ANCA serotype and histopathological classification for the prediction of renal outcome in ANCA-associated glomerulonephritis

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ABSTRACT

Background. The phenotype of renal involvement in anti-neutrophil cytoplasmic antibodies (ANCA) vasculitis has a major influence on survival, and histological subgrouping of diagnostic renal biopsies has been proposed to aid in the prediction of renal outcome. We aimed to validate this histological subgrouping and to investigate the additional value of ANCA serotype in the prediction of renal outcome.

Methods. Data were retrospectively collected from the time of diagnosis by systematic review of medical records from 136 patients with renal biopsies recruited to cohorts from the UK and Spain, over 15 years. The end point, renal survival, was the composite of end-stage renal disease (ESRD) or death from any cause. The occurrence of ESRD, Stage 4 Kidney Disease Outcomes Quality Initiative-Chronic Kidney Disease, was assessed separately, in order to establish a severity index risk of chronic kidney disease.

Results. Renal survival at 5 years was 96% in the focal, 86% in the crescentic, 81% in the mixed and 61% in the sclerotic subgroups (P = 0.03). Myeloperoxidase (MPO)-ANCA was associated with more severe disease when compared with PR3-ANCA, as demonstrated by a lower frequency of focal and higher frequency of sclerotic subgroups, by more advanced interstitial fibrotic change and by lower glomerular filtration rate at diagnosis and worse renal function at 1 and 2 years.

Conclusions. We have confirmed the predictive value for renal survival of the ANCA vasculitis histology classification in a multi-centre study. We found a worse renal outcome in patients with tubulointerstitial fibrosis and atrophy. MPO-ANCA positive patients had a worse renal prognosis due to more severe glomerular injury. These results contribute to patient stratification in renal vasculitis for therapeutic, epidemiological and basic research.

Keywords: ANCA, glomerulonephritis, histopathology, outcome, vasculitis

INTRODUCTION

The prevalence of kidney involvement in anti-neutrophil cytoplasmic antibodies (ANCA)-associated vasculitis [AAV, including granulomatosis with polyangiitis, (GPA) and microscopic polyangiitis, (MPA)] is 75–90%, and impacts patients’ quality of life, morbidity and mortality, particularly among those with end-stage renal disease (ESRD) [1]. The clinical phenotype of vasculitis correlates with ANCA specificity. The majority of patients with kidney-limited disease have myeloperoxidase (MPO)-ANCA positivity (81%), and those with destructive lesions of the upper airways have proteinase 3 (PR3)-ANCA positivity (94%). When vasculitis was expanded from the kidney-limited variant to involve the gastrointestinal or respiratory tract, MPO-ANCA positivity was less frequent and PR3-ANCA positivity increased [2]. Classification of AAV according to ANCA specificity is more strongly associated with clinical outcomes than classification by clinical subgrouping [3]. Moreover, Lyons et al. confirmed that genetic associations are more strongly associated with antigenic specificity of ANCA, than with clinical syndrome (GPA versus MPA) [4]; however, the extent to which any gene might influence the clinical phenotype of vasculitis is not known [2]. Animal
models have confirmed the pathogenicity of MPO-ANCA, whereas this is less clear for PR3-ANCA [5]. PR3-ANCA positivity is predominant in northern Europe, whereas MPO-ANCA positivity is predominant in southern Europe, Japan and China [6–7] and AAV associated with environmental factors, usually demonstrates a predominance of an MPO-ANCA [8–9].

The renal histology of AAV is characterized by cellular crescents, fibrinoid necrosis and interstitial inflammation. In patients with moderately impaired renal function, active lesions such as cellular crescents and fibrinoid necrosis were associated with renal recovery [10]. In patients with severely impaired renal function, the presence of normal glomeruli on biopsy was a positive predictor of dialysis independence and improved renal function after 12 months [11].

