Anti-PLA2R Antibodies as a Prognostic Factor in PLA2R-Related Membranous Nephropathy

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Abstract
Background: The natural course of idiopathic membranous nephropathy (MN) varies, as it is known through favorable outcomes in most patients. However, one third of patients with idiopathic MN will slowly progress to end-stage renal disease (ESRD). To prevent disease progression, patients at high risk to develop ESRD are treated with immunosuppressive agents. Therefore, a correct selection of patients who need immunosuppressive treatment is important. Methods: Here, we evaluated the prognostic value of anti-phospholipase A2 receptor 1 antibody (anti-PLA2R) levels regarding clinical outcome in a well-defined cohort of 73 PLA2R-related MN patients with long-term follow-up. At baseline, patients were subdivided into patients with either low or high antibody levels based on ELISA testing. Results: Spontaneous remission rates were highest among patients with low anti-PLA2R levels (79%; hazard ratio 2.72 (95% CI 1.22–6.08), p = 0.02) after a median follow-up of 2.9 (95% CI 0.8–5.0, p < 0.001) years, whereas high anti-PLA2R levels were associated with persistent proteinuria (p = 0.04) and/or the need for immunosuppressive therapy (p < 0.001). Renal survival rates were 97% at 5 years, 93% at 10 years, and 89% at 15 years; however, this was not different between the anti-PLA2R groups. ESRD occurred significantly faster in patients with severe proteinuria as compared to patients with either mild (p = 0.02) or moderate proteinuria (p = 0.05). Conclusions: Low anti-PLA2R levels may predict spontaneous remissions in patients with PLA2R-related MN. Therefore, we suggest that quantification of anti-PLA2R is of value to monitor these patients.

Introduction

Idiopathic membranous nephropathy (MN) is the most common cause of the nephrotic syndrome in the adult Caucasian population [1, 2]. Beck et al. [3] identified a circulating autoantibody reactive with the trans-
membrane glycoprotein M-type anti-phospholipase A2 receptor 1 (anti-PLA2R) on the human podocyte. Anti-PLA2R has been demonstrated to be highly specific for idiopathic MN in which autoantibodies can be found in about 70% of patients [3–5] and hence, these patients can be considered to have PLA2R-related MN.

The natural course of idiopathic MN varies. Spontaneous remissions occur in about 30% of patients, whereas the other two thirds of patients will suffer equally from either persistent proteinuria with long-term preservation of renal function or slow progression to end-stage renal disease (ESRD) [6–8]. To prevent ESRD, patients may be treated with immunosuppressive agents. Since a favorable outcome has been observed in at least 30% of patients treated with ACE inhibitors and/or angiotensin II receptor blockers, that is, ‘conservative therapy’ [9, 10], it is important to predict which patients need immunosuppressive therapy (IST) and which patients will benefit from conservative therapy. Accordingly, the Kidney Disease Improving Global Outcomes (KDIGO) guidelines recommend immunosuppressive agents only in patients at high risk for developing ESRD [11]. Therefore, studies of predictive markers to select patients with idiopathic MN at high risk to develop ESRD are warranted.

We postulate that the level of anti-PLA2R antibodies at the time of renal biopsy may predict the disease course. Therefore, we evaluated retrospectively the prognostic value of these antibodies in a well-defined cohort of PLA2R-related MN patients.

Material and Methods

Patient Cohort

Consecutive patients who underwent renal biopsy between November 1979 and March 2011 in the province of Limburg, the Netherlands, were identified as having idiopathic MN [12]. Only those patients with idiopathic MN and either anti-PLA2R serum reactivity or glomerular PLA2R deposits, that is, patients with PLA2R-related MN, were included. Patients with secondary MN were excluded [12]. According to our Limburg Renal Registry protocol, renal biopsies were obtained as soon as it was feasible. In practice, the time from established proteinuria to renal biopsy was at maximum 6 weeks. Furthermore, we included only those patients for whom baseline serum samples, obtained at the time of renal biopsy, were available.

No IST was allowed before inclusion in this study. Other medications to control blood pressure and proteinuria were allowed. After inclusion, the reason to start IST was generally based on either serum creatinine or the composition of urinary proteins such as IgG, β2-microglobulin, and/or α1-microglobulin [13, 14].

