Kidney biopsy is a sensitive tool for retrospective diagnosis of PLA2R-related membranous nephropathy

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Abstract

Background. Antibodies against M-type phospholipase A₂ receptor (PLA2R) are serological markers of disease activity in patients with idiopathic membranous nephropathy (iMN). To determine the most sensitive test for the diagnosis of PLA2R-related membranous nephropathy (MN) irrespective of sampling time, we investigated the presence of PLA2R in glomerular immune deposits and assessed circulating anti-PLA2R antibodies in a retrospective cohort of Czech patients with idiopathic, lupus and other few secondary MN.

Methods. We tested archival paraffin-embedded kidney biopsies of 84 consecutive patients with biopsy-proven MN, for the presence of PLA2R in glomerular immune deposits and assessed circulating anti-PLA2R antibodies in a retrospective cohort of Czech patients with idiopathic, lupus and other few secondary MN.

Results. In 45 of 65 (69%) patients with iMN, PLA2R was detected in a finely granular pattern in sub-epithelial deposits along glomerular capillary loops. Circulating anti-PLA2R antibodies were detected in 20 of 31 (65%) sera from patients sampled during active disease. Six patients with active disease were negative for circulating anti-PLA2R antibodies despite PLA2R antigen positivity in the kidney biopsies. Only 8 of 37 (22%) sera sampled at the time of remission were PLA2R positive while PLA2R antigen was found in 22 of the 37 (59%) corresponding biopsies. PLA2R was found in immune deposits in 3 patients with secondary MN (2 with hepatitis B, and 1 with sarcoidosis) but in none of the 16 patients with lupus.

Conclusions. In case of delayed serum sampling, assessment of PLA2R antigen in biopsy specimens is more sensitive than the serological test for the diagnosis of PLA2R-related MN which can be established retrospectively.

Introduction

Membranous nephropathy (MN) is an organ-specific autoimmune disease, which targets the kidney glomerulus, resulting in the formation of immune deposits on the outer regions of the glomerular basement membrane (GBM) and complement mediated proteinuria. Eighty per cent of cases are referred to as ‘idiopathic’ MN (iMN), while about 20% are classified as ‘secondary’ because they occur in patients presenting with associate clinical conditions such as infections (hepatitis B), lupus erythematosus, cancer and drug intoxication [1–3]. MN is the most common cause of nephrotic syndrome (defined by massive urinary protein loss) in Caucasian adults. This is very heterogenous disease with variable outcome and sensitivity to treatment [4–6].

The M-type phospholipase A₂ receptor (PLA2R) was recently identified as a major target antigen in adult iMN [7]. In addition, the strong association of the PLA2R1 gene with iMN in a recent genome-wide association study suggests that genetic variants of PLA2R1 are at risk for the disease [8]. Circulating antibodies against PLA2R were found in up to 70–80% of patients with iMN but not in any other glomerular diseases or healthy people, and very rarely in secondary MN [7, 9, 10]. Levels of circulating anti-PLA2R revealed a strong correlation with clinical disease activity and can be used to monitor response to treatment [11–13]. Our recent study in a small French cohort of 42 consecutive patients with iMN whose sera were sampled at the time of biopsy before immunosuppressive treatment (i.e. during active disease), suggested that assessment of both circulating PLA2R antibodies and PLA2R in biopsy might better categorize patients into different groups than only assessing anti-PLA2R antibodies. This study
showed that the absence of circulating PLA2R antibody at the time of kidney biopsy and proteinuria was probably not sufficient to rule out a diagnosis of PLA2R-related MN [14]. Because therapeutic strategies are different for patients with iMN and secondary MN, discriminating between these two groups of patients is of utmost clinical importance. The goal of the present study was to investigate the presence of PLA2R in glomerular immune deposits and to assess circulating anti-PLA2R antibodies in a large retrospective cohort of Czech patients with idiopathic, lupus and other secondary MN. Because in this cohort, serum samples were taken at different time points, often during remission, we wanted to investigate the feasibility and value of studies on archival kidney biopsies to retrospectively establish the diagnosis of PLA2R-related MN, which is of particular importance for those patients who will need a transplant.

MATERIALS AND METHODS

We tested a total of 84 paraffin-embedded tissue sections from patients (57/27 M/F; median age 54.5 years, range 19–77 years) with biopsy-proven MN collected between 1996 and 2011, including 65 patients with iMN and 19 patients with secondary MN (hepatitis B, 2; sarcoidosis, 1; lupus nephritis, 16). Twenty-three sera (idiopathic MN/secondary MN 20/3) sampled before treatment at the time of renal biopsy were available. In patients with iMN, we also tested 8 sera sampled several months after biopsy at the time of relapse or in patients with persistent proteinuria, as well as 37 sera sampled at the time of partial or complete remission induced by treatment or occurring spontaneously (Figure 1). Detailed clinical characteristics of the patients included are given in Tables 1, 2 and 3 in the Supplementary Appendix. Secondary causes of MN were excluded after extensive clinical workup including detailed medical history and patient examination, serological analysis (lupus autoantibodies, hepatitis B and C and HIV) and at least chest X-ray, abdominal ultrasound and tumour serological markers.