An international working group of renal pathologists proposed a classification system for ANCA-associated glomerulonephritis (GN) comprising four subgroups: focal, crescentic, mixed and sclerotic [12]. This classification takes into account that glomerular lesions assessed by a light microscopy, but tubulointerstitial features were not found to improve the prognostic value. The subgroups corresponded with the severity of renal impairment [estimated glomerular filtration rate (eGFR)] at presentation, and after 1 and 5 years [12]. Subsequent study confirmed that the focal subgroup had the best renal survival and that the system could be optimized by recording the percentage of normal glomeruli in the biopsy [13].

We sought to validate the AAV GN classification system for the prediction of renal outcome in an inclusive, multi-centre European cohort as opposed to a cohort selected from clinical trial recruits. We also sought to test the additional value of ANCA serotype and to revisit the potential predictive role of tubulointerstitial fibrosis in a larger study.

MATERIALS AND METHODS

Study design

This was a retrospective study. Consecutive patients with AAV GN undergoing biopsy were studied from the Vasculitis and Lupus Clinic at the Renal Unit in Addenbrooke’s Hospital, Cambridge, UK and the Department of Nephrology, Hospital Clinic, Barcelona, Spain between April 1996 and July 2012.

Patients and data

Patients with a renal biopsy showing pauci-immune necrotizing GN consistent with AAV and at least 1 year of follow-up and, not incorporated in previous analyses regarding renal outcome in relation to renal histopathology, were included. Clinical diagnoses were based on the 2012 Chapel Hill consensus conference on the nomenclature of systemic vasculitis [3]. Patients with ANCA-negative GN were classified as MPA.

The therapeutic regimens were similar in both centres reflecting the current standard of care of AAV. They included pulsed intravenous cyclophosphamide, 15 mg/kg for every 2–3 weeks or daily oral cyclophosphamide, 2 mg/kg/day, with a switch to azathioprine at remission, between 3–6 months; combined with prednisolone, 1 mg/kg/day reducing in steps to 12.5–15 mg by 3 months. All data were retrospectively collected from the time of diagnosis and throughout follow-up by a systematic review of medical records. The study was approved by the local ethics committees in the UK and Spain. All patients enrolled in this study gave their consent, and all treatment decisions were made prior to our evaluation.

Evaluation of clinical parameters

At the time of renal biopsy, patient age, sex, renal function, ANCA specificity and diagnosis were recorded. Renal function was defined by eGFR [14], and was assessed at 1, 2 and 5 years, and corrected for eGFR at baseline. Corrected glomerular filtration rate (GFR) was defined as the difference between the observed eGFR and its linear prediction on the basis of baseline eGFR [15]. Renal function at 1 year was classified according to the Kidney Disease Outcomes Quality Initiative classification for chronic kidney disease [16]. The primary outcome for this analysis was the composite of ESRD or death from any cause. ESRD was defined as requiring dialysis for >12 weeks. The composite was chosen, because death acts as a competing event for ESRD. The occurrence of ESRD, Stage 4 Kidney Disease Outcomes Quality Initiative-Chronic Kidney Disease (K/DOQI-CKD) and death were assessed, separately.

Histopathology

A minimum of eight glomeruli was considered adequate for a biopsy to be included. All biopsies were examined using the previously developed scoring protocol for patients with AAV and GN [17]; biopsies were classified into one of four subgroups [12], those with ≥50% of globally sclerosed glomeruli were classified as sclerotic, those with ≥50% of normal glomeruli as focal and those with ≥50% of glomeruli with cellular crescents were classified as the crescentic. Samples that did not meet these criteria were classified as mixed. Tubulointerstitial fibrosis and atrophy (IFTA) were assessed using the Banff score, briefly IFTA 0 = 0%, IFTA 1 = <25%, IFTA 2 25–50% and IFTA 3 > 50% [18].

Statistical analysis

Descriptive data are presented as mean (SD) for continuous variables and as frequency (%) for categorical data. Renal survival was assessed using the Kaplan–Meier test, comparing the different histological classes using the log-rank test. The Cox proportional hazard model was used for the regression study. Coefficients were expressed as a hazard ratio (HR) with a 95% confidence interval (CI). All P-values were two-tailed and were considered significant <0.05. The software used for statistical analyses was SPSS 22 version for windows (SPSS, Inc., Chicago, IL, USA).

