Immunostaining findings in IgA nephropathy: correlation with histology and clinical outcome in the Oxford classification patient cohort

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Abstract

Background. IgA nephropathy is defined by the presence of IgA-dominant glomerular deposits. Within this definition, there is variation in the location of IgA and the presence of other immunoglobulins. The Oxford classification of IgA nephropathy identifies four histological features that are independent predictors of clinical outcome but does not include immunostains. Here, we investigate the potential clinical significance of immunostaining data.

Methods. Original biopsy reports from the patients in the Oxford classification study were reviewed. The location of IgA deposits (mesangial versus mesangial + capillary wall) and the presence of IgG > trace were correlated with histological and clinical features.

Results. Original biopsy reports were available for 211 of 265 patients in the Oxford classification cohort, of which 175 included sufficient details to subclassify immunostaining findings. The presence of capillary wall IgA deposits was associated with a higher mesangial cellularity score (1.3 ± 0.6 versus 0.9 ± 0.5 for mesangial-only IgA, P = 0.007) and endocapillary proliferation (per cent of patients with any endocapillary proliferation of 62 versus 35% for mesangial-only IgA, P = 0.01). Similarly, the presence of IgG was associated with a higher mesangial cellularity score (1.2 ± 0.6 versus 0.9 ± 0.5, P = 0.03) and endocapillary proliferation (per cent of patients with endocapillary proliferation of 57 versus 31% with no IgG, P = 0.009). There was no significant association between the location of IgA or the presence of IgG and rate of loss of renal function and association between the location of IgA and renal survival although patients with these immunofluorescence findings tended to receive more immunosuppression. There was a trend towards poorer renal survival in those patients with glomerular IgG (hazard ratio of 2.1, 95% confidence interval, 1.0–4.6, P = 0.06).

Conclusions. We conclude that the location of glomerular IgA and the presence of IgG correlate with mesangial and endocapillary cellularity. This supports the role of IgG and capillary wall IgA in the development of proliferative changes in IgA nephropathy.

Introduction

IgA nephropathy is the most common primary glomerular disease worldwide. It is defined by the presence of IgA-dominant or codominant immune deposits within glomeruli as demonstrated by immunohistochemistry or immunofluorescence. The disease has diverse clinical manifestations, reflecting a wide range of histological changes, from near normal appearance on light microscopy to severe necrotizing lesions with crescents. The recent Oxford classification of IgA nephropathy identified four histological features, mesangial cellularity, endocapillary proliferation, segmental sclerosis and tubular atrophy/interstitial fibrosis, which are independent predictors of clinical outcome [1, 2] but did not include pattern of immunostaining in the analysis.

Following the seminal article by Berger et al. [3, 4], there have been several case series detailing the immunohistology of IgA nephropathy, reviewed by Haas [5]. D’Amico [6] has critically analysed the results of 23 clinicopathological studies, some of which have also discussed immunohistology.

IgA deposits are typically mesangial, although capillary wall IgA is seen in around one-third of cases (Figure 1). Glomerular IgG, in addition to IgA, is also a frequent finding. Both capillary wall IgA and IgG deposits have been associated with an adverse clinical outcome, but it is unclear whether the immunohistology is of predictive value, independent of other histological features. Here, we investigate the association with histology and clinical impact of immunohistological findings in the Oxford classification cohort.

Materials and methods

Patients

The patient cohort used in the development of the Oxford classification of IgA nephropathy included 206 adults and 59 children from eight countries.
Fig. 1. Immunoperoxidase staining for IgA showing two different distributions of deposits: (A) the most common, mesangial, pattern and (B) capillary wall staining, associated with endocapillary hypercellularity, a pattern frequently associated with systemic features of Henoch–Schönlein purpura.

