Renal histology in ANCA-associated vasculitis: Differences between diagnostic and serologic subgroups

Herbert A. Hauer, Ingeborg M. Bajema, Hans C. van Houwelingen, Franco Ferrari, Laure-Hélène Noël, Rüdiger Waldherr, David R.W. Jayne, Niels Rasmussen, Jan A. Bruijn, and E. Christiaan Hagen, on behalf of the European Vasculitis Study Group (EUVAS)

Departments of Pathology and Medical Statistics, Leiden University Medical Center, Leiden, Department of Pathology, Erasmus University Medical Center, Rotterdam, and Department of Internal Medicine, Eemland Hospital, Amersfoort, the Netherlands; Renal Immunopathology Center, Ospedale San Carlo Borromeo, Milan, Italy; INSERM U507, Hôpital Necker, Paris, France; Department of Pathology, University of Heidelberg, Heidelberg, Germany; Renal Unit, Addenbrooke’s Hospital, Cambridge, England, United Kingdom; and Department of Otolaryngology, Rigshospitalet, Copenhagen, Denmark

Renal histology in ANCA-associated vasculitis: Differences between diagnostic and serologic subgroups.

Background. Differences in renal histopathology between microscopic polyangiitis (MPA) and Wegener’s granulomatosis (WG), and between anti-neutrophil cytoplasm antibody (ANCA) test results in patients with ANCA-associated vasculitis may provide insight into the differences in pathogenesis and raise the opportunity of classifying the vasculitides more accurately. The possible differences in histopathology are investigated in this study.

Methods. We report an analysis of 173 patients with renal disease in microscopic polyangiitis or Wegener’s granulomatosis. A total of 173 renal biopsies, performed at diagnosis, were scored by two observers separately, using a previously standardized protocol. Consensus on each biopsy was achieved during a central review.

Results. Normal glomeruli were more common in WG than in MPA (P < 0.001). Glomerulosclerosis was more prominent in MPA than in WG (P = 0.003). Interstitial fibrosis (P < 0.001), tubular atrophy (P < 0.001), and tubular casts (P = 0.005) were more frequently present and more severe in MPA than in WG. Presence of glomerulosclerosis was more extensive in patients with myeloperoxidase (MPO)-ANCA than with proteinase 3 (PR3)-ANCA (P = 0.022). Interstitial fibrosis (P = 0.008), tubular necrosis (P = 0.030), tubular atrophy (P = 0.013), and intra-epithelial infiltrates (P = 0.006) were more frequently present and more severe in MPO-ANCA than in PR3-ANCA.

Conclusions. Glomerulonephritis in relation to MPA has more characteristics of chronic injury at the time of presenta-


80
Table 1. Inclusion criteria for CYCAZAREM (1, 2 and 3 are required)

1. A diagnosis of new or previously untreated MPA, RLV, or WG with or without histological confirmation.
2. Either Renal involvement attributable to active MPA, RLV, or WG with one or more of:
   1) Elevated serum creatinine
   2) Hematuria (>30 red blood cells per high power field)
   3) Red cell casts
   4) Proteinuria (>1 gram/24 h) and/or Other severe organ involvement or imminent loss of vital organ function, attributable to active MPA or WG.
3. ANCA-positivity; that is, one of the following possibilities:
   1) C-ANCA+
   2) PR3-ANCA+
   3) MPO-ANCA+
   ANCA-negativity is allowed if the disease is confirmed histologically.

Abbreviations are in the Appendix.

Table 2. Inclusion criteria for MEPEX (1, 2 and 3 are required)

1. A diagnosis of new or previously untreated MPA, RLV, or WG with active vasculitis, as indicated by the presence of active necrotizing glomerulonephritis on renal biopsy.
2. ANCA-positivity; that is, one of the following possibilities:
   1) C-ANCA+
   2) PR3-ANCA+
   3) MPO-ANCA+
   ANCA-negativity is allowed if the disease is confirmed histologically.
3. Biopsy-proven necrotizing and/or crescentic glomerulonephritis, in the absence of another defined glomerulopathy, with severe renal impairment; that is, one of the following possibilities:
   1) Oliguria (<400 mL/24 h)
   2) Intention to commence dialysis within 48 hours of admission
   3) Creatinine ≥500 μmol/L