RESULTS

One hundred and thirty-six patients with ANCA-associated GN were included in the analysis (Table 1). There were differences in clinical characteristics between centres, reflecting the known variability associated with the geographic area.
The mean age at baseline was 62.1 years. The female-to-male ratio was 65:71. Eighty-five (58.8%) patients had MPA and 44 (32.4%) GPA. Fifty-six per cent were MPO-ANCA and 37.5% were PR3-ANCA positive and there were differences in age between ANCA serotypes; 65.1 and 58.5 years, respectively (P = 0.01).

Thirty-two patients (23.5%) reached ESRD within 29.8 months, with 13 of 32 reaching ESRD and forty-seven (34.5%) developed the composite end point of death or Stage 4 K/DOQI-CKD during the first year. Twenty-seven (19.9%) died with a mean time to death of 48.8 months, and 48 (35%) developed the composite end point of death or ESRD. Fifty-four (40%) either died or developed Stage 4 K/DOQI-CKD after 1 year. The mean follow-up was 57.4 months.

The mean number of glomeruli was 18 (range 8–40). Thirty-five biopsies (25.7%) were classified as focal, 31 of 136 (22.8%) as crescentic and 17 (12.5%) as sclerotic. Fifty-three per cent were PR3-ANCA positive and there were differences with respect to the composite outcome of ESRD or death according to glomerular subtyping (P = 0.08). In the Cox regression model, the following covariates were included: age, sex, ANCA subtype, diagnosis, baseline eGFR and histological subgroups and IFTA score. Baseline eGFR was associated with renal survival (ESRD) (HR 0.933, 95% CI 0.883–0.985; P = 0.01), and patients in the mixed subgroup were at decreased risk of developing ESRD when compared with the sclerotic subgroup (HR 0.985; P < 0.01).

PR3-ANCA was less frequent than MPO-ANCA in the mixed and sclerotic subgroups (P < 0.05) (Table 3), and IFTA scores were higher in MPO-ANCA patients (P < 0.01).

Renal survival at 5 years was 96% in the focal, 86% in the crescentic, 81% in the mixed and 61% in the sclerotic subgroups (Figure 1) (P = 0.03), but there were no differences with respect to the composite outcome of ESRD or death according to glomerular subtyping (P = 0.08). In the Cox regression model, the following covariates were included: age, sex, ANCA subtype, diagnosis, baseline eGFR and histological subgroups and IFTA score. Baseline eGFR was associated with renal survival (ESRD) (HR 0.933, 95% CI 0.883–0.985; P = 0.01), and patients in the mixed subgroup were at decreased risk of developing ESRD when compared with the sclerotic subgroup (HR 0.985; 95% CI 0.997–1.000; P < 0.01).

Table 1. The main clinical cohort characteristics and distribution between centres

<table>
<thead>
<tr>
<th>Centre</th>
<th>Hospital clinic (Barcelona, Spain) (N = 36)</th>
<th>Addenbrooke’s Hospital (Cambridge, UK) (N = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical diagnosis (%)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPA</td>
<td>55</td>
<td>60</td>
</tr>
<tr>
<td>GPA</td>
<td>17</td>
<td>38</td>
</tr>
<tr>
<td>Renal limited</td>
<td>19</td>
<td>1</td>
</tr>
<tr>
<td>No diagnosis</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>ANCA type (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR3</td>
<td>22</td>
<td>43</td>
</tr>
<tr>
<td>MPO</td>
<td>72</td>
<td>50</td>
</tr>
<tr>
<td>negative</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>17/19</td>
<td>54/46</td>
</tr>
<tr>
<td>Age, mean (SD)</td>
<td>61 (14.28)</td>
<td>62 (12.41)</td>
</tr>
<tr>
<td>GFR, mean (SD)*</td>
<td>32 (24.03)</td>
<td>23 (24.16)</td>
</tr>
<tr>
<td>Outcomes, n</td>
<td>36</td>
<td>100</td>
</tr>
<tr>
<td>Deceased, n (%)</td>
<td>5 (14)</td>
<td>22 (22)</td>
</tr>
<tr>
<td>Mean time to death (SD), mo</td>
<td>59 (78.59)</td>
<td>41 (41.61)</td>
</tr>
<tr>
<td>ESRD, n (%)</td>
<td>7 (20)</td>
<td>25 (25)</td>
</tr>
<tr>
<td>Mean time to ESRD (SD), mo</td>
<td>33 (31.77)</td>
<td>29 (40.92)</td>
</tr>
<tr>
<td>Mean (SD) follow-up, mo</td>
<td>63 (46.99)</td>
<td>56 (42.24)</td>
</tr>
</tbody>
</table>