Baseline characteristics that were obtained from all patients included anti-PLA2R status, proteinuria, serum creatinine, and estimated GFR at the time of renal biopsy. From all patients, clinical data were prospectively collected [1, 2]. Medical records were retrospectively reviewed for outcome. Since our previous publication [5], outcome data have been expanded.

The Limburg Renal Registry was approved according to the guidelines of the Helsinki declaration by the local ethics committee of the Maastricht University Medical Center. All patients gave written informed consent for their participation.

Outcome

The primary outcome was the appearance of a spontaneous remission, defined as induction of either a complete or partial remission (PR) without the use of immunosuppressive agents. Secondary outcomes were (i) induced remission, defined as induction of either a complete or PR with the use of immunosuppressive agents; (ii) relapse of proteinuria, defined as recurrent proteinuria within the nephrotic range; (iii) ESRD, defined as the need of renal replacement therapy (i.e. dialysis or renal transplantation), and (iv) mortality.

Complete remission (CR) was defined by proteinuria of <0.2 g/day. PR was defined by proteinuria of <2.0 g/day but ≥0.2 g/day [15, 16].

Quantification of Circulating Anti-PLA2R

Circulating anti-PLA2R antibodies were determined using a commercially available ELISA (EUROIMMUN AG, Lübeck, Germany) that contained PLA2R1-coated microplates [5, 17]. Briefly, plates were incubated with human sera diluted 1:101 in sample buffer (0.05% (w/v) Tween-20, 1% (w/v) casein in PBS) for 30 min. After incubation, antibodies were detected with anti-human IgG horse radish peroxidase conjugate (EUROIMMUN AG) diluted 1:1,000 in sample buffer for 30 min. After washing, chromogen substrate solution (EUROIMMUN AG) was added for 15 min after which the reaction was stopped with the stopping solution. The optical density was read by using an automated microplate absorbance reader (iMark, Bio-Rad, Veenendaal, the Netherlands). Values higher than 2 RU/ml were considered positive [5].

PLA2R Antigen Staining

Renal biopsies from patients without circulating anti-PLA2R were stained for the PLA2R antigen, since the assessment of both is more sensitive than the serological test alone for the diagnosis of PLA2R-related MN [18, 19]. PLA2R was detected in 2 μm frozen sections using rabbit polyclonal anti-PLA2R1 antibodies (Atlas Antibodies, Stockholm, Sweden) at a dilution of 1:200 followed by goat Alexa 488 conjugated anti-rabbit IgG (Life Technologies, Carlsbad, Calif., USA) at a dilution of 1:100. PLA2R staining was considered positive if there was positive granular capillary loop staining in glomeruli and negative if there was no staining in the glomeruli (online suppl. fig. S1; for all online suppl. material, see www.karger.com/doi/10.1159/000437236).

Statistical Analysis

Statistical analysis was performed using SPSS, version 17.0 for Windows (IBM, Chicago, Ill., USA). Differences in continuous and categorical variables were checked using the independent samples t test or Mann–Whitney U test and the chi-square or Fisher’s exact test, respectively. A survival curve was assessed using the Kaplan–Meier method. Differences in estimated survival curves were assessed using the log-rank test. The influence of anti-PLA2R test results was analyzed by Cox regression, where the effect is ad-
justed for potential confounders, such as, age, male gender, rate of proteinuria, and serum creatinine [7]. A 2-sided p ≤ 0.05 was considered statistically significant.

For the analysis, patients were subdivided into patients with either low or high anti-PLA2R levels. The former comprised patients with antibody levels in the lowest tertile and seronegative patients, whereas high anti-PLA2R levels were defined as antibody levels in the highest tertiles. Furthermore, patients were subdivided into patients with either mild (<4 g/day), moderate (4–8 g/day), or severe proteinuria (≥8 g/day) [7, 11].

### Results

#### Patient Characteristics and Treatment

One-hundred and nine patients (69 men and 40 women) were identified as having idiopathic MN. Twenty-one patients were excluded because the lack of sufficient follow-up and/or laboratory data; furthermore, patients without PLA2R-related MN were excluded (online suppl. fig. S2). The mean age ± SD of the remaining 73 patients was 52.4 ± 14.0 years. Sixty-five (89%) out of 73 patients were seropositive. Patients were subdivided based on anti-PLA2R levels (table 1); of note, baseline characteristics did not differ between the patient groups that have been combined into either low or high anti-PLA2R levels (data not shown). Median proteinuria and serum creatinine were 6.7 (interquartile range (IQR) 4.0–9.7) g/day and 86 (IQR 79–109) μmol/l, respectively. Patients with high anti-PLA2R levels had more proteinuria than patients with low antibody levels (p = 0.01). Patients were followed for a median period of 11.3 (IQR 5.4–16.9) years.

