Analysis of glomerular PLA2R efficacy in evaluating the prognosis of idiopathic membranous nephropathy in the background of different serum anti-PLA2R levels

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Prognostic evaluation of renal PLA2R for membranous nephropathy.

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Abstract

Objective To verify serum PLA2R antibody and glomerular PLA2R antigen expression in membranous nephropathy as well as to explore their relationship with clinical presentation and disease prognosis. *Methods* We retrospectively analyzed 155 patients clinical figures who were diagnosed with primary membranous nephropathy by kidney biopsy. Patients were divided into 6 groups according to their serum PLA2R antibody or glomerular PLA2R antigen positiveness and the level of serum PLA2R antibody titer. Both clinical features and pathological characteristics were recorded, and remission rate as well as time to response were compared among groups. Correlation between clinical figures and titer of PLA2R antibody or semi-quantity of PLA2R antigen were detected. *Results* Among patients with positive serum PLA2R antibody and tissue PLA2R antigen, higher baseline PLA2R antibody levels were associated with lower remission rate and longer remission time. A positive correlation between time to partial remission and serum PLA2R antibody titer was found. Among patients with serum PLA2R antibody titer <150U/L, there were shorter remission time in negative tissue PLA2R antigen group compared with positive tissue PLA2R antigen, and a positive correlation between time to complete remission and semi-quantity of tissue PLA2R antigen was found. *Conclusion* Both glomerular PLA2R antigen and serum PLA2R antibody play a role in disease presentation and prognosis in primary membranous nephropathy. Glomerular PLA2R antigen has a major role on disease prognosis when serum PLA2R antibody titer is less than 150U/L, while serum PLA2R antibody has predominant role in MN prognosis when serum PLA2R antibody titer is above 150 U/L.

What is already known about this topic?

Several studies have shown that the anti-PLA2R antibody titer reflected the activity of idiopathic membranous nephropathy (IMN). Previous studies mainly focused on the antibody status of patients with IMN and showed that the low antibody titer in antibody-positive patients indicated a high disease remission rate, and thus, this antibody can be used as a predictor of clinical efficacy.

What does this article add?

However, there are few studies on glomerular PLA2R antigen in evaluating the prognosis of the disease, especially the relationship between different levels of glomerular PLA2R antigen combined with serum PLA2R antibody and disease risk. Based on the stratification of serum PLA2R antibody levels, our study analyzed the different effects of glomerular PLA2R antigen on the prognosis assessment of IMN, and further explained how to evaluate the disease risk by combining serum PLA2R antibody and glomerular PLA2R antigen.

Previous studies of serum PLA2R antibody for disease prediction mainly used the parameter of remission rate, ignoring the difference of remission time under the same remission rate. Our study compared the difference of remission time between different groups, and more accurately analyzed the evaluation efficacy of glomerular PLA2R antigen on the prognosis of the disease in the background of different serum anti-PLA2R levels.

1. Introduction

Membranous nephropathy (MN) is characterized by the deposition of immune complexes in the subepithelial space and the diffuse thickening of the glomerular basement membrane, which is now the leading cause of nephrotic syndrome in adults[1]. Membranous nephropathy can be categorized as primary and secondary forms according to its pathogeny. About 80% cases are renal limited and described as idiopathic membranous nephropathy (IMN), while other 20% are associated with secondary causes such as systemic autoimmune diseases, infections, malignancies or the chronic consumption of drugs such as NSAIDs and penicillamine[2].The M-type phospholipase A2 receptor (PLA2R), first discovered by Beck, which located on cell surface of podocytes, is the major auto-antigen in most patients with IMN[3]. Serum PLA2R antibodies in adult patients with MN have been widely studied by many nephrologists, which is generally believed that the level of antibody titer are correlated with disease severity and prognosis[4][5][6], in which higher titer predicted more severe proteinuria and less chance to remission. However, some patients with negative serum PLA2R antibodies can have positive glomerular deposits of PLA2R, which received less focus and whether the combination of the two standards plays a role in prediction of the clinical outcome has yet to be explored. So in this study, we intend to verify the correlation between serum anti-PLA2R as well as glomerular PLA2R antigen and clinical manifestation and prognosis.