Methods

Patients

Patients in the present study were derived from two of the first-wave trials of the EUVAS [10]: CYCAZAREM, a randomized trial of cyclophosphamide versus azathioprine during remission of ANCA-positive systemic vasculitis [10], and MEPEX, a randomized trial of adjunctive therapy for severe glomerulonephritis in ANCA-associated systemic vasculitis that examined plasma exchange versus intravenous administration of methylprednisolone [10]. Patients only were included into the present study if a renal biopsy, performed at study entry, was available. Inclusion criteria for CYCAZAREM and MEPEX are listed in Tables 1 and 2, respectively. Exclusion criteria for both trials included age under 18, pregnancy, previous malignancy, known HIV positivity, administration of any cytotoxic drug within a year before entry, and hepatitis B antigenemia (only if HepB-ag positive). An additional exclusion criterion for CYCAZAREM was serum creatinine >500 μmol/L. Additional exclusion criteria for MEPEX were serum creatinine <500 μmol/L, life-threatening non-renal manifestations of vasculitis, including alveolar hemorrhage requiring mechanical ventilation within 24 hours of admission, on dialysis for more than two weeks prior to referral, significant baseline renal impairment (creatinine >200 μmol/L, one year or more before presentation), or a previous episode of biopsy-proven necrotizing and/or crescentic glomerulonephritis [10].

Renal histopathology

Paraffin sections were stained with silver, periodic acid-Schiff (PAS), hematoxylin & eosin (H&E), and tri-
chrome, and were forwarded to two of five participating pathologists (IMB, LHN, FF, RW, JAB). Both pathologists scored the biopsies separately, blinded to patient data and the scores of the other observer, and according to a previously standardized protocol for scoring renal biopsies of patients with ANCA-associated systemic vasculitis [11, 12]. In short, each glomerulus had to be scored separately on the presence of fibrinoid necrosis, crescents (cellular/fibrous and segmental/circumferential), glomerulosclerosis (local/segmental/global), periglomerular infiltrates, granulomatous reactions, as well as a number of other lesions. The presence of glomerular lesions was calculated as the percentage of the total number of glomeruli in a biopsy. Most interstitial infiltrates were scored as present or absent, except for interstitial infiltrates \((-/+)+/+\) and tubular atrophy \((-/+)+\), which were scored semiquantitatively. Together, 39 histological parameters were examined. The scores were entered into a central database (MSAccess) and discrepancies between observers were resolved by conference during central reviews, achieving consensus for each biopsy.

Immunofluorescence for immunoglobulins and complement components, and electron microscopy were performed locally in all participating centers. For logistical reasons, these procedures were not standardized or reviewed by the EUVAS, but the local conclusions were reported.

Diagnosis

Meeting the inclusion criteria was needed to enter patients in either CYCAZAREM or MEPEX. Diagnostic categories were distinguished (always with respect to the inclusion criteria, Tables 1 and 2) by the following criteria [10] and as such determined by the local physicians, without review by the EUVAS:

- **Microscopic polyangiitis**: Systemic vasculitis, predominantly affecting small vessels, with extrarenal symptoms, but without airway symptoms compatible with Wegener’s granulomatosis.
- **Renal limited vasculitis**: Idiopathic rapidly progressive glomerulonephritis without systemic disease manifestations.
- **Wegener’s granulomatosis**: Inflammation of the respiratory tract together with necrotizing vasculitis affecting small- to medium-sized vessels.

ANCA testing

Indirect immunofluorescence (IIF) and ELISA were performed for local ANCA testing in all participating centers. The staining pattern in the IIF test was scored as perinuclear (P-ANCA), cytoplasmic (C-ANCA), atypical, or negative. Sera positive for ANCA directed against MPO and PR3 were reported as MPO-ANCA and PR3-ANCA, respectively. To determine the different patterns of renal lesions in ANCA test subgroups, the ANCA test results also were classified as a combination of the IIF and ELISA results, as the specificity of ANCA testing increases by using this combination, compared to IIF or ELISA alone [5].

Statistics

Differences of quantitative parameters between groups were assessed with the \(t\) test for independent samples. Differences of semiquantitative results were tested by using the Mann-Whitney \(U\) test. Difference of gender between groups was analyzed by the chi-square test.

RESULTS

Patients

Renal biopsies were performed in 132 of 157 patients that were recruited in CYCAZAREM. As a renal biopsy was an inclusion criterion for MEPEX, a renal biopsy was performed in all 124 patients in this study. Of the combined 256 biopsies from these two studies, 180 were received for re-evaluation. Seven of these 180 biopsies were excluded: five biopsies because of the absence of cortical tissue, and two because patients appeared to have antibodies directed against the glomerular basement membrane. Together, 173 biopsies (98 from CYCAZAREM and 75 from MEPEX) were available for evaluation in the present study. Patient diagnoses (as defined in the Methods section) were microscopic polyangiitis \((N = 80)\), renal limited vasculitis \((N = 19)\), and Wegener’s granulomatosis \((N = 73)\). The diagnosis of one patient remained uncertain (doubt about whether extrarenal manifestations were attributable to vasculitis). ANCA test results are listed in Table 3. The mean age of all patients was 59 years (range 21 to 83 years). Patients with MPO-ANCA were older than patients with PR3-ANCA \((63 \text{ vs.} 58 \text{ years, } P = 0.033)\). Patients included 56% females and 44% males. The gender distribution was comparable in the various groups. Mean serum creatinine and GFR of all patients were 453 \(\mu\text{mol/L}\) and 31 \(\text{mL/min, respectively. Patients with microscopic polyangiitis and renal limited vasculitis had a significantly lower mean GFR than patients with Wegener’s granulomatosis (20 and 22 vs. 44 mL/min, } P < 0.001)\). Patients with a P-ANCA IIF had a significantly lower mean GFR than patients with a C-ANCA IIF \((21 \text{ vs.} 40 \text{ mL/min, } P < 0.001)\). Patients with MPO-ANCA had a significantly lower mean GFR than patients with PR3-ANCA \((21 \text{ vs.} 38 \text{ mL/min, } P = 0.002)\).