Table 1. Correlation of histological features with immunostaining patterns

<table>
<thead>
<tr>
<th></th>
<th>Mesangial-only IgA (n = 149)</th>
<th>Capillary wall IgA (n = 26)</th>
<th>P-value</th>
<th>No/trace IgG (n = 119)</th>
<th>IgG &gt; trace (n = 30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesangial cellularity score</td>
<td>0.9 ± 0.5</td>
<td>1.3 ± 0.6</td>
<td>0.007</td>
<td>0.9 ± 0.5</td>
<td>1.2 ± 0.6</td>
<td>0.03</td>
</tr>
<tr>
<td>% Glomerular global glomerulosclerosis</td>
<td>8 (0–82)</td>
<td>11 (0–55)</td>
<td>&gt;0.1</td>
<td>11 (0–82)</td>
<td>8 (0–63)</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>% Glomerular segmental glomerulosclerosis</td>
<td>6 (0–44)</td>
<td>5 (0–33)</td>
<td>&gt;0.1</td>
<td>4 (0–38)</td>
<td>9 (0–38)</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>% Glomerular endocapillary proliferation</td>
<td>0 (0–54)</td>
<td>6 (0–47)</td>
<td>0.003</td>
<td>0 (0–50)</td>
<td>4 (0–47)</td>
<td>0.005</td>
</tr>
<tr>
<td>% Glomerular cellular + fibrocellular crescents</td>
<td>0 (0–55)</td>
<td>0 (0–39)</td>
<td>&gt;0.1</td>
<td>0 (0–55)</td>
<td>0 (0–39)</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>% Tubulointerstitial atrophy</td>
<td>10 (0–70)</td>
<td>10 (0–30)</td>
<td>&gt;0.1</td>
<td>10 (0–70)</td>
<td>10 (0–60)</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>% Interstitial fibrosis</td>
<td>10 (0–70)</td>
<td>10 (0–30)</td>
<td>&gt;0.1</td>
<td>10 (0–70)</td>
<td>10 (0–60)</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Arteriosclerosis score</td>
<td>0 (0–2)</td>
<td>0 (0–2)</td>
<td>&gt;0.1</td>
<td>0 (0–2)</td>
<td>0 (0–2)</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Arteriolar hyalinosis score</td>
<td>0 (0–3)</td>
<td>0 (0–3)</td>
<td>&gt;0.1</td>
<td>0 (0–3)</td>
<td>0 (0–3)</td>
<td>&gt;0.1</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SD for normally distributed data or median (range) for non-parametric distributions.

and four continents [1, 2]. Inclusion criteria were estimated glomerular filtration rate (eGFR) ≥30 mL/min/1.73m² and proteinuria >0.5 g/24 h in adults and ≥0.5 g/24 h in children at the time of diagnosis. Patients with Henoch–Schönlein purpura were excluded. eGFR was estimated using the four-variable MDRD formulae in adults and the Schwartz formula in children. End-stage renal disease (ESRD) was defined as glomerular filtration rate (eGFR) <15 mL/min/1.73m². Immunosuppressive treatment is reported as intent-to-treat, regardless of the type or duration of therapy. Two clinical outcomes were studied to address the predictive value of pathology variables: (i) the rate of renal function decline (slope of eGFR) and (ii) survival from a 50% reduction in renal function or ESRD (combined event).

Immunostaining data

Original biopsy reports were available for 211 patients, of which 175 included sufficient detail to subclassify the immunostaining findings and 119 cases included details of IgG staining. The staining method used was immunofluorescence in 174 cases and a single case by immunoperoxidase staining. These reports were reviewed and the following features noted:

1. Pattern of glomerular IgA staining: mesangial versus mesangial + capillary wall.
2. Presence or absence of glomerular IgG staining: > trace when present was taken as positive.

These immunohistological findings were compared with patient demographics, histological features and clinical outcome.