PLA2R was assessed in glomerular deposits by confocal microscopy in the paraffin-embedded biopsy sample from each patient with rabbit affinity purified specific anti-PLA2R antibodies (Atlas Antibodies) followed by goat Alexa 488 conjugated anti-rabbit Fab IgG (Molecular Probes). Circulating antibodies against PLA2R were assessed by commercially available indirect immunofluorescence test (Euroimmun). Kidney biopsy studies were performed in Paris while antibodies were assessed in Prague without knowledge of the clinical details or the results of the corresponding test.

RESULTS

Immunofluorescence staining for PLA2R showed a very weak positivity in normal kidneys (Figure 2A). In 45 of 65 of patients with iMN, PLA2R was detected in a finely granular pattern in sub-epithelial deposits along glomerular capillary loops as visualized by confocal microscopy (Figure 2B). In the 20 remaining biopsies from iMN, there was no PLA2R in glomerular immune deposits (Figure 2C). Importantly, there was no significant difference in any relevant clinical parameters such as proteinuria, serum creatinine and age between patients with and patients without PLA2R in immune deposits (Table 1). None of the 16 lupus patients had PLA2R in immune deposits (Figure 2D). However, three patients with secondary MN (two with hepatitis B and one with sarcoidosis) also had PLA2R in immune deposits (Figures 2E and F). PLA2R was not detected in glomeruli in five patients with sarcoidosis but without MN. The secondary anti-rabbit antibody did not label the immune deposits (data not shown).

Circulating anti-PLA2R antibodies were detected in 14 of 20 (70%) sera from patients with iMN sampled at the time of renal biopsy. Three patients were negative for circulating anti-PLA2R antibodies although PLA2R antigen was detected in glomerular deposits (Table 2). Of the eight patients first sampled at the time of relapse or several months after the kidney biopsy when disease was still active with persistent proteinuria, four had circulating anti-PLA2R antibodies while the other four were negative. Seven of these eight patients had PLA2R in glomerular immune deposits (Table 2). In summary, circulating anti-PLA2R antibodies were detected in 18 of 28 (64%) sera from patients with iMN sampled during...
active disease. Six patients were negative for circulating anti-PLA2R antibodies despite PLA2R antigen positivity in the kidney biopsy. In addition, anti-PLA2R antibodies were detected in two of the three patients with secondary MN (one with hepatitis B, the other with sarcoidosis) (Table 2).

In the group of 37 iMN patients with spontaneous or drug-induced remission whose serum was sampled several months after the kidney biopsy, 22 (59%) showed PLA2R in glomerular immune deposits in the kidney biopsy taken at the time of initial disease presentation. Among these 22 patients, seven (32%) with partial remission had circulating anti-PLA2R antibodies while the remaining 15 patients with partial or complete remission had no detectable PLA2R in serum (Table 3). Overall, only 8 of 37 (22%) sera were positive for PLA2R antibodies at the time of remission when compared with 64% at the time of active disease.

As previously reported in the French cohort [14], we found in the Czech cohort two patients who had circulating anti-PLA2R antibodies but did not have detectable PLA2R in glomerular deposits.

**DISCUSSION**

This retrospective study conducted in Czech patients with MN shows for the first time that detection of PLA2R in biopsy samples can have a major diagnostic importance in cases where only a biopsy, in the absence of serum, is available at initial presentation. Secondly, detection of PLA2R in kidney biopsies can discriminate between iMN and lupus MN but not always with other forms of secondary MN although we cannot exclude a coincidence of iMN with the associated disease.

However, more cases of secondary MN should be analysed before reaching a final conclusion as to the exact prevalence of PLA2R-related MN in those patients.

Although the exact role of PLA2R in the pathogenesis of iMN is still not yet well understood, the presence of circulating anti-PLA2R is highly specific for iMN [7, 9, 10]. Several recent studies have shown that anti-PLA2R antibody levels correlate with the clinical manifestations of disease activity, decreasing during a spontaneous or treatment-induced remission and

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**Table 1. Clinical characteristics of iMN patients with and without PLA2R expression in glomerular immune deposits**