Histological diagnoses

Figure 1 shows the histopathological diagnoses on the basis of the renal biopsy findings. Crescentic necrotizing glomerulonephritis was observed in 112 biopsies (65%);
crescentic glomerulonephritis without fibrinoid necrosis was present in 40 cases (23%). Two biopsies showed interstitial vasculitis only, one biopsy was normal, and nine biopsies (5%) showed diffuse global glomerulosclerosis. Other biopsies included focal global glomerulosclerosis, interstitial fibrosis, and mesangial matrix increase.

### Histological parameters in all patients

The histological findings in the entire patient cohort showed that a mean of 29% of glomeruli per biopsy were normal, 45% of glomeruli had (predominantly cellular) crescents, and 23% were globally sclerotic. Fibrinoid necrosis of the glomerular tuft was present in 22% of glomeruli. In five biopsies fibrinoid necrosis was present in glomeruli without crescents, although crescents were found in other glomeruli in the same biopsy. Interstitial edema was present in 34% of biopsies. Interstitial infiltrates were present in 92% of biopsies; in half of those scored as mild (+), in the other half as severe (++). Mononuclear inflammatory cells were always present, and usually, they formed the predominant part of the infiltrates. Neutrophils were present in 65% of biopsies, but predominant in only a few cases. Although eosinophils were seen in 22% of biopsies, they were never scored as predominant. Interstitial fibrosis was present in 83% of biopsies and in approximately half of those cases it was scored as diffuse (++). Tubular casts and tubular necrosis were present in 87% and 66% of biopsies, respectively. Tubular atrophy was present in 86% of biopsies and in approximately one third of those cases was scored as diffuse (++). Tubular intra-epithelial infiltrates were seen in 64% of biopsies. Interstitial vasculitis was present in only 12% of biopsies. Arteriosclerosis and arteriolosclerosis were present in 70% and 32% of biopsies, respectively. Pauci-immunity was concluded in all patients. Electron microscopy was performed in some patients, but histopathological conclusions remained unchanged with this procedure.

### Differences between diagnoses

Table 4 shows the mean percentage of the main glomerular parameters in microscopic polyangiitis, renal limited vasculitis, and Wegener’s granulomatosis. In microscopic polyangiitis, 21% of glomeruli per biopsy were normal, whereas in Wegener’s granulomatosis 40% of glomeruli were normal (Fig. 2A, \(P < 0.001\)). Glomerulosclerosis was present in 30% of patients who were diagnosed with microscopic polyangiitis versus only 16% of patients with Wegener’s granulomatosis (Fig. 2A, \(P = 0.002\)). The percentage of glomeruli with fibrinoid necrosis or crescents did not distinguish between the diagnoses. In microscopic polyangiitis, interstitial fibrosis (Fig. 2B) and tubular atrophy (Fig. 2C) were more frequently and severely present than in Wegener’s granulomatosis (\(P < 0.001\) for both differences). Interstitial infiltrates (Fig. 2D) and tubular necrosis (Fig. 2E) tended to occur more frequently in microscopic polyangiitis than in Wegener’s granulomatosis \(\left(P = 0.030\right)\). Tubular casts were more often reported in microscopic polyangiitis than in Wegener’s granulomatosis \(\left(P = 0.005\right)\). However, there were no differences in proteinuria between the diagnostic groups (data not shown). Arteriosclerosis was present more often in microscopic polyangiitis than in Wegener’s granulomatosis \(\left(P = 0.021\right)\). No difference was observed in the occurrence of interstitial vasculitis. Biopsies from the 19 patients with renal limited vasculitis were more or less comparable to those from the patients with microscopic polyangiitis (Table 4 and Fig. 2).