All patients received conservative therapy; 59 (81%) patients had been treated with ACE inhibitors and/or angiotensin II receptor blockers. During follow-up, 26 patients had also been treated with immunosuppressive agents, that is, cyclophosphamide (n = 19), cyclosporine (n = 2), prednisolone monotherapy (n = 2), chlorambucil (n = 1), mycophenolate mofetil (n = 1), or rituximab (n = 1). Remarkably, patients with either anti-PLA2R levels in the highest tertiles or severe proteinuria required IST more often than patients with low antibody levels (53 vs. 10%, p < 0.001) or mild proteinuria (54 vs. 16%, p = 0.005), respectively.

#### Remission of Proteinuria

Fifty-two (71%) out of 73 patients showed a remission of proteinuria during follow-up – 23 of these had a PR and 29 a CR (table 2). Cumulative remission rates were 4, 42, and 53% at 1, 3, and 5 years of follow-up, respectively. Thirty-five (67%) out of the 52 remitting patients achieved a remission after immunosuppressive treatment.

Remarkably, spontaneous remission rates were higher in anti-PLA2R seronegative patients as compared to seropositive patients: 7 (88%) and 28 (43%) for the seronegative and seropositive groups, respectively.
tive and positive group, respectively (p = 0.02). Interestingly, in those patients seropositive for anti-PLA2R, spontaneous remissions were significantly more frequently occurring in patients with antibody levels in the lowest tertile compared to patients with anti-PLA2R levels in the highest tertiles: 16 (76%) and 12 (27%), respectively (p < 0.001). In both seronegative patients and patients with antibody levels in the lowest tertile (i.e. patients with low anti-PLA2R levels), spontaneous remissions occurred in 23 (79%) out of 29 patients after a median follow-up of 2.9 (95% CI 0.8–5.0) years, while spontaneous remissions were only observed in 12 (27%) out of 44 patients with anti-PLA2R levels in the highest tertiles (fig. 1a; p < 0.001).

Also, spontaneous remission rates were higher among both patients with mild and moderate proteinuria compared to patients with severe proteinuria: 13 (68%, p = 0.003) and 12 (52%, p = 0.05) vs. 7 (25%) patients, respectively (table 2). Median follow-up until spontaneous remission was 2.7 (95% CI 0.8–4.6, p = 0.02) years and 5.0 (95% CI 0.1–9.9, p = 0.05) years in patients with mild and moderate proteinuria, respectively; this was shorter as compared to patients with severe proteinuria (fig. 1b).

The clinical significance of both anti-PLA2R status and rate of proteinuria was analyzed by a multivariate Cox regression (table 3). Interestingly, a low antibody level at baseline was the most pronounced independent predictor of a spontaneous remission: hazard ratio (HR) was 2.72 (95% CI 1.22–6.08, p = 0.02). Predictive characteristics of anti-PLA2R and proteinuria regarding subsequent spontaneous remission are presented in online supplementary table S1.

**Relapse of Proteinuria**

Relapses occurred in 14 (27%) of 52 patients after a remission was induced, 5 of whom had been previously treated with immunosuppressive agents. At baseline, neither anti-PLA2R status nor rate of proteinuria differed between patients with or without a relapse.

**ESRD and Mortality**

During follow-up, 10 patients required renal replacement therapy after a mean follow-up period of 25.3 ± 1.7 (95% CI 21.9–28.6) years. Eight patients received a renal transplant. Renal survival was 97, 93, and 89% after 5, 10, and 15 years, respectively (fig. 1c, d). Of note, the occurrence of ESRD did not differ between patients treated with immunosuppressive agents and those that were not treated. Twelve patients deceased during follow-up after a mean follow-up period of 24.1 ± 1.8 (95% CI 20.5–27.7) years. Of these patients, 3 patients suffered from ESRD. ESRD was observed in 7 (25%) out of 28 patients with severe proteinuria and 3 (7%) out of 42 patients with either mild or moderate proteinuria (p = 0.08). During follow-up, ESRD occurred earlier in patients who presented with severe proteinuria as compared to patients with mild (p = 0.02) or moderate proteinuria (p = 0.08). During follow-up, ESRD occurred earlier in patients who presented with severe proteinuria as compared to patients with mild (p = 0.02) or moderate proteinuria (fig. 1d; p = 0.05). The clinical significance of severe proteinuria was analyzed by a multivariate Cox regression (online suppl. table S2), which revealed a HR of 15.0 (95% CI 1.03–218.05, p = 0.05).