2. Methods

2.1 Patients

A total of 155 patients with biopsy-proven MN collected between June 2018 and June 2019 were

examined for serum PLA2R antibody and renal tissue PLA2R antigen as baseline data at the same time of biopsy (Figure 1). Patients with secondary MN, including autoimmune diseases (lupus nephritis, rheumatoid arthritis, etc.), infection related MN (HBV-MN), and MN with malignancies or exposure to toxic agents, were excluded. Patients were divided into 6 groups according to their serum PLA2R antibody or glomerular PLA2R antigen positiveness and the level of serum PLA2R antibody titer. Of the included individuals, baseline clinical features at kidney biopsy were compared among 6 groups, including urinary protein excretion, serum albumin level. Of these patients, the proportions of receiving immunosuppressive therapy or supportive therapy were compared between PLA2R-associated and non-PLA2R-associated MN patients whose clinical data were available after 12 months since the biopsy. Remission rate and remission time after the start of immunosuppressive therapy was also compared in patients with data available. Immunosuppressive therapy was commonly started in high-risk patients with severe nephrotic syndrome, proteinuria not responding to supportive therapy or deteriorated renal function. Time to remission was recorded according to the following criteria: Complete remission (CR) was defined as urinary protein excretion <0.5g/day (urine protein-to-creatinine ratio [Upcr] <500mg/g); partial remission (PR) was defined as a 50% or greater reduction from peak values of urinary protein and urinary protein excretion <3.5g/day (Upcr <3500mg/g).

2.2 Measurement of serum PLA2R antibody, detection of PLA2R antigen and IgG Subclasses in renal biopsy

Circulating PLA2R antibodies were measured in 155 patients with IMN simultaneously at the time of biopsy. Serum samples were measured by anti-PLA2R ELISA kit (EUROIMMUN AG, Lübeck, Germany). The results were considered as negative for <20RU/ml and positive for ≥20RU/ml. Renal biopsy specimens were divided and processed for light, immunofluorescence (IF) and electron microscopy (EM) analysis. To detect presence of PLA2R antigen, sections of biopsied tissue were de-paraffinized, hydrated, and heated for 10 minutes at 120°C before being blocked with 10% FBS for 10 minutes. The antigens were then conjugated using a rabbit polyclonal anti–human PLA2R antibody (Atlas Antibodies) followed by an FITC–conjugated swine anti–rabbit IgG antibody (Dako). Renal biopsy specimens were analyzed by three pathologist independently and the final fluorescence intensity value was determined by the average value of the results. Semi-quantity of PLA2R antigen and IgG subclass was calculated by the fluorescence intensity on glomerular staining as follows: negative, 0; very weak, 0.5; weak, 1; moderate, 2; strong, 3.

2.3 Statistical analysis

The statistical analyses were conducted using SPSS version 19.0. As for the data description, continuous variables with symmetric distribution were presented as mean ± standard deviation (SD), while nonnormally distributed variables as medians (25–75% interquartile range). The *t*-test was used for parametric analysis and the Mann-Whitney U-test was used for nonparametric analysis. Categorical variables were described as frequencies or percentages, and the data were analyzed with Pearson's Chi-square test or Fisher's exact test. The differences were considered statistically significant with a $P<0.05$.

3. Results

3.1 Clinical and histopathological differences between anti-PLA2R positive and negative patients with IMN

Clinical differences between serum anti-PLA2R positive and negative patients with IMN were examined (Table 1). Serum anti-PLA2R negative patients had lower urinary protein (*P=0.75*) and higher serum albumin level (*P=0.09*) compared with serum anti-PLA2R positive patients, though the difference was not significant. Besides, there were higher PLA2R antigen (*P=0.048*) and IgG4 (*P=0.057*) expression in glomeruler tissue in serum anti-PLA2R positive group compared with anti-PLA2R negative group.