### Differences between MPO-ANCA and PR3-ANCA

The distribution of histological parameters among the ELISA test result groups is presented in Table 5. The mean percentage glomerulosclerosis was 15% in patients with PR3-ANCA, 25% in patients with MPO-ANCA, and 35% in patients with negative ELISA test results \(\left(P = 0.022\right)\) for the difference between PR3-ANCA and MPO-ANCA, \(P = 0.005\) for the difference between negative test results and PR3-ANCA; Fig. 3A). The occurrence of fibrinoid necrosis and crescents was similar in all groups. In patients with MPO-ANCA positivity, interstitial fibrosis (Fig. 3B) and tubular atrophy (Fig. 3C) were more frequently and more severely present than in patients with PR3-ANCA positivity \(\left(P = 0.008\right)\) and

---

### Table 3. ANCA test results

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>MPA RLV WG Uncertain</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-ANCA</td>
<td>35 11 9 0</td>
</tr>
<tr>
<td>PR3-ANCA</td>
<td>1 0 0 0</td>
</tr>
<tr>
<td>MPO-ANCA &amp; PR3-ANCA</td>
<td>2 0 0 0</td>
</tr>
<tr>
<td>Negative</td>
<td>5 0 0 0</td>
</tr>
<tr>
<td>Not reported</td>
<td>5 2 1 0</td>
</tr>
<tr>
<td>C-ANCA</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>MPO-ANCA</td>
<td>17 2 40 0</td>
</tr>
<tr>
<td>PR3-ANCA</td>
<td>0 0 2 0</td>
</tr>
<tr>
<td>MPO-ANCA &amp; PR3-ANCA</td>
<td>2 0 4 0</td>
</tr>
<tr>
<td>Negative</td>
<td>2 0 4 0</td>
</tr>
<tr>
<td>Not reported</td>
<td>5 1 6 0</td>
</tr>
<tr>
<td>Atypical ANCA</td>
<td>1 0 0 0</td>
</tr>
<tr>
<td>MPO-ANCA</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>PR3-ANCA</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>MPO-ANCA &amp; PR3-ANCA</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>Negative</td>
<td>1 1 1 0</td>
</tr>
<tr>
<td>Not reported</td>
<td>1 0 2 0</td>
</tr>
<tr>
<td>Negative</td>
<td>0 0 1 0</td>
</tr>
<tr>
<td>Not reported</td>
<td>1 0 0 0</td>
</tr>
</tbody>
</table>

Abbreviations are in the Appendix.
Table 4. Main glomerular lesions in MPA, RLV, and WG

<table>
<thead>
<tr>
<th>Lesions</th>
<th>MPA (N=80)</th>
<th>RLV (N=19)</th>
<th>WG (N=73)</th>
<th>MPA vs. RLV</th>
<th>RLV vs. WG</th>
<th>MPA vs. WG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal glomeruli</td>
<td>Mean 21 SD 22</td>
<td>Mean 27 SD 25</td>
<td>Mean 40 SD 34</td>
<td>0.291</td>
<td>0.137</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fibrinoid necrosis</td>
<td>Mean 21 SD 25</td>
<td>Mean 21 SD 26</td>
<td>Mean 23 SD 26</td>
<td>0.976</td>
<td>0.656</td>
<td>0.496</td>
</tr>
<tr>
<td>Crescents</td>
<td>Mean 47 SD 28</td>
<td>Mean 49 SD 33</td>
<td>Mean 42 SD 32</td>
<td>0.790</td>
<td>0.365</td>
<td>0.257</td>
</tr>
<tr>
<td>Cellular crescents</td>
<td>Mean 45 SD 28</td>
<td>Mean 33 SD 6</td>
<td>Mean 45 SD 9</td>
<td>0.239</td>
<td>0.205</td>
<td>0.902</td>
</tr>
<tr>
<td>Fibrous crescents</td>
<td>Mean 7 SD 10</td>
<td>Mean 3 SD 6</td>
<td>Mean 4 SD 9</td>
<td>0.361</td>
<td>0.265</td>
<td>0.002</td>
</tr>
<tr>
<td>Glomerulosclerosis</td>
<td>Mean 30 SD 29</td>
<td>Mean 23 SD 25</td>
<td>Mean 16 SD 23</td>
<td>0.121</td>
<td>0.033</td>
<td>0.680</td>
</tr>
<tr>
<td>Periglomerular infiltrates</td>
<td>Mean 10 SD 16</td>
<td>Mean 16 SD 20</td>
<td>Mean 9 SD 12</td>
<td>0.566</td>
<td>0.918</td>
<td>0.379</td>
</tr>
<tr>
<td>Granulomatous reaction</td>
<td>Mean 4 SD 13</td>
<td>Mean 2 SD 6</td>
<td>Mean 2 SD 8</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The mean percentage of cellular crescents and fibrous crescents together is usually different from the mean percentage of crescents, as the former two lesions were not scored if crescents were absent.