ESRD was observed in 6 (14%) out of 44 patients and 4 (14%) out of 29 patients with high and low antibody levels, respectively. Half the patients with low anti-PLA2R levels presented with severe proteinuria; data regarding the clinical course have been provided in the online sup-

### Table 2. Outcome data

<table>
<thead>
<tr>
<th>Anti-PLA2R levels</th>
<th>Proteinuria</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>low</td>
<td>mild</td>
<td>moderate</td>
</tr>
<tr>
<td>Patients</td>
<td>29</td>
<td>19</td>
</tr>
<tr>
<td>Spontaneous remission</td>
<td>23a</td>
<td>13a</td>
</tr>
<tr>
<td>CR</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>PR</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Induced remission</td>
<td>1a</td>
<td>16</td>
</tr>
<tr>
<td>CR</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>PR</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Persistent proteinuria*</td>
<td>1b</td>
<td>10</td>
</tr>
<tr>
<td>ESRD*</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

a p ≤ 0.005 low vs. high anti-PLA2R levels, mild/moderate vs. severe proteinuria.
b p ≤ 0.05 low vs. high anti-PLA2R levels, mild vs. severe proteinuria.

*Immunosuppressive agents were used in 5 out of 11 and 2 out of 10 patients with persistent proteinuria and ESRD, respectively.

### Table 3. Multivariate Cox regression analysis of spontaneous remission in PLA2R-related MN

<table>
<thead>
<tr>
<th>HR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low anti-PLA2R levels</td>
<td>2.72 (1.22–6.08)</td>
</tr>
<tr>
<td>Age</td>
<td>1.00 (0.97–1.03)</td>
</tr>
<tr>
<td>Male</td>
<td>0.75 (0.35–1.63)</td>
</tr>
<tr>
<td>Mild proteinuria</td>
<td>Reference</td>
</tr>
<tr>
<td>Moderate proteinuria</td>
<td>0.68 (0.29–1.61)</td>
</tr>
<tr>
<td>Severe proteinuria</td>
<td>0.43 (0.16–1.16)</td>
</tr>
<tr>
<td>Serum creatinine</td>
<td>0.99 (0.98–1.01)</td>
</tr>
</tbody>
</table>
Discussion

In our study, we found that spontaneous remission rates were higher in patients with PLA2R-related MN with low anti-PLA2R levels (i.e. both seronegative and seropositive patients with antibody levels in the lowest tertile) as compared to those patients with antibody levels in the highest tertiles. Remarkably, low anti-PLA2R levels appeared to be the best predictor of a spontaneous remission, whereas spontaneous remissions were less frequently observed in patients with severe proteinuria. Furthermore, patients with high antibody levels were more prone to suffer from persistent proteinuria and were more often treated with immunosuppressive agents, suggesting that those patients may have had a profile that is associated with a high risk to develop ESRD. Our findings therefore suggest that quantification of anti-PLA2R is of clinical relevance.

The clinical relevance of anti-PLA2R in PLA2R-related MN has been addressed in several cross-sectional studies. As observed in our study, antibody levels were found to be associated with the amount of proteinuria [20–23]. Furthermore, we observed that spontaneous remissions occurred frequently in patients with low anti-PLA2R levels. Our data corroborate Hofstra et al. [22] who found that spontaneous remissions were only occasionally observed in patients with high anti-PLA2R titers, whereas in patients with low antibody titers spontaneous remissions
occurred in 38% of patients. Of note, almost half of Hofstra’s patients received immunosuppressive agents and, as a consequence, could not have reached the endpoint of a spontaneous remission, whereas only 10% of our patients with low anti-PLA2R levels received IST. Remarkably, the cumulative incidence of spontaneous remissions in these patients was even 79%, suggesting that spontaneous remissions may occur more frequently than previously thought.

