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J Rheumatol 2014;41;1140-1146
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ABSTRACT. Objective. Antineutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis is a vasculitis affecting the glomerular capillaries and small renal arteries. Although crescent formation has been reported to be characteristic of this condition, the significance of coexisting vasculitis affecting the small renal arteries has not been investigated.

Methods. Fifty patients with ANCA-positive rapidly progressive glomerulonephritis whose renal biopsy specimens contained arterioles and/or interlobular arteries were retrospectively evaluated. Cellular crescents and/or necrotizing glomerulonephritis were noted in all 50 patients. Ten patients had vasculitis of the small renal arteries (group A) and 40 patients were without such vasculitis (group B). The clinical features of these 2 groups were compared.

Results. Group A comprised 4 patients who had granulomatosis with polyangiitis (GPA) and 6 with microscopic polyangiitis (MPA), while group B included 1 patient with GPA and 39 with MPA. No patient in either group had eosinophilic granulomatosis with polyangiitis. The C-reactive protein (CRP) level was significantly higher in group A compared with group B (11.58 ± 6.19 vs 2.7 ± 3.55 mg/dl, p < 0.05), and pulmonary involvement was more frequent in group A than group B (80% vs 37.5%, p < 0.05).

Conclusion. In patients with ANCA-positive glomerulonephritis, vasculitis of small renal arteries may be associated with systemic vasculitis (including pulmonary involvement) because of elevated CRP, a systemic inflammatory marker related to overproduction of interleukin 6. (First Release April 15 2014; J Rheumatol 2014;41:1140–6; doi:10.3899/jrheum.130657)

Key Indexing Terms: VASCULITIS OF SMALL RENAL ARTERIES GLOMERULONEPHRITIS ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES

The classification of vasculitis was developed by the first international Chapel Hill consensus conference in 1994 (CHCC 1994)1. Combining the American College of Rheumatology criteria2 and the Lanham criteria for Churg-Strauss syndrome3, an algorithm for differential diagnosis was proposed by the European Medicines Agency in 20074. This classification was updated by the second international CHCC in 20125,6. At that time, small vessel vasculitis was divided into antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) and immune complex-type vasculitis. The former type comprises microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), and eosinophilic granulomatosis with polyangiitis (Churg-Strauss syndrome; EGPA). Renal involvement in patients with AAV (ANCA-associated glomerulonephritis) features vasculitis that affects both the glomerular capillaries and the small arteries, including the arterioles and the interlobular and arcuate arteries. However, previous studies of ANCA-associated glomerulonephritis have largely focused on glomerular capillary lesions, such as necrotizing glomerulonephritis with crescent formation, and the significance of small artery vasculitis has not been investigated in much detail. In fact, only a few studies have assessed vasculitis of the small renal arteries7,8,9,10,11. Accordingly, we performed a retrospective investigation to determine whether the presence of small artery vasculitis had a clinical effect in patients with ANCA-associated glomerulonephritis.
MATERIALS AND METHODS

Patients. Sixty-one patients were diagnosed by renal biopsy as having ANCA-positive glomerulonephritis at our hospital from 1991 to 2011. Eleven patients were excluded because they had secondary vasculitis such as lupus vasculitis, antiglomerular basement membrane disease, or biopsy errors (specimens without cellular crescents and/or necrotizing glomerulonephritis and small arteries). The remaining 50 patients with ANCA-associated glomerulonephritis were enrolled in this retrospective study. They were divided into group A (with concomitant vasculitis of small arteries including the arterioles, interlobular arteries, or arcuate arteries) and group B (without small artery vasculitis), and various clinical characteristics were compared between the 2 groups (Table 1).

Classification of vasculitis. According to the European Medicines Agency algorithm, the patients were divided into 3 subgroups: EGPA, GPA, and MPA, including renal-limited AAV (RLV; defined as no evidence of vasculitis outside the kidneys).

Measurement of ANCA. ANCA was measured at the time of presentation before renal biopsy. The first 3 patients were positive for perinuclear (p)ANCA or cytoplasmic (c)ANCA by indirect immunofluorescence, while the remaining 47 patients were positive for myeloperoxidase (MPO) ANCA or proteinase 3 (PR3)-ANCA by ELISA (Nipro).

Histological evaluation of renal biopsy specimens. Renal biopsy specimens were fixed in 6% formalin, embedded in paraffin, cut into 1–2 μm sections, and stained with H&E, periodic acid Schiff, Weigert’s elastica-van Gieson, Masson trichrome, periodic acid methenamine silver (PAM), or PAM-Masson stain for light microscopy. All 50 renal biopsy specimens were reviewed by at least 2 pathologists. A cellular crescent was defined as a lesion consisting of proliferative epithelial cells and inflammatory cells that occupied 25% or more of Bowman’s space, and included at least 2 layers of proliferating cells (Figure 1A). Vasculitis of the small arteries was defined as fibrinoid necrosis (red on Masson trichrome staining and/or H&E staining) accompanied by infiltration of leukocytes (Figure 1B).