Note: MPA, microscopic polyangiitis; GPA, granulomatosis with polyangiitis; GFR, baseline glomerular filtration rate.

*P < 0.05.

Table 2. ANCA-associated GN classification and distribution between centres

<table>
<thead>
<tr>
<th>Histological subgrouping</th>
<th>Centre</th>
<th>Hospital Clinic (Barcelona, Spain) (N = 36)</th>
<th>Addenbrooke’s Hospital (Cambridge, UK) (N = 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal, n (%)</td>
<td>7 (19)</td>
<td>28 (28)</td>
<td>35 (26)</td>
</tr>
<tr>
<td>Crescentic, n (%)</td>
<td>13 (36)</td>
<td>18 (18)</td>
<td>31 (23)</td>
</tr>
<tr>
<td>Mixed, n (%)</td>
<td>10 (28)</td>
<td>43 (43)</td>
<td>33 (39)</td>
</tr>
<tr>
<td>Sclerotic, n (%)</td>
<td>6 (17)</td>
<td>11 (11)</td>
<td>17 (13)</td>
</tr>
</tbody>
</table>

Table 3. ANCA serotype frequency among glomerular lesion categories

<table>
<thead>
<tr>
<th>Histological subgrouping</th>
<th>PR3</th>
<th>MPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal, n (%)</td>
<td>17 (33)</td>
<td>13 (18)</td>
</tr>
<tr>
<td>Crescentic, n (%)</td>
<td>14 (28)</td>
<td>16 (21)</td>
</tr>
<tr>
<td>Mixed, n (%)</td>
<td>18 (35)</td>
<td>32 (44)</td>
</tr>
<tr>
<td>Sclerotic, n (%)</td>
<td>2 (4)</td>
<td>12 (16)</td>
</tr>
</tbody>
</table>

*There were differences in the frequency of mixed and sclerotic classes between MPO and PR3-ANCA patients (P < 0.05).
compared with PR3-ANCA (glomerular subgrouping was omitted from the analysis 95% CI 1.03–4.04, P = 0.04) is shown in Figure 2. For the glomerular subgroups, the risk of Stage 4 CKD for the crescentic, mixed and sclerotic subgroups was 4.30, 6.54 and 7.04, respectively (P < 0.02) (Table 4).

Factors that were associated with eGFR at 12 months were glomerular subgroup and ANCA serotype (P = 0.00).

DISCUSSION

The phenotype of renal involvement in AAV has influences on renal survival, and histological subgrouping of diagnostic renal biopsies has been proposed to aid in prognosis [12]. We aimed to validate this subgrouping and to investigate the additional value of ANCA serotype and tubulointerstitial fibrosis in the prediction of renal outcome in 136 patients with renal biopsies in cohorts from the UK and Spain.

In common with the observations by Berden et al. [12] the focal subgroup had a more favourable renal outcome and better preserved renal function during the follow-up, and the sclerotic subgroup was associated with worse renal function at 1 and 2 years when compared with the other subgroups and also had the highest risk for developing ESRD, and a low rate of renal recovery compared with the focal subgroup. Although the crescentic subgroup had the lowest eGFR at baseline, it had the highest chance of recovery of renal function by 1 and 5 years, indicating the reversibility of cellular crescents, consistent with the previous studies where cellular and segmental crescents were positively correlated with renal recovery [10]. In contrast to the Berden study, we did not find differences in ESRD between the mixed and crescentic subgroups but did observe differences by regression analysis using the composite outcome of death/advanced CKD (stages >4) between all four histological subgroups. Categorization between mixed and crescentic subgroups is subject to more observer variation than for the other subgroups and was not strictly controlled by the nature of this multi-centre retrospective analysis; similar findings were reported in two recent studies from China and the USA [19, 20].