$P = 0.013$, respectively). Tubular necrosis (Fig. 3E), tubular intra-epithelial infiltrates (Fig. 3F), and arteriosclerosis were more frequently present in patients with MPO-ANCA than in patients with PR3-ANCA ($P = 0.030$, $P = 0.006$, and $P = 0.027$, respectively). The histopathology in the group of patients with negative ELISA test results was more or less similar to that of MPO-ANCA positive patients (Table 5 and Fig. 3). It is questionable whether these results are independent of the diagnostic subgroup. To address this issue, we tested whether ANCA specificity influenced renal histology independently of diagnosis. The observed differences in the occurrence of tubular necrosis ($P = 0.021$) and tubular intra-epithelial infiltrates ($P = 0.005$) between MPO-ANCA and PR3-ANCA positive patients remained significant after this correction. No differences between the groups were observed in the occurrence of interstitial vasculitis. We also investigated the distribution patterns of histological features in patients with P-ANCA versus patients with C-ANCA, and of P- and MPO-ANCA–positive patients versus C- and PR3-ANCA–positive patients. Results of these analyses were similar to the above-described histological distribution patterns in patients with MPO-ANCA versus patients with PR3-ANCA (data not shown).

**Sample size**

Reported results are based on analyses without a minimum for the number of glomeruli in the biopsies (biopsies without cortical tissue were excluded). We tested whether the presence of only a small number of glomeruli—less than five and less than ten—would influence our results, but this was not the case (data not shown).

**DISCUSSION**

We present the results of a prospective multicenter study of 173 renal biopsies from a homogeneous group of patients with newly diagnosed renal disease in relation to ANCA-associated vasculitis. Our aim was to determine (1) the differences in the occurrence of renal histological lesions in microscopic polyangiitis, Wegener’s granulomatosis, and renal limited vasculitis; and (2) the differences in the occurrence of renal histological lesions in patients with ANCA-associated vasculitis with various ANCA test results.
Fig. 2. Renal histology in patients with microscopic polyangiitis, renal limited vasculitis, or Wegener’s granulomatosis. Differences in presence of glomerular lesions (A), interstitial fibrosis (B), tubular atrophy (C), interstitial infiltrates (D), tubular necrosis (E), and tubular intra-epithelial infiltrates (F), between the diagnostic categories are presented. Symbols in panel A are: (■) other; (□) sclerosis; (▲) crescents; (●) normal glomeruli. Symbols in panels B and C are: (■) diffuse; (□) focal; (▲) absent. Panel D symbols are: (■) very dense; (□) dense; (▲) mild; (▲) absent. Symbols in panels E and F are: (■) present; (□) absent.
Glomerulosclerosis, interstitial fibrosis, tubular atrophy, tubular casts, and arteriosclerosis occurred more often and more pronounced in microscopic polyangiitis and renal limited vasculitis than in Wegener’s granulomatosis. However, none of these lesions were specific for one of the diagnostic subgroups, which limits the role of renal histopathology in further sub-categorizing ANCA-associated vasculitis. Except for tubular casts, the above-mentioned lesions are representative of a chronic disease phase, reflecting the result of longer existing inflammation of the kidney, in which irreversible scars develop. So-called active lesions (such as tubular intra-epithelial infiltrates and glomerular crescents) did not discriminate between the three diagnostic subgroups. We believe that the explanation for these results is that in patients with microscopic polyangiitis and renal limited vasculitis, the diagnosis is established later than in patients with Wegener’s granulomatosis. In over 90% of cases, Wegener’s granulomatosis presents with upper airway or pulmonary symptoms, or both [13], which probably reduces the patient’s and doctor’s delay. Alternatively, although such a mechanism is not known in vasculitis, our results could also indicate that, at the same time point after onset of the disease, patients with renal limited vasculitis and with microscopic polyangiitis may have developed more irreversible lesions in the kidney than patients with Wegener’s granulomatosis. This may be explained by a faster progression of active lesions to chronic lesions in microscopic polyangiitis and renal limited vasculitis or, alternatively, by development of chronic lesions without the obligatory presence of active lesions. An argument in favor of the occurrence of the latter mechanisms would be that, after correction for the diagnostic delay, the observed differences in renal histology between the diagnoses are still present. Therefore, we re-analyzed our results by correcting for the diagnostic delay (by correcting for the GFR), but no significant differences were observed (data not shown).