<table>
<thead>
<tr>
<th></th>
<th>PLA2R positive</th>
<th>PLA2R negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>45</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>52.6 ± 13.6</td>
<td>55.0 ± 14.3</td>
<td>0.525</td>
</tr>
<tr>
<td>Gender (m:f)</td>
<td>31:14</td>
<td>16:4</td>
<td>0.363</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL (mean ± SD)</td>
<td>1.22 ± 0.58</td>
<td>1.10 ± 0.28</td>
<td>0.409</td>
</tr>
<tr>
<td>Proteinuria, g/24 h (mean ± SD)</td>
<td>10.1 ± 7.6</td>
<td>9.0 ± 6.9</td>
<td>0.571</td>
</tr>
</tbody>
</table>

**FIGURE 2:** Expression of PLA2R in glomeruli. Confocal microscopic analysis of paraffin kidney biopsy specimens show: a very weak expression of PLA2R in normal kidneys podocytes (A); the presence of PLA2R in sub-epithelial deposits along glomerular capillary loops in patient with iMN (one representative image from 45 patients) (B); and its absence in other group of patients with iMN (one representative image from 20 patients) (C); patients with lupus associated MN had no PLA2R in immune deposits (D); patients with secondary MN as hepatitis B and sarcoidosis had PLA2R in immune deposits (E and F).
reappearing during a relapse [7, 9–13]. Changes in circulating levels of anti-PLA2R antibody often precede changes in proteinuria [12, 13]. Because PLA2R antibody titres can fluctuate, the absence of circulating PLA2R antibody at the time of kidney biopsy does not rule out a diagnosis of PLA2R-related MN. Indeed in this study, we found that six patients had no circulating anti-PLA2R antibody although they had PLA2R detected in glomerular immune deposits. These six patients had proteinuria at the time of serum collection (initial disease or relapse). These findings could be explained by the rapid clearance of antibodies from the circulation and deposition in glomeruli, or by late sampling of patients when proteinuria persisted because of severe alteration of the glomerular capillary wall, supporting the hypothesis that proteinuria does not necessarily imply immunological activity. On the other hand, we also identified two patients with circulating anti-PLA2R antibodies but without detectable PLA2R in glomerular deposits. These two cases suggest that antibodies were not pathogenic or that epitopes were poorly accessible at the time of kidney biopsy. Altogether, these results obtained in a Czech cohort validate and contribute to our previous data in a French cohort of patients with iMN [14].

Because in iMN, PLA2R antibodies follow disease activity [11] and usually dramatically decrease in treatment responsive patients [13], the kidney biopsy seems to be of utmost clinical importance for the retrospective diagnosis of PLA2R-related MN. We showed that in the patients sampled at distance from the biopsy at the time of spontaneous or drug-induced remission, the percentage of positive sera was 22% when compared with 59% positive biopsies. Of note, among these patients, the double positive cases for PLA2R antigen in the biopsy and anti-PLA2R antibodies were the patients with partial clinical remission only and a mean proteinuria of 1.99 g/24 h, while the group with positive PLA2R in the biopsy, but no detectable anti-PLA2R antibody at the time of sampling, had a mean proteinuria of 0.65 g/24 h and 9 of the 15 remissions were complete clinical remissions (Table 2, Supplementary appendix). These observations support the concept of a lag time between immunological and clinical remission in iMN, with the antibodies disappearing before proteinuria [12, 13]. The diagnosis

Table 2. PLA2R in glomerular immune deposits and circulating anti-PLA2R autoantibodies in sera sampled at the time of active disease

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>PLA2R</th>
<th>Time from renal biopsy to PLA2R-AB (months)</th>
<th>Proteinuria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biopsy</td>
<td>Serum</td>
<td></td>
</tr>
<tr>
<td>iMN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>−</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>−</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>−</td>
<td>−</td>
<td>0</td>
</tr>
<tr>
<td>iMN relapse or PP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+</td>
<td>16–45</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
<td>−</td>
<td>4–85</td>
</tr>
<tr>
<td>1</td>
<td>−</td>
<td>−</td>
<td>85</td>
</tr>
<tr>
<td>Secondary MN</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HepB1</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>HepB2</td>
<td>+</td>
<td>−</td>
<td>0</td>
</tr>
<tr>
<td>Sarc 1</td>
<td>+</td>
<td>+</td>
<td>5</td>
</tr>
</tbody>
</table>
| PP, persistent proteinuria; HepB, hepatitis B; Sarc, sarcoidosis.