The patients of serum anti-PLA2R positive were further divided into high titer and low titer group defined by anti-PLA2R titer above or below 150U/L, and it showed lower serum albumin in high PLA2R antibody titer group compared with low PLA2R antibody group (*P=0.032*). However, there was no difference among 3 groups regarding to urinary protein or PLA2R/IgG4 expression in renal tissue.

Correlation of clinical parameters of serum albumin and urinary protein with the serum anti-PLA2R titer was analyzed in patients with primary MN. Analyzed by Spearman's correlation coefficient, It showed a slightly negative correlation between serum albumin and serum anti-PLA2R titer (R=-0.19 *P=0.021*) (Figure 2). However, no correlation was detected between quantity of urinary protein and serum anti-PLA2R titer, and serum anti-PLA2R titer was not correlated to PLA2R antigen expression in tissue.

In order to explore the role of PLA2R antibody in prognosis of membranous nephropathy, we compared remission rate between serum PLA2R antibody positive and negative. As shown in Table 1, there were higher proportion of patients achieving complete remission (40.0% vs 24.4%, *P=0.067*) and partial remission (64.4% vs 52.4%, *P=0.27*) in negative anti-PLA2R group, though the statistic did not reach significance.

3.2 Glomerular PLA2R antigen expression in idiopathic membranous nephropathy

The immunofluorescence of glomerular PLA2R and IgG4 of IMN were shown in Figure 1. The urinary protein levels tended to be lower in glomerular PLA2R antigen negative IMN patients than in positive patients (*P=0.017*), while there was no significant difference with respect to serum albumin between the two groups. Histopathologically, there was higher expression intensity of IgG4 along with PLA2R antigen in glomerular capillary in positive PLA2R group than the negative group (*P=0.007*), but no significant differences were observed in the extent of EM stages.

It showed a positive correlation between urinary protein and the intensity of PLA2R antigen expression in renal tissue, though the correlation coefficient was low but significant (R=0.23, *P=0.004*) (Figure 3). What's more, renal IgG4 expression was positive correlated to PLA2R intensity in renal tissue, (r=0.22, *P=0.006*). The remission rate was not different between 2 groups (64.4% vs 52.4%, *P>0.05*) (Table 2).

3.3 Combination of serum PLA2R antibody and renal tissue PLA2R antigen to evaluate clinical outcome in primary MN

3.3.1 Comparison of remission rates among groups with different baseline levels of serum PLA2R antibody and renal tissue PLA2R

Patients were divided into 6 groups according to their serum PLA2R antibody or glomerular

PLA2R antigen positiveness and the level of serum PLA2R antibody titer, so as to detect the relationship between serum PLA2R antibody or glomerular PLA2R antigen and clinical remission rate. Patients with both negative serum PLA2R antibody and glomerular PLA2R antigen presented with higher complete remission rate compared with other groups (*P<0.05*). Among patients with positive glomerular PLA2R antigen, there was higher remission rate in group of lower serum PLA2R antibody titer (<150U/L) compared with higher titer group (150U/L). Results showed that patients who were non-PLA2R related MN experienced a higher complete remission rate than PLA2R related patients (*P<0.001*) (Table 3).

3.3.2 Comparison of the effects on remission time of serum PLA2R antibody combined with renal tissue PLA2R at different baseline levels

Among 40 patients achieved complete remission, there was shorter time to complete remission in patients with negative tissue PLA2R antigen and positive serum PLA2R antibody but less than 150U/L compared with PLA2R antibody above 150U/L (*P=0.032*). There was shorter remission time in group of serum PLA2R antibody <150U/L and negative PLA2R antigen in renal tissue compared with the group of negative serum PLA2R antibody and positive tissue PLA2R antigen (*P=0.014*). However, no differences were found in partial remission time (Table 4).

3.4 Correlation between time to remission and serum PLA2R antibody or tissue PLA2R antigen

In order to explore the role of PLA2R antibody in prediction of MN prognosis, we further detect whether there is a correlation between serum anti-PLA2R titer or renal tissue intensity and time to remission. Among 74 patients achieved partial remission in proteinuria, a positive correlation was found between time to partial remission and serum anti-PLA2R titer (R=0.25, *P=0.03*) (Figure 4). Among 40 patients achieved complete remission in proteinuria, a positive correlation was found between time to complete remission and semi-quantity of tissue PLA2R antigen. (R=0.385, *P=0.01*) (Figure 5).