Treatment. Patients were treated with various regimens. Nineteen patients received oral prednisolone (PSL; 0.8 mg/kg) with concomitant intravenous methylprednisolone pulse therapy (500 mg/day for 3 days). Thereafter, cyclophosphamide was added for 6 patients. Among the other 31 patients, only oral PSL was given to 4 patients, while 3 patients received oral PSL with oral cyclophosphamide and 24 patients received oral PSL with intravenous cyclophosphamide (500 mg/day for 3 days).

Relapse. Relapse was defined as the clinical requirement for an increase in the dose of PSL because of deterioration of renal function with nephritic sediment or symptoms of vasculitis.

Statistical analysis. Data were summarized as proportions or as the mean (± SD). Categorical variables were analyzed with the chi-square test or Fisher’s exact test as appropriate, while continuous variables were
Pulmonary involvement showed a significant difference in prevalence between group A and group B, being found in 9 patients (80%) and 15 patients (37.5%), respectively (p < 0.05). In group A, the breakdown of pulmonary involvement was pulmonary hemorrhage in 1 patient, interstitial lung disease in 5 patients, and pulmonary nodules in 2 patients, while group B had pulmonary hemorrhage in 5 patients and interstitial lung disease in 10 patients.

**Glomerular changes.** There were no significant differences between the 2 groups in the percentage of glomeruli with crescent formation and fibrinoid necrosis. However, the percentage of glomeruli with global sclerosis was lower in group A than in group B (15.0 ± 15.7% vs 35.0 ± 29.1%). When the number of glomeruli per specimen (as a surrogate for the amount of cortex sampled) was compared between groups A and group B, there was no significant difference (27.0 ± 19.6 and 23.2 ± 14.4, respectively). This finding suggested that the number of arteries affected by vasculitis was not correlated with the number of affected glomeruli including small arteries. Vasculitis was not detected in the medullary region.

**Prognosis.** The relapse rate was significantly higher in group A than in group B (15.0 ± 15.7% vs 35.0 ± 29.1%). When the number of glomeruli per specimen (as a surrogate for the amount of cortex sampled) was compared between groups A and group B, there was no significant difference (27.0 ± 19.6 and 23.2 ± 14.4, respectively). This finding suggested that the number of arteries affected by vasculitis was not correlated with the number of affected glomeruli including small arteries. Vasculitis was not detected in the medullary region.
Figure 1. A. Periodic acid methenamine silver–Masson staining shows a cellular crescent (*) with fibrin-rich material (small arrows) and fibrillary material (large arrow). B. H&E stain shows fibrinoid necrosis extending from the interlobular artery to arcuate artery.
Among many studies of ANCA-related glomerulonephritis, vasculitis of the small arteries, including interstitial capillaries, arterioles, interlobular arteries, and arcuate arteries, has been reported by several authors. Bajema, et al performed a metaanalysis of 349 patients with Wegener’s granulomatosis (now called GPA) reported in the literature from 1979 to 1997, including evaluation of data on 134 renal biopsies. The most frequent lesion revealed by renal biopsy was extracapillary proliferation (70%), followed by fibrinoid necrosis of the glomerular tufts (54%). Vasculitis of the interstitial arteries and arterioles was present in almost 20%, whereas renal granulomas were seen in only 7 biopsy specimens. However, the details of vasculitis of the small arteries were not analyzed. In addition, Hauer, et al analyzed 173 patients with MPA (n = 80), GPA (n = 73), or renal-limited vasculitis (n = 19). Glomerular lesions (fibrinoid necrosis of the glomeruli, crescents, glomerulosclerosis, periglomerular infiltrates, and granulomatous reaction) and tubulointerstitial lesions (interstitial infiltrates, interstitial fibrosis, and tubular atrophy) were evaluated. They reported that 12% of the patients had interstitial vasculitis, but we could not determine whether such interstitial vasculitis corresponded to vasculitis of the small renal arteries from the description in their article. Further, Chen, et al evaluated renal biopsies from 61 patients with GPA and 44 patients with MPA, focusing on vascular lesions. Among the 39 patients with MPO-ANCA-positive GPA, necrotizing lesions of interlobular arteries were found in 4 (10.3%). Among the 22 patients with PR3-ANCA-positive GPA, necrotizing lesions of the arterioles were found in only 1 case. Out of the 44 patients with MPO-ANCA-positive MPA, 2 had necrotizing lesions of the arterioles and another 2 had necrotizing lesions of the interlobular arteries. However, the characteristics of these 9 cases with small artery vasculitis were not reported.