In this study, ANCA positivity was present in 149 of 164 patients in which ANCA test results were reported. Patients with MPO-ANCA had significantly more glomerulosclerosis, interstitial fibrosis, tubular atrophy, and arteriosclerosis, but also significantly more tubular necrosis and tubular intra-epithelial infiltrates. In other words, a mixture of both chronic and active lesions was more abundantly present in patients with MPO-ANCA than in patients with PR3-ANCA. The observed differences between the occurrence of tubular necrosis and tubular intra-epithelial infiltrates remained significant after correction for diagnosis. One should realize, however, that the observed differences are small, that none of the lesions is specific for one of the groups, and that active glomerular lesions (that is, fibrinoid necrosis and cellular crescents) were present to the same extent. Our results may be explained by the finding that circulating soluble MPO, released by activated polymorphonuclears, may bind to resting “innocent bystander polymorphonuclears,” thereby making them reactive to anti-MPO antibodies, which leads to perpetuating inflammation and tissue destruction [14]. In addition, anti-MPO antibodies may inhibit the inactivation of MPO by ceruloplasmin, its natural inhibitor [15], resulting in persistent generation of reactive oxygen radicals with enhanced potential for endothelial cytotoxicity [16]. These processes may lead to a more active disease, and in the long term, to more chronic damage in MPO-ANCA-positive patients than in PR3-ANCA-positive patients. The previously reported tendency to a more frequent renal involvement in patients with MPO-ANCA than in patients with PR3-ANCA is further evidence for a stronger renal pathogenic effect of MPO-ANCA compared to PR3-ANCA [12, 17].

### Table 5. Main glomerular lesions in patients with MPO-ANCA, PR3-ANCA, double positivity, and negative ELISA

<table>
<thead>
<tr>
<th>Lesions</th>
<th>MPO- &amp; PR3- ANCA (N = 5)</th>
<th>PR3- ANCA (N = 63)</th>
<th>MPO- ANCA (N = 58)</th>
<th>Negative ELISA (N = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal glomeruli</td>
<td>20 (Mean ± SD)</td>
<td>20 (Mean ± SD)</td>
<td>20 (Mean ± SD)</td>
<td>20 (Mean ± SD)</td>
</tr>
<tr>
<td>Fibrinoid necrosis</td>
<td>15 (Mean ± SD)</td>
<td>15 (Mean ± SD)</td>
<td>15 (Mean ± SD)</td>
<td>15 (Mean ± SD)</td>
</tr>
<tr>
<td>Crescents</td>
<td>45 (Mean ± SD)</td>
<td>45 (Mean ± SD)</td>
<td>45 (Mean ± SD)</td>
<td>45 (Mean ± SD)</td>
</tr>
<tr>
<td>Cellular crescents</td>
<td>46 (Mean ± SD)</td>
<td>46 (Mean ± SD)</td>
<td>46 (Mean ± SD)</td>
<td>46 (Mean ± SD)</td>
</tr>
<tr>
<td>Fibrous crescents</td>
<td>10 (Mean ± SD)</td>
<td>10 (Mean ± SD)</td>
<td>10 (Mean ± SD)</td>
<td>10 (Mean ± SD)</td>
</tr>
<tr>
<td>Glomerulosclerosis</td>
<td>15 (Mean ± SD)</td>
<td>15 (Mean ± SD)</td>
<td>15 (Mean ± SD)</td>
<td>15 (Mean ± SD)</td>
</tr>
<tr>
<td>Periglomerular infiltrates</td>
<td>8 (Mean ± SD)</td>
<td>8 (Mean ± SD)</td>
<td>8 (Mean ± SD)</td>
<td>8 (Mean ± SD)</td>
</tr>
<tr>
<td>Granulomatous reaction</td>
<td>1 (Mean ± SD)</td>
<td>1 (Mean ± SD)</td>
<td>1 (Mean ± SD)</td>
<td>1 (Mean ± SD)</td>
</tr>
</tbody>
</table>

*The mean percentage of cellular crescents and fibrous crescents together is usually different from the mean percentage of crescents as the former two lesions were not scored if crescents were absent

*P* values of negative, MPO-ANCA, and PR3-ANCA vs. MPO-ANCA positivity are not reported (in general, *P* values were over 0.100)
Fig. 3. Renal histology in patients with PR3-ANCA, MPO-ANCA, PR3- and MPO-ANCA, or negative ELISA. Differences in presence of glomerular lesions (A), interstitial fibrosis (B), tubular atrophy (C), interstitial infiltrates (D), tubular necrosis (E), and tubular intra-epithelial infiltrates (F) between the ELISA test result groups are illustrated. Symbols in panel A are: (■) other; (□) sclerosis; (▲) crescents; (▲) normal glomeruli. Symbols in panels B and C are: (■) diffuse; (□) focal; (▲) absent. Panel D symbols are: (■) very dense; (□) dense; (▲) mild; (▲) absent. Symbols in panels E and F are: (■) present; (▲) absent.