Histological scoring

Details of histological scoring of these biopsies have been previously described [2]. Other than mesangial, arteriolar and arteriolar scores, the histological lesions in Table 1 are expressed as the percentage of glomeruli showing the lesion, or for tubulointerstitial lesions, the percentage of renal cortex present showing atrophy/fibrosis. The mesangial cellularity score was obtained by scoring each glomerulus in the biopsy 0–3 according to the number of mesangial cell nuclei in the most cellular segment (<4 mesangial cells per mesangial area = 0; 4–5 mesangial cells = 1; 6–7 mesangial cells = 2 and ≥8 mesangial cells = 3) and taking the mean score of all glomeruli. Arteriosclerosis was scored variously 0–2 according to intimal thickening in the worst affected artery: normal (0) and thickened to less (1) or more than (2) the thickness of the media. Arteriolar hyaline was scored according to the proportion of arterioles affected and scored variously 0–3 (0; 1, 1–25%; 2, 26–50%; 3, ≥50%).

Statistics

Normally distributed variables were expressed as mean ± SD and compared using student’s t-test. Non-parametric variables were expressed as median and range and compared using Mann–Whitney U-test. Categorical variables were expressed in percentages and compared using the Pearson’s chi-square test.

The rate of renal function decline was determined by fitting a straight line through the calculated eGFR using the principle of least squares. Cox regression was performed to test the association between immunofluorescence findings and survival from a combined event. This was illustrated using Kaplan–Meier curves. All P-values were two tailed and values <0.05 were considered statistically significant. Analyses were carried out using SPSS software (version 11; SPSS Inc., Chicago, IL).

Results

Correlation of immunostaining with histology

Of the 175 cases, mesangial and capillary wall IgA staining was noted in 26 cases, whereas 149 showed pure mesangial IgA staining. The histological features in these two groups are summarized in Table 1. The presence of capillary wall IgA deposits was associated with a higher mesangial
cellularity score (1.3 ± 0.6 versus 0.9 ± 0.5 for mesangial-only IgA, \( P = 0.007 \)) and the presence of endocapillary proliferation (per cent of patients with any endocapillary proliferation 62 versus 35% of those with mesangial-only IgA, \( P = 0.01 \)). Of the 149 cases that included sufficient details of IgG staining, 30 showed >trace IgG. Most of the reports did not include the details of the location of IgG in the glomeruli. IgG staining was associated with the presence of endocapillary proliferation (per cent of patients with any endocapillary proliferation 57 versus 31% of those with no IgG, \( P = 0.009 \)) and a higher mesangial cellularity score (1.2 ± 0.6 versus 0.9 ± 0.5, \( P = 0.03 \)).

The location of IgA deposits and the presence of IgG were not associated with the presence of crescents, focal and segmental glomerulosclerosis, interstitial or vascular lesions (data not shown). There was a trend towards the presence of capillary wall IgA in those biopsies with IgG staining (chi square \( P = 0.08 \)).

Correlation of immunostaining with clinical features at presentation and follow-up

There was no significant association between the location of IgA or presence of IgG and initial mean arterial pressure, urine protein excretion or eGFR at the time of diagnosis (data not shown). Biopsies from paediatric patients were more likely to show capillary wall IgA, which was present in 26.8% of children versus 11.2% of adults (\( P = 0.014 \)). The mean age of patients with capillary wall IgA was 26 years versus 31 years for those with mesangial-only IgA (\( P = 0.087 \)). There was no association between the presence of IgG and the age at diagnosis.

Capillary wall IgA and the presence of IgG were associated with trends towards greater immunosuppression (IS). A total of 35% of patients with capillary wall IgA received IS versus 23% with mesangial-only IgA (\( P = 0.207 \)); 37% of patients with IgG staining received IS versus 21% with no IgG staining (\( P = 0.079 \)). These patients did not receive less renin angiotensin blockade or antihypertensive medication (data not shown). The trends towards greater IS in patients with capillary wall IgA and glomerular IgG deposits were not merely due to greater endocapillary proliferation in these groups. In patients whose biopsies did not show endocapillary proliferation, capillary wall IgA was associated with a higher frequency of IS; 40% of patients with capillary wall IgA received IS, compared to 12.5% of those with mesangial-only IgA (\( P = 0.021 \)). In the same group, 31% of patients with glomerular IgG received IS, compared to 14% of those without IgG deposits (\( P = 0.116 \)).

