>
<td>16</td>
<td>60</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>8/55/M ENT, Syst</td>
<td>-</td>
<td>-</td>
<td>27</td>
<td>59</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>9/29/F ENT, Chest</td>
<td>2</td>
<td>Nose</td>
<td>10</td>
<td>22</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>10/54/F ENT</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>17</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

ND = Not done (See Table 3 for other definitions).

Figure 2 Box plots indicating the mean value and the standard deviation (error bars) of levels of antineutrophil cytoplasmic antibodies directed to proteinase-3 (PR3-ANCA) in patients with ANCA-associated vasculitis after a rise in ANCA as measured by antigen-specific enzyme-linked immunosorbent assay (ELISA; n = 40). The numbers above the horizontal axis indicate the number of patients who randomized for no treatment (n=20, upper numbers) or pre-emptive treatment (n=20, lower numbers) who were still at risk for a relapse at 3, 6, 9, and 12 months after the rise in antibody levels as detected by ELISA. P value was calculated by Mann-Whitney U test (* P < 0.05, ** P < 0.01, *** P < 0.001).
ANCA

ANCA levels declined in the 20 pre-emptive treated patients (group II) during the first 3 months after a rise in ANCA titer, and temporarily became undetectable in one patient. Afterwards, ANCA levels rose above baseline levels. In contrast, immediately after randomization ANCA levels rose further in the 20 patients randomized to no treatment (group I). ANCA levels in untreated patients were significantly higher from 1.5 months up until 10.5 months after randomization (Figure 2). Remarkably, 1.5 months prior to relapse ANCA levels of patients who would be assigned to pre-emptive treatment were significantly higher compared to patients who would be assigned to no treatment.

Discussion

To address the question whether ANCA based treatment is beneficial, we started a large multi-center randomized study in 100 patients with ANCA associated vasculitis in which we evaluated whether treatment with azathioprine and prednisolone based on rises in ANCA levels as measured by ELISA is effective in preventing relapses in patients with Wegener’s granulomatosis, and whether this pre-emptive therapy outweighs the risk of unnecessary exposure to immunosuppressive drugs in some patients. Over 36 months, PR3-ANCA (ELISA) significantly rose in 40 out of 100 patients. Twenty of these 40 patients were randomly assigned to receive a 9-months course of azathioprine and a 4.5-months course of prednisolone at the time of the PR3-ANCA rise, and 20 patients were randomized to receive no treatment. In our ongoing study, 4 of 20 (20%) with pre-emptive treatment relapsed 13.6 months after randomization (median, range 10.1 to 14.8) and 10 of 20 (50%) patients without pre-emptive treatment relapsed 10.8 months after randomization (median, range 0.2 to 29.1). Actuarial disease-free survival with pre-emptive treatment was 100% and 89% whereas without pre-emptive treatment survival was 90% and 73% at 3 and 12 months, respectively. Therefore, this study demonstrates that although imminent relapses can be prevented by early treatment with prednisolone and azathioprine, late relapses still occur.

In our previous study in 1990 we reported that treatment with prednisolone and cyclophosphamide based on rises in C-ANCA (IIF) could prevent the occurrence of early as well as late relapses (Cohen Tervaert et al., 1990a). Over 24 months, C-ANCA rose in 20 out of 58 patients in this study. Nine patients were randomly assigned to receive a 9-month course of cyclophosphamide and a 3-month course of prednisolone at the time of the C-ANCA rise. Six of the 11 untreated patients relapsed within 3 months of the C-ANCA rise and 3 of the remaining 5 patients relapsed after 3 months during long-term follow-up. There were no early or late relapses in the nine patients randomized for treatment. Therefore, treatment with cyclophosphamide and prednisolone seems to be more effective in preventing relapses. However, starting treatment with cyclophosphamide and prednisolone in patients with an ANCA rise exposes a number of patients to the unnecessary risk of drug-related morbidity.
since in the present study in 10 out of 20 patients a rise in ANCA was not followed by a 
relapse during an extended period of follow-up ranging up to 29 months. This implies some 
degree of overtreatment when treatment is based only on changes in ANCA levels. But 
replacing cyclophosphamide by azathioprine is therefore to be preferred since the long-term 
toxicity of azathioprine is considered to be lower (Bouroncle et al., 1967; Norton et al., 1968).
Furthermore, analysis of rises in ANCA in the present study revealed that patients randomized 
for no treatment generally had lower rises in ANCA compared to patients randomized for pre-
emptive treatment (99% vs. 161%). Since we previously demonstrated that lower rises in 
ANCA are associated with a decreased sensitivity for an ensuing relapse (Boomsma et al., 
2000), this may have resulted in a decreased incidence of relapses in patients randomized for 
no treatment and an increased incidence of relapses in patients randomized for pre-emptive 
treatment.