Previous investigations on the occurrence of renal histopathological differences between patients with MPO-ANCA and PR3-ANCA led to contradictory results. In three reports, no differences between these groups were found (abstract;  

ibid) [7, 8]. Franssen et al described in a retrospective analysis of 36 patients with ANCA-associated vasculitis, that patients with PR3-ANCA positivity had more fibrinoid necrosis and a higher activity index than MPO-ANCA positive patients, and that patients with MPO-ANCA had more glomerulosclerosis.
and a higher chronicity index [9], which may be explained by the inhibitory effect by PR3-ANCA binding to PR3 on the irreversible inactivation of PR3 by α1-antitrypsin [18], similar to the mechanism by which MPO-ANCA inhibits inactivation of MPO by ceruloplasmin [19, 20]. In addition, the respiratory burst following activation of tumor necrosis factor-α (TNF-α) primed neutrophils in vitro by PR3 may be more potent than activation by MPO [21], though this difference is not evident in other reports [22–24].

It seems that, although differences exist between PR3-ANCA– and MPO-ANCA–associated vasculitis [6], current knowledge on the mechanisms by which these ANCs are involved in the pathogenesis of vasculitis is insufficient for the explanation of these differences. Alternatively, the action of ANCA is just an epiphenomenon.

In summary, we conclude that glomerulonephritis in relation to microscopic polyangiitis has more characteristics of chronic injury at the time of presentation than glomerulonephritis in relation to Wegener’s granulomatosis. We suggest that this difference is due to a delayed establishment of diagnosis in patients with microscopic polyangiitis compared to patients with Wegener’s granulomatosis. The finding that both active and chronic lesions are more abundantly present in MPO-ANCA-positive patients than in patients with PR3-ANCA positivity may be an argument in favor of the existence of different routes in the pathogenesis of renal disease in these ANCA subsets, although the mechanism remains unclear. In addition, our results suggest that ANCA test results may be useful in further classifying ANCA-associated vasculitides. Within the framework of the EUVAS, the phenotype of patients with systemic disease, differentiation of the leukocyte infiltration, and the predictive value of the renal histopathological parameters in this patient cohort will be investigated in the near future.

ACKNOWLEDGMENTS

The EUVAS group was supported by grants from the EU (Contract nos. BMH1-CT93-1078, CIPDCT94-0307, BMH4-CT97-2328, and ERBIC20-CT97-0019).