Vizjak, et al reported on the prevalence of vasculitis in biopsies from patients with ANCA-associated glomerulonephritis. Active extraglomerular vasculitis was observed in 22.2%, while chronic vascular lesions indicative of previous vasculitis were present in 11.9%. Extraglomerular renal vasculitis was identified in 38.3% of the patients with systemic vasculitis and in 10.0% of the patients with renal-limited vasculitis. They hypothesized that extraglomerular vasculitis of small renal vessel may be a predictor of systemic involvement, although they did not find any difference between PR3-positive patients and MPO-positive patients. The report by Yamagata, et al on Japanese patients with AAV may be useful for assessing the true value of the present study. They investigated 46 patients with GPA, 344 patients with MPA, and 745 patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group A, n = 10</th>
<th>Group B, n = 40</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>67.6 ± 9.05</td>
<td>61.4 ± 15.5</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, men/women</td>
<td>3/7</td>
<td>11/29</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of disease, mos</td>
<td>57.7 ± 57.6</td>
<td>78.8 ± 63.0</td>
<td>NS</td>
</tr>
<tr>
<td>MPO-ANCA positive</td>
<td>8 (80)</td>
<td>42 (95.4 )</td>
<td>NS</td>
</tr>
<tr>
<td>PR3-ANCA positive</td>
<td>2 (20)</td>
<td>3 (6.7)</td>
<td>NS</td>
</tr>
<tr>
<td>MPO-ANCA (EU)</td>
<td>377.0 ± 294.1</td>
<td>272.0 ± 336.7</td>
<td>NS</td>
</tr>
<tr>
<td>PR3-ANCA (EU)</td>
<td>70.0 ± 84.9</td>
<td>39.3 ± 51.9</td>
<td>NS</td>
</tr>
<tr>
<td>C-reactive protein, mg/dl</td>
<td>11.58 ± 6.19</td>
<td>2.7 ± 3.55</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>3.8 ± 3.2</td>
<td>3.7 ± 3.0</td>
<td>NS</td>
</tr>
<tr>
<td>Urine protein, g/day</td>
<td>0.94 ± 0.65</td>
<td>2.08 ± 2.25</td>
<td>NS</td>
</tr>
<tr>
<td>Hematuria, &gt; 6/HPF</td>
<td>9 (90)</td>
<td>40 (100)</td>
<td>NS</td>
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<tr>
<td>Glomerular lesions; crescents (%)</td>
<td>41.1 ± 29.6</td>
<td>42.9 ± 29.4</td>
<td>NS</td>
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<tr>
<td>Glomerular sclerosis (%)</td>
<td>15.0 ± 15.7</td>
<td>35.0 ± 29.1</td>
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<tr>
<td>Fibrinoid necrosis</td>
<td>6 (60)</td>
<td>16 (40)</td>
<td>NS</td>
</tr>
<tr>
<td>Pulmonary involvement</td>
<td>8 (80)</td>
<td>15 (37.5)</td>
<td>0.016</td>
</tr>
<tr>
<td>Others (neuron, ear, skin)</td>
<td>4 (40)</td>
<td>7 (17.5)</td>
<td>NS</td>
</tr>
<tr>
<td>GPA (patients)</td>
<td>4 (40)</td>
<td>1 (2.5)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MPA (RLV)</td>
<td>n = 6 (n = 2)</td>
<td>n = 39 (n = 21)</td>
<td>—</td>
</tr>
</tbody>
</table>

HPF: high-power field; MPO: myeloperoxidase; ANCA: antineutrophil cytoplasmic antibody; PR3: proteinase 3; GPA: granulomatosis with polyangiitis; MPA: microscopic polyangiitis; RLV: renal-limited AAV; AAV: ANCA-associated vasculitis; NS: not significant.
Figure 2. A. The relapse rate was significantly higher in group A than in group B (p < 0.05; group A vs group B: p = 0.04 by log-rank). B. The renal survival rate did not show a significant difference between the 2 groups (group A vs group B: p = 0.66 by log-rank). C. The prognosis also showed no significant difference between the 2 groups (group A vs group B: p = 0.12 by log-rank).
with RLV. Among the 745 patients with RLV, 656 patients (88.1%) had MPO and 55 patients (7.4%) had PR3. In addition, there were 316 patients (91.8%) who had MPO and 21 patients (6.1%) who had PR3 among the 344 patients with MPA, while 10 patients (22.7%) had MPO and 33 patients (71.1%) had PR3 among the 46 patients with GPA. It should be noted that the number of patients with GPA and/or PR3 is smaller in Japan than in Western countries.

Finally, Inoue, et al. clarified the 3-dimensional morphology of the renal interlobular arteries in 6 autopsy cases of acute inflammatory MPA with necrotizing vasculitis by examination of serial paraffin sections. All 19 lesions that were detected had microaneurysms, of which 18 were sausage-shaped and 1 was saccular. A possibility of a risk of rupture was suggested in patients with microaneurysms.

Our study showed that small renal artery lesions are closely related to elevation of CRP and pulmonary involvement. Because CRP is a systemic inflammatory marker that indicates the overproduction of interleukin 6, elevation of CRP may be involved in the process by which inflammation progresses from intraglomerular capillaries to small renal arteries and/or small systemic vessels, including the pulmonary arteries. This hypothesis is supported by the findings of Vizjak, et al.10

ACKNOWLEDGMENT
We thank Dr. Akira Yamada (Division of Nephrology, Department of Internal Medicine, Kyorin University School of Medicine) for his helpful advice.

REFERENCES