Analysis of the follow-up data revealed no significant association between the location of IgA or the presence of IgG and rate of renal function decline, follow-up proteinuria and blood pressure and no association between the location of IgA and survival from a combined event. There was a trend towards poorer survival in those patients with glomerular IgG (hazard ratio of 2.1, 95% confidence interval, 1.0–4.6, \( P = 0.06 \)) (Figure 2). The presence of IgG did not significantly correlate with outcome in multivariate analysis that included mesangial cellularity. However, the hazard ratio associated with IgG did not change greatly when adding the mesangial score (2.1 to 1.9), suggesting little relationship between the two variables.

Discussion

In his original article, Berger described the classical pattern of staining on immunohistology as diffuse mesangial staining for IgA whose intensity of staining is either greater than or equal to that of other immunoglobulins present [3]. Berger also noted that mesangial IgA deposits were accompanied by IgG deposits in all but 2 of the 55 cases. There are several studies subsequently describing the intensity and pattern of IgA staining and its significance [5–9]. In a published composite review comprising 2000 patients from 13 different series, 50% of cases of IgA nephropathy showed mesangial IgG deposits [5]. D’Amico identified and critically analysed the 23 most valid studies published in the preceding two decades [6].
Some of these included immunohistological features. Four of the eight studies that used univariate analysis and one study that used multivariate analysis confirmed that extension of IgA deposition from the mesangial area to the peripheral capillary wall was a significant adverse risk factor. In addition, two studies, both univariate, included in the same series noted that co-deposition of IgG was an independent risk factor affecting renal outcome. IgG deposits have also been associated with greater inflammation in an animal model [10].

The presence of glomerular capillary wall IgA deposits is reported to be associated with greater proteinuria and histological severity [11–13]. In one study, children with glomerular basement membrane IgA deposition had higher urinary protein at diagnosis and more severe histological alterations, including more frequent crescent formation, segmental and global sclerosis, tubular atrophy and interstitial fibrosis, as compared to children with mesangial-only IgA deposition. Furthermore, these children were more likely to show persistent proteinuria and progressive renal failure [9]. In our cohort of 175 patients, capillary wall IgA staining was noted in 15% of cases. The presence of capillary wall IgA deposits was associated with greater mesangial and endocapillary cellularity. Of 119 cases with sufficient details of glomerular IgG staining, 25% showed trace IgG. Similar to capillary wall IgA positivity, the presence of IgG was associated with greater mesangial and endocapillary proliferation.

The patient cohort was not controlled for immunosuppressive therapy and a trend to greater IS in those patients whose biopsies showed capillary wall IgA or the presence of IgG is a potential source of bias when interpreting the impact of immunostaining findings on clinical outcome. Analysis of the follow-up data revealed no significant association between the location of IgA or the presence of IgG and rate of loss of renal function and renal survival, although there was a trend towards poorer renal survival in those patients with glomerular IgG.

We conclude that the location of glomerular IgA and the presence of IgG correlate with greater histological activity. A correlation between immunostaining pattern and clinical outcome has not been demonstrated in this cohort and there is insufficient evidence to include immunostaining data in the Oxford classification at the present time. However, validation of these findings is required in other cohorts, particularly in view of the potential bias in the outcome data resulting from immunosuppressive therapy in some patients. It is recommended that the location and intensity of IgA and IgG staining is routinely included in the renal biopsy report.

Conflict of interest statement. None declared.

References
11. Andreoli SP, Yum MN, Bergstein JM. Significance of glomerular basement membrane deposition of IgA. Am J Nephrol 1986; 6: 28–33

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