Table 3. PLA2R in glomerular immune deposits and circulating anti-PLA2R antibodies in sera sampled from iMN patients at the time of partial or complete remission

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>PLA2R</th>
<th>Time from renal biopsy to PLA2R-AB (months)</th>
<th>Proteinuria</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biopsy</td>
<td>Serum</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+</td>
<td>3–44</td>
</tr>
<tr>
<td>15</td>
<td>+</td>
<td>−</td>
<td>6–168</td>
</tr>
<tr>
<td>1</td>
<td>−</td>
<td>+</td>
<td>48</td>
</tr>
<tr>
<td>14</td>
<td>−</td>
<td>−</td>
<td>5–164</td>
</tr>
<tr>
<td>7PR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6PR, 9CR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4PR, 10PR</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Because in iMN, PLA2R antibodies follow disease activity [11] and usually dramatically decrease in treatment responsive patients [13], the kidney biopsy seems to be of utmost clinical importance for the retrospective diagnosis of PLA2R-related MN. We showed that in the patients sampled at distance from the biopsy at the time of spontaneous or drug-induced remission, the percentage of positive sera was 22% when compared with 59% positive biopsies. Of note, among these patients, the double positive cases for PLA2R antigen in the biopsy and anti-PLA2R antibodies were the patients with partial clinical remission only and a mean proteinuria of 1.99 g/24 h, while the group with positive PLA2R in the biopsy, but no detectable anti-PLA2R antibody at the time of sampling, had a mean proteinuria of 0.65 g/24 h and 9 of the 15 remissions were complete clinical remissions (Table 2, Supplementary appendix). These observations support the concept of a lag time between immunological and clinical remission in iMN, with the antibodies disappearing before proteinuria [12, 13]. The diagnosis
of PLA2R-related MN is of special interest in patients who will reach end-stage renal failure and benefit from a kidney transplant. Although we could not find a tight correlation between the presence of anti-PLA2R antibodies and recurrence of MN on the kidney graft [15], we think that monitoring would benefit from whether the native kidney MN was related or unrelated to PLA2R. This question can be easily addressed on paraffin sections of archival biopsies.

In secondary MN, pathophysiological mechanisms leading to immune complex deposition are most likely different involving mainly exogenous antigens, which is very much in keeping with the common absence of circulating anti-PLA2R autoantibodies [9, 10]. Consistent with this statement, circulating anti-PLA2R autoantibodies were not detected in lupus MN patients (this study and [16]) except in one Chinese patient [9]. For the first time, we have tested PLA2R in the biopsy of 16 Czech patients with lupus MN, but we failed to detect PLA2R antigen in glomerular immune deposits. Similarly, the 12 French patients with lupus MN from our series had no PLA2R antigen in glomerular deposits (Debiec and Ronco, unpublished observations). These findings strongly suggest that lupus MN is not related to PLA2R.

In contrast, in two cases of hepatitis B virus-associated MN, PLA2R was detected in glomerular immune deposits and in one patient, circulating anti-PLA2R autoantibodies were also present. In addition, we identified the second case of sarcoidosis-associated MN with circulating anti-PLA2R antibodies and PLA2R antigen in immune deposits whereas the five patients with sarcoidosis but without MN were all negative. Although in these cases, coincidental occurrence of iMN with the associated disease cannot be excluded, there is increasing evidence that other autoimmune diseases such as antineutrophil cytoplasmic antibody-associated glomerulonephritis, anti-GBM disease, sarcoidosis and overlap syndrome can coexist with MN [17–20]. Therefore, extended screening for the presence of PLA2R in glomerular immune deposits in those patients is recommended.

Anti-PLA2R autoantibodies or PLA2R antigen in immune deposits were not found in all cases of iMN. The recently described antigens SOD2 and alpha enolase [21, 22] probably do not play a role in our PLA2R negative patients because they were not detected in archival biopsies from those patients (Debiec and Ronco, unpublished). iMN is not a uniform disease and might have different target antigens that have not been identified yet. Moreover, some patients who were negative for PLA2R might have been misclassified as idiopathic when they actually had a secondary form of MN. It would be very interesting to assess on a much larger sample of patients if there is a difference in natural course, long-term outcome, and response to treatment between PLA2R- positive and -negative patients as defined by the presence of antibodies in the serum and/or antigen in the biopsy.

In conclusion, assessment of PLA2R antigen in biopsy specimens is more sensitive than the serological test alone for the diagnosis of PLA2R-related MN, in case of delayed serum sampling. PLA2R staining in glomeruli should be examined in all new biopsies of patients with idiopathic MN if they have negative anti-PLA2R activity in the serum. Search for PLA2R antigen in archival kidney biopsies is also important for the monitoring of patients who will benefit from a kidney transplant. Detection of PLA2R in kidney biopsies can discriminate between iMN and lupus M.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES


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Unilateral renal agenesis: a systematic review on associated anomalies and renal injury

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Keywords: congenital anomalies of the kidney and urinary tract, renal injury, solitary functioning kidney, systematic review, unilateral renal agenesis

ABSTRACT

Background. Unilateral renal agenesis (URA) is associated with other congenital anomalies of the kidney and urinary tract (CAKUT) and extra-renal anomalies. However, the reported prevalences of these anomalies are highly variable. We estimated the prevalence of associated CAKUT and extra-renal anomalies in patients with URA. Furthermore, we determined the prevalence of renal injury in URA patients.

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