We recently reported that measuring ANCA by ELISA is superior to measuring ANCA by IIF 
for the prediction of an ensuing relapse in patients with WG (Boomsma et al., 2000). Disease-
free survival at 12 months after a rise in ANCA by ELISA was 41% as compared to 50% after 
a rise in ANCA by IIF (Boomsma et al., 2000). This is, however, considerably lower 
compared to the present study in which disease controlled survival after a rise in ANCA 
without pre-emptive treatment was 73% at 12 months. A reasonable explanation of the 
observed differences between our current and the previous study may be the height of ANCA 
rises. Comparison in hindered by differences with respect to frequency of ANCA testing 
(current study once every six weeks; previous study at least once every 12 weeks). ANCA 
rose by 3.4% / [range 1.3 - 51.9] in the previous study whereas ANCA rose by 2.0% / day 
[range 1.5 - 5.0] (P = 0.02) in patients randomized for no treatment (Group I) in the present 
study. Furthermore, in the previous study patients were included as soon as complete 
remission was induced (no restrictions with regard to immunosuppressives) whereas in the 
present study eligibility required complete remission and immunosuppressives had to be 
tapered to 50 mg / day or less of cyclofosfamide and 15 mg / day or less of prednisolone.

In the present study, 10 patients with a relapse were not identified by a preceding rise in 
ANCA during the study. Based on the relapse rate in Group I (10 relapses in patients 
randomized for no treatment), we hypothesize that without pre-emptive treatment we would 
have also observed 10 relapses in Group II. Therefore, 10 of 30 (33%) relapses would have 
been missed despite serial measurement of ANCA. In our previous study 6 of 33 (18%) 
relapses were not preceded by a rise in ANCA of 75% or more (Boomsma et al., 2000). However, 2 out of 10 relapses in Group III were preceded by a rise in ANCA of 75% or more 
before inclusion in the study. Additionally, 7 patients in Group III had a rise of 50-75% 
preceding the relapse. Since in our previous study the patients were sampled with intervals of 
up to 3 months, as compared to intervals of 6 weeks in the present study, these rises may have 
very well have been 75% or more when the time of interval should have been lengthened.
The possible pathophysiologic role of ANCA has been examined by many groups but remains unclear. As indicated in studies involving experimental models of vasculitis (Heeringa et al., 1998), it is clear that ANCA in itself are not sufficient for disease induction. However, the close relation between antibody titer and clinical disease supports a pathogenetic role. Patients may, however, have high and stable ANCA levels without disease activity. Therefore we postulate that differences in qualities of antibodies are important with respect to the development of a relapse. The IgG3 ANCA / total IgG ANCA ratio (Mulder et al., 1995), the capacity of anti-PR3 antibodies to inhibit complexation of PR3 with α1-antitrypsin (Dolman et al., 1993), and the inhibitory activity of anti-PR3 antibodies to PR3 (Daouk et al., 1995) rather than the titer of the anti-PR3 antibodies alone may be important. All these studies support the idea that not only the quantity, but also the quality of ANCA might influence the pathogenic potential of the antibodies.

Based on our preliminary data, we conclude that pre-emptive treatment with a 9-months course of azathioprine and a 4.5-months of prednisolone effectively protects patients against imminent relapses of disease activity. Unfortunately, four treated patients developed a relapse 1 to 6 months after pre-emptive treatment was ended and after an initial decline of ANCA levels, these eventually rose above baseline values in the patients after prednisolone treatment was stopped. Pre-emptive treatment based on a rise in ANCA was only instituted once. Three patients (75%) randomized for pre-emptive treatment who developed a relapse had (a) subsequent rise(s) in ANCA prior to relapse. Whether these relapses could have been prevented by repeated courses of pre-emptive treatment, or whether pre-emptive treatment should have been prolonged remains unclear. Further studies upon the value of such strategies should answer this question. Pre-emptive treatment cannot reduce the occurrence of all relapses since in some patients relapses occur without a rise in ANCA as was also found in our previous study (Boomsma et al., 2000).

In conclusion, quantification of PR3-ANCA by ELISA can be used as a therapeutic guideline in antibody directed treatment of patients with ANCA associated vasculitis. Combined courses of azathioprine in combination with prednisolone are capable of postponing but in contrast to cyclophosphamide and prednisolone does not prevent relapses of disease activity. Further studies are needed to identify which patients might benefit most of pre-emptive treatment based a rise in ANCA, and which treatment protocol combines low toxicity with effective protection against early and late relapses.

Acknowledgements

We thank Dr EC Hagen and Drs E. van Gurp (Eemland Hospital, Amersfoort), Dr. C. Halma (Medical Center, Leeuwarden) for the care of one or more patients who participated in this study; M.J. van der Leij (University Hospital, Groningen) for technical support; A Mellema (University Hospital, Groningen) for providing secretarial assistance. This study was supported by a grant from the University Hospital Groningen (AZG-stimuleringsgelden).
Chapter 7

Literature