The following investigators participated in the European Vasculitis Study Group (EUVAS): Co-ordinator and project leader: N. Rasmussen (Rigshospitalet, Copenhagen, Denmark); Co-project leader: D.R.W. Jayne (Addenbrooke’s Hospital, Cambridge, UK). EUVAS Members: I. Neumann (Wilhelmina Hospital, Vienna, Austria), D. Abramowicz (Erasmus Hospital, Bruxelles, Belgium), Ph. Madhoun (Edith Cavell Medical Institute, Bruxelles, Belgium), J. Sennesaal (Academic Hospital of the Free University, Bruxelles, Belgium), V. Teras, I. Ryhik, J. Bartunkova, J. Lukas (Charles University Hospital, Prague, Czech Republic), B. Ravn Juhl, J. Petersen, C.B. Andersen, and W. Szpirt (Rigshospitalet, Copenhagen, Denmark), A. Wiik (Statens Serum Institut, Copenhagen, Denmark), H. Løkkegaard, H. Nielsen (Herlev County Hospital, Denmark), T. Ring (Aalborg Hospital, Aalborg, Denmark), S. Fretesleben Sørensen (Biøpsherg Hospital, Biøpsherg, Denmark), P.A. Bacon, A. Exley, C.O.S. Savage (University Hospital Birmingham, Birmingham, UK), G. Gaskin, C. Pusey (Hammersmith Hospital, London, UK), C.M. Lockwood† (Addenbrooke’s Hospital, Cambridge, UK), R. Luqmani (Western General Hospital, Edinburgh, UK), C. Grønahren-Riska, A. Ekstrand (Helsinki University Hospital, Helsinki, Finland), L. Guillemin, F. Lhote (Hôpital Avicenne, Bobigny, France), Ph. Lesavre, L.H. Noël, P. Landais (Hôpital Necker, Paris, France), Ph. Vanhille (Centre Hospitalier de Valenciennes, Valenciennes, France), P. Bataille (Centre Hospitalier General, Bologne sur Mer, France), V. Ensaust (CHU Hotel Dieu, Nantes, France), F. van der Woude, R. Waldherr, W. Schmitt, K. Andressy, O. Hergecssi, R. Nowack (Heidelberg University Hospital, Heidelberg, Germany), K. De Visscher, L. Herlyn-Nel, G. Grootenboer (Dijkzigt Hospital, Rotterdam, The Netherlands), D. S. Emmanouel (University Hospital Crete, Heraklion, Greece), C. Feighery (Saint James’s Hospital, Dublin, Ireland), F. Ferrario, R.A. Simico (Ospedale San Carlo Borromeo, Milan, Italy), G. Gregorini (Spedale Civili, Brescia, Italy), J. Dadoniene (University Hospital Vilnius, Vilnius, Lithuania), E.C. Hagen (Umeå University Hospital, Umeå, Sweden), C.G.M. Kallenberg, C. Stegeman (University Hospital Groningen, Groningen, The Netherlands), J.W. Cohen Tervaert (University Hospital Maastricht, Maastricht, The Netherlands), C.A. Verburgh, C.E.H. Siegert, J.A. Brujin, H.A. Hauer, J. Hermans, J.C. Van Houwelingen, C.E. Vergunst (Leiden University Medical Center, Leiden, The Netherlands), E. van Gump (University Medical Center Utrecht, Utrecht, The Netherlands), E.M. Bajema (Dijkzigt Hospital, Rotterdam, The Netherlands), C. Vasconcelos (Hospital San Antonio, Porto, Portugal), E. Mirapeix, M. Solé (Hospital Clinico I Provincial, Barcelona, Spain), E.E. Pettersson, A. Bruchfeld (Huddinge University Hospital, Huddinge, Sweden), K.W.A. Westman (University Hospital of Lund, Lund, Sweden), Z. Heigl (Karolinska Hospitai, Stockholm, Sweden), M. Carreras (University Hospital of Lund, Lund, Sweden), E. Gaffney (University Hospital Leuven, Leuven, Belgium), A. Stejskalova, Z. Vernevarova (Charles University, Prague, Czech Republic), T. Tormoth (University of Helsinki, Helsinki, Finland), A.C. Feller (University of Luebeck, Luebeck, Germany), E. Gaffney (Saint James’s Hospital, Dublin, Ireland), R. Tardanico (Ospedale Civili, Brescia, Italy), R. Consolancier (Ospedale Maggiore CA Granda, Milan, Italy), G. Garibotto (ISL, Genova, Italy), A.T.M.G. Tiebosch (Academic Hospital Groningen, Groningen, The Netherlands), C.D. Kooijman (Eemland Hospital, Amersfoort, The Netherlands), M. Sole Arques (Hospital Clinic I Provincial de Barcelona, Barcelona, Spain), F. Algba (Puigvert, Barcelona, Barcelona, Spain), M. Carreras (Hospital de Bellvitge, Barcelona, Spain), M. Vaquero Perez (Hospital Universitari Germans Trias I Pujol, Badalona, Spain), L. Bernardo (Hospital Dr. Josep Trueta, Girona, Spain), B. Sundelin (Karolinska Hospital, Stockholm, Sweden), P. Alm (University Hospital of Lund, Lund, Sweden), A. Wernersson (Huddinge University Hospital, Sweden), B. Veress (University Hospital Malmö, Malmö, Sweden), W. Landells (Saint Helier Hospital, London, UK), A.J. Howie (University Hospital Birmingham, Birmingham, UK), S. Fleming (University Department of Pathology, Edinburgh, UK), A.P. Griffith (Morriston Hospital, Swansea, UK), P.N. Furness (Leicester Area Histopathology Service, Leicester, UK), H.T. Cook (Hammersmith Hospital, London, UK).

Reprint requests to H.A. Hauer, M.D., Department of Pathology, Leiden University Medical Center, P.O. Box 9600, Building 1, LI-Q, 2300 RC Leiden, The Netherlands.

E-mail: H.A.Hauer@LUMC.nl

APPENDIX

Abbreviations used in this study are: ANCA, anti-neutrophil cytoplasm autoantibodies; C-ANCA, ANCA with a cytoplasmic staining pattern; P-ANCA, ANCA directed against perinuclear antigens; CYCAZAREM, randomized trial of CYClophosphamide versus AZAthioprine during REMission of ANCA-positive systemic vasculitis; ELISA, enzyme-linked immunosorbsant assay; EUVAS, European VAsculitis Study group; GFR, glomerular filtration rate; IIF, indirect immunofluorescence; MEPEx, randomized trial of adjunctive therapy for severe glomerulonephritis in ANCA-associated systemic vasculitis–intravenous administration of Methylprednisolone versus Plasma EXchange; MPA, microscopic polyangiitis; MPO, myeloperoxidase; MPO-ANCA, ANCA directed against myeloperoxidase as determined by ELISA; P-ANCA, ANCA with a perinuclear staining as determined by IIF; PR3, proteinase-3; RLV, renal limited vasculitis; SD, standard deviation; WG, Wegener’s granulomatosis.
REFERENCES