4. Discussion

In this retrospective study in a Chinese cohort, we detected positive serum PLA2R antibody or glomerular PLA2R antigen in 77.42% patients with IMN, classified as PLA2R-associated IMN. Our data confirmed that serum or renal PLA2R negative IMN patients, classified as non-PLA2R-associated IMN, were similar in baseline levels of proteinuria and serum albumin compared with PLA2R-associated IMN patients. However, patients with lower titer of serum PLA2R antibody exhibit higher response rate and shorter remission time compared with higher titer PLA2R antibody. Glomerular PLA2R antigen plays a major role on disease prognosis when serum PLA2R antibody titer is low.

It has been reported about the value of either serum PLA2R antibody or tissue PLA2R antigen to diagnosis of primary MN with 50%-80% sensitive and almost 100% specific[7]-[8], now our study displays 75.3% patients with IMN had serum PLA2R antibody or tissue PLA2R antigen and no PLA2R positivity in secondary MN or other primary glomerulus. As regard to patients with no expression in serum or renal tissue, we assume that other antigens are responsible for disease development such as Thrombospondin Type-1 Domain–Containing 7A (THSD7A)[9] or recently recognized Exostosin 1 and Exostosin 2[10]. Among patients diagnosed with PLA2R

related membranous nephropathy, we also observed the discrepancy of the PLA2R positivity between serum and tissue, with 23/97 cases presented positive serum PLA2R and negative PLA2R staining in renal tissue while 14/97 cases presented opposite results. A possible explanation to this divergence is the timing of biopsy in relation to the disease course[Error: Reference source not found][11]. In the former scenario, serum PLA2R antibody may be negative because of the early stage in the disease course, on the other hand, the patients may already achieved immunological remission. The latter scenario is less common, which has been reported earlier[12,13] and the possible explanations may be that these antibodies were not pathogenic or that some specific epitope of PLA2R antigen were poorly detectable in kidney biopsy[14].

While some studies found an association between the degree of proteinuria or serum albumin and serum PLA2R antibody titer at a defined time point, which is consistent to our results[15][16][17][18][19], others found only a weak or no association[20][21][22]. Such variability likely reflects the time lag between immunologic and clinical activity, and indeed, a latency period as long as 8 months has been observed between the presence of PLA2R antibody in serum and the first clinical manifestations of MN.

Several studies demonstrated that strong immunofluorescence staining of glomerular IgG2 is frequently seen with cancer-associated MN, whereas the dominance of deposits of IgG4 generally favors IMN[23][24]. In primary MN, the dominant IgG subclass of PLA2R antibody is IgG4[Error: Reference source not found][25][26], which can be observed co-deposition of PLA2R and IgG4 in renal tissue. Consistent with that conclusion, we observed 49 cases with both deposition of PLA2R and IgG4 in our study. However, the remaining 43 cases showed predominant IgG4 but no PLA2R, which may indicate some other antigen can also bind to IgG4 antibody such as THSD7A[27]. As for cases showed PLA2R but no predominant IgG4 deposition, Hofstra's study [Error: Reference source not found]

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revealed that the anti-PLA2R antibody was not confined to IgG4 subclass in 5-7% cases which may give explanation to this scenario.

Lots of studies had confirmed that low baseline PLA2R antibody levels predict subsequent spontaneous remission[Error: Reference source not found,28], whereas high baseline PLA2R antibody levels are associated with development of nephrotic syndrome in patients with initial non-nephrotic proteinuria and with progressive loss of kidney function[29,30,31]. Consistent to that conclusion, we observed higher complete remission rate in PLA2R antibody titer <150U/L among PLA2R-associated MN. What's innovative of this research is that we observed shorter remission time in serum PLA2R antibody <150U/L and negative PLA2R antigen in renal tissue. Our results indicated that glomerular PLA2R antigen had a major role on disease prognosis when serum PLA2R antibody titer was less than 150U/L, which means with PLA2R antigen intensity in renal tissue increased, the time to clinical remission became longer. However, when serum PLA2R antibody titer was high (150U/L), serum PLA2R antibody played a predominant role in the disease remission. As serum PLA2R antibody titer increased, the time to clinical remission became longer. Further prospective cohort researches are needed to confirm this conclusion.