Also, we assessed the presence of the PLA2R antigen in glomeruli of patients without anti-PLA2R serum reactivity at the time of diagnosis. Our results confirm previous studies [18, 19], which demonstrated that renal PLA2R staining increases the sensitivity for the diagnosis of PLA2R-related MN. Although a favorable outcome has been assumed in these patients, data are lacking. In our study, the cumulative incidence of spontaneous remissions was 88%, in seronegative patients with PLA2R-related MN. One patient without anti-PLA2R serum reactivity, however, progressed to ESRD. Remarkably, anti-PLA2R antibodies became detectable and proteinuria persisted during follow-up, providing further evidence that seroconversion can occur in seronegative patients with PLA2R-related MN [24]. Although the overall prognosis of seronegative patients is favorable, the development of circulating antibodies may be associated with a poor prognosis.

Unfortunately, we were not able to collect serum samples at fixed time points during follow-up. Beck et al. [25] and Hoxha et al. [26], evaluated the course of anti-PLA2R in patients with PLA2R-related MN who were treated with immunosuppressive agents and observed that the decrease of anti-PLA2R preceded the decrease in proteinuria. This suggests that proteinuria may be present when autoantibody levels are already undetectable [27]. Because most patients were treated with immunosuppressive agents, these data do not allow us to conclude that antibody levels can help to identify patients who will develop a spontaneous remission. However, Hoxha et al. [26] observed spontaneous remissions in 5 (42%) out of 12 patients who did not receive immunosuppressive treatment. In those patients, anti-PLA2R levels were significantly lower during follow-up as compared to antibody levels in patients not experiencing a remission. Our data suggest that most patients with low or no detectable anti-PLA2R levels have a less active disease and hence, are more prone to develop a spontaneous remission. Therefore, low anti-PLA2R levels may predict spontaneous remissions in PLA2R-related MN.

Another interesting finding of our study is that renal survival during long-term follow-up was favorable (fig. 1c, d). Although only 26 (36%) out of 73 patients were treated with immunosuppressive agents, renal survival was similar to the dialysis-free survival in the intervention groups of the trials by Ponticelli et al. [15] and Jha et al. [16]. As described previously [9, 28], these findings suggest that a substantial number of patients with idiopathic MN do not need IST. On the other hand, patients at high risk to develop ESRD will benefit from IST [29] and therefore, the decision to start therapy should be based on reliable predictors of clinical outcome. In current clinical practice, the decision to start immunosuppressive treatment is mainly based on the rate of proteinuria [11], since severe proteinuria, as observed in the present study, has been associated with ESRD [7]. On the contrary, a substantial number of patients with idiopathic MN and severe proteinuria will develop a spontaneous remission with excellent outcome during long-term follow-up [14, 30]. Because unequivocal results are presented in the literature, the Glomerulonephritis Work Group questioned whether or not anti-PLA2R should be used as guide for treatment decisions in PLA2R-related MN [11].

Our data suggest that measurement and quantification of anti-PLA2R at diagnosis is an easy tool for early identification of patients who will likely develop a spontaneous remission. Because spontaneous remissions could be observed after a prolonged follow-up [31], we suggest that immunosuppressive agents should be withheld in patients with low anti-PLA2R levels and a well-preserved renal function despite the presence of (sometimes prolonged) proteinuria within the nephrotic range. However, because most patients with severe proteinuria did not achieve a spontaneous remission and even more importantly are at risk for ESRD, it is important to emphasize the need for close and careful monitoring of these patients [30]. Of note, late initiation of immunosuppressive treatment in high-risk patients is not associated with worse renal outcome [32], supporting the use of a restrictive treatment strategy and hence, avoiding unnecessary drug exposure risks in those patients who would otherwise develop a spontaneous remission.

In summary, we found that low anti-PLA2R levels at diagnosis are associated with spontaneous remissions in PLA2R-related MN, whereas persistent proteinuria and/or the need for immunosuppressive agents were less frequently observed in these patients. From these data we postulate that combined assessment of circulating anti-PLA2R and proteinuria may be instrumental to monitor disease activity and guide treatment decisions in PLA2R-
related MN. As suggested by Hofstra et al. [33], immunosuppressive agents should be withheld in patients with low antibody levels and a well-preserved renal function, whereas in patients with high antibody levels and/or severe proteinuria (i.e. patients in whom the occurrence of a spontaneous remission is unlikely), closer monitoring of renal function is warranted. To obtain further proof of this hypothesis, prospective trials are, however, needed.

Conclusion

Anti-PLA2R quantification as determined at the time of diagnosis is of predictive value in patients with PLA2R-related MN. Low antibody levels are associated with spontaneous remissions in these patients.

References


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