Overall, we have confirmed the predictive value for the composite outcome Stage ≥4 K/DOQI-CKD or death of the ANCA-associated GN classification in a multi-centre patient cohort, but this glomerular subgrouping performed less well in predicting the composite outcome of death or ESRD.

We observed geographic variability in the clinical phenotype and ANCA serotype between northern and southern Europe and confirmed the association of MPO-ANCA with more severe and chronic disease, as demonstrated by a lower frequency of focal and higher frequency of mixed and sclerotic subgroups, by more advanced fibrotic changes in the interstitium, by lower eGFR at diagnosis and worse renal function at 1 and 2 years. In accordance with our study, Chang et al. [19] have recently reported a higher frequency of the sclerotic subgroup in Chinese MPO-ANCA patients, but they were not able to demonstrated differences in renal outcome between ANCA serotype due to the scarce prevalence of PR3-ANCA patients in that cohort.

Long-term studies have shown a trend to worse renal and patient survival in those with MPO-ANCA, and studies of
baseline biopsies have reported more severe fibrosis in MPO when compared with PR3-ANCA patients, but ANCA subtype has not been shown to be an independent predictor of renal outcome [10, 17, 21, 22]. We confirmed an association of MPO-ANCA serotype with a higher IFTA score and found worse renal outcome in patients with an IFTA score of 2 or 3 when compared with those with no tubular damage. But, an IFTA of 1, the most frequent score, carried no prognostic value. A well-defined tubulitis and tubulointerstitial injury score combined with immunostaining for CD3, CD79a, CD20, CD138 and a periodic acid–schiff counterstain to identify inflammatory cells located within and outside the boundaries of the tubular basement membranes, merits further prognostic evaluation and might increase the value of the glomerular subgrouping [23].

Thus, the serotype, MPO-ANCA and PR3-ANCA appear to define differing renal phenotypes at diagnosis, with MPO-ANCA patients being older and with more advanced fibrosis on renal biopsy. These factors are of a prognostic value because they associate with more frequent occurrence of Stage 4 K/DOQI-CKD and a lower chance of renal recovery at 1 and 5 years. Moreover, these results correlate with the stronger genetic associations with ANCA serotype than diagnostic subgroup ANCA [4].

In this study, treatment received after the diagnosis was not taken into account when considering long-term renal survival, but differences in practice in the choice and duration of therapy between centres was small. Follow-up renal biopsies in AAV GN have suggested that therapy could facilitate the partial reversal of active lesions [24] and that vasculitis activity may regress under therapy, while chronicity progresses despite treatment [25], so treatment received by the patients in the study was likely to have influenced renal outcomes. The study is limited by its retrospective design and data collection from two centres. Although histopathological parameters on renal biopsies were assessed by experienced pathologists following a scoring protocol developed for AAV GN, inter-observer variation was not assessed, although this has been determined in the previous studies. Inter-observer variation for the classification into the four categories was not assessed either.

Renal biopsy and baseline eGFR remain the most important outcome predictors available for ANCA-associated renal vasculitis. The severity of proteinuria after the diagnosis is also a marker of the degree of injury, and urinary monocyte chemoattractant protein 1 correlates with histological activity [26]. But no other plasma or urine biomarkers reliably contribute to prognosis or management of patients with renal vasculitis.

In conclusion, we have found evidence that inclusion of tubulointerstitial fibrosis may enhance the prognostic value of the glomerular subgrouping scheme. We have confirmed that MPO-ANCA positive patients have a worse renal prognosis due to more severe glomerular injury. These results contribute to the stratification of patients into more consistent disease groups for therapeutic, epidemiological and basic research.

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**CONFLICT OF INTEREST STATEMENT**

The authors have no conflict of interest.

**REFERENCES**


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