Moreover, we found a positive correlation between time to partial remission and serum PLA2R antibody titer among patients achieved partial remission in proteinuria. All together, these findings indicated that serum PLA2R antibody titer in primary MN may reflect disease activity and prognosis. The reason why high level of PLA2R antibody titer is associated with inferior prognosis in primary MN had attracted much attention. A study by Seitz[Error: Reference source not found]demonstrated high level PLA2R antibodies were associated with epitope spreading, which means the auto-antibodies reactive with epitopes in PLA2R beyond the immunodominant N-terminal epitope. What's more, epitope spreading is also a risk factor for disease progression, by which serum antibody activity restricted to the cysteine-rich epitope of the PLA2R molecule is associated with a high rate of spontaneous remission, whereas epitope spreading during follow-up associates with worsening of the disease[32][33]. Unfortunately, we did not detect the epitope of PLA2R antibodies in patients with high titer and resistant to therapy.

PLA2R antigen in kidney tissue can be detected in a large proportion of patients who were negative for serum PLA2R antibody and provide a more reliable means by which to diagnose IMN [34][35][36], though there was no agreement on the role of renal tissue PLA2R antigen in prognosis of MN. One research[37] found higher remission rate after immunosuppressant therapy in the PLA2R antigen positive group. On the contrary, another Chinese cohort[38] found higher remission rates in non-PLA2R-associated MN compared with PLA2R-associated MN at both 3rd month and 6th month landmark analysis. We found no differences in remission rate between baseline positive and negative tissue PLA2R antigen group. However, as mentioned above, among patients with serum PLA2R antibody titer <150U/L, our results revealed shorter remission time in negative tissue PLA2R antigen group compared with positive tissue PLA2R antigen, which suggests that negative tissue PLA2R antigen with low titer of serum antibody may be associated with a better response to therapy.

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Only a few research explored the relationship between the semi-quantity of PLA2R antigen in renal tissue and MN prognosis. A Chinese center[39] included MN patients with repeated biopsy found that patients with increasing or stable expression of renal tissue PLA2R in second biopsy tended to achieve no remission or disease relapse. Consistent to their findings, our results revealed a positive correlation between time to complete remission and semi-quantity of tissue PLA2R antigen at baseline. These results confirmed the prognosis role of PLA2R antigen in MN and PLA2R antigen expression in renal tissue may trigger immune response and leads to disease deterioration.

In conclusion, based on the stratification of serum PLA2R antibody levels, our study analyzed the different effects of glomerular PLA2R antigen on the prognostic assessment of IMN, and further explained how to evaluate the disease risk by combining serum PLA2R antibody and glomerular PLA2R antigen. The patients with negative tissue PLA2R antigen and low titer of serum PLA2R antibody seem to have lower proteinuria and higher serum albumin level as well as better response to immunosuppressive therapy. Certainly, our research still exists some limitations. Firstly, it is only a single center clinical research with limited cases, so selection bias could not be avoided. Secondly, we did not detect serial PLA2R antibody titer and had not conducted repeated kidney biopsy, so we could not dynamically monitor the tendency of PLA2R antibody titer and PLA2R antigen expression. Further study of large cohort and basic researches at the molecular level are needed to confirm the pathological role of PLA2R antigen in kidney tissue, so as to provide accurate judgement to the prognosis of the disease as well as provide more effective and individualized therapy for patients with primary membranous nephropathy.

Declarations

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Conflicts of interest

The authors declare that there is no conflicts of interests.

Data Availability Statement

All data generated or analysed during this study are included in this published article and its supplementary information files.

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