Anti-phospholipase A$_2$ receptor antibodies in membranous nephropathy and clinical correlation

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Anti-phospholipase A$_2$ receptor antibodies in membranous nephropathy and clinical correlation

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To anyone I may omitted, my apologies: I appreciated your efforts nonetheless.
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ABSTRACT

Anti-phospholipase A2 receptor antibodies in membranous nephropathy and clinical correlation

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The Graduate School, Yonsei University

(Directed by Professor Shin-Wook Kang)

Circulating autoantibody to M-type phospholipase A2 receptor (PLA2R) is known as an important pathogenic antibody of idiopathic membranous nephropathy (MN) in adults. However, there has been a discrepancy on relationship between anti-PLA2R and clinical disease activity. Previous studies had several flaws including insufficient study subjects and different time-points and methods for anti-PLA2R measurement. In order to overcome the limitations, I measured anti-PLA2R with western blotting using serum samples obtained at the time of kidney biopsy from relatively large number of MN patients. Anti-PLA2Rs were detected in 69 (69.0%) idiopathic MN patients at initial diagnosis. However, the prevalence of the autoantibodies in patients who entered remission after treatment was lower (15.8%) compared with patients in an initial diagnostic phase. There was a significant correlation between anti-PLA2R reactivity and clinical disease activity. Proteinuria and hypoalbuminemia were more severe in patients with anti-PLA2R than those without the autoantibodies (2.95 g/g vs. 6.85 g/g, \( P=0.003 \), 3.1 g/dL vs. 2.5 g/dL).
g/dL, \( P=0.004 \)). Furthermore, these clinical severities were increased proportionally as anti-PLA\(_2\)R levels increased (\( P=0.015 \) and \( P \) for trend <0.001 for proteinuria and hypoalbuminemia, respectively). However, neither presence of anti-PLA\(_2\)R nor anti-PLA\(_2\)R levels showed a significant correlation with clinical outcomes including remission rate and time to remission. In conclusion, anti-PLA\(_2\)R is pathognomonic for idiopathic MN in Korean ethnic group as well, and reflects a clinical disease activity. However, both the presence of anti-PLA\(_2\)R and the levels of anti-PLA\(_2\)R cannot predict the clinical outcomes in current clinical practice.

Key words : phospholipase A2 receptors, membranous nephropathy, autoantibodies
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I. INTRODUCTION

Membranous nephropathy (MN) is one of the autoimmune diseases and a leading cause of nephrotic syndrome in adults¹. In situ formation of immune complexes along the subepithelial space of the glomerular basement membrane is the distinct pathologic feature of MN²-⁴. Recently, M-type phospholipase A₂ receptor (PLA₂R) was identified as the target antigen of autoantibodies in adult idiopathic MN⁵. Several studies have reported that the autoantibodies against PLA₂R are sensitive and specific marker for idiopathic MN. However, there have been discrepancies regarding the relationship between the anti-PLA₂R levels and clinical presentations⁶-¹⁰.

There could be several reasons for the discrepancy. First, the nature of most previous study groups was heterogeneous in terms of the time point of anti-PLA₂R measurement. Thus, comparing the data measured at different phases of the disease could lead to inappropriate interpretation. Second, methods for detection and titration of anti-PLA₂R were different among the studies. Third,
most of previous studies could not include a large number of patients enough to expect a valid generalization.

In addition, the correlation between the autoantibodies and clinical status has not been investigated thoroughly in Asian patients with MN particularly, even though race or ethnic background are associated with the incidence or prognosis of the disease\textsuperscript{11-15}. To overcome these limitations, I measured anti-PLA\textsubscript{2}R levels at the time of kidney biopsy in relatively large number of patients with MN, and explored the correlation between anti-PLA\textsubscript{2}R and clinical disease activity and outcomes.

In this study, I investigated the prevalence of anti-PLA\textsubscript{2}R in Korean idiopathic MN patients using western blotting and validated the reproducibility of the method to detect autoantibodies. And then, I examined the difference of clinical presentation according to the presence and levels of anti-PLA\textsubscript{2}R, and evaluated the possibility that anti-PLA\textsubscript{2}R could be a biomarker to predict clinical outcomes.
II. MATERIALS AND METHODS

1. Study subjects and serum samples

The research was conducted under the approvals of the institutional review boards of Seoul National University Hospital (Seoul, Korea) and Yonsei University Severance Hospital (Seoul, Korea). Biopsy-proven MN patients diagnosed between 2002 and 2011 were evaluated. A total of 100 idiopathic MN patients and 10 secondary MN patients whose serum samples corrected at the time of kidney biopsy were included in this study. To compare the prevalence of anti-PLA2R between active-phase MN patients and patients in remission, additional 19 idiopathic MN patients who had entered remission were also included.

The diagnosis of idiopathic MN was confirmed by the histologic findings including subepithelial electron dense deposits on electron microscopy and a diffuse granular pattern of IgG and C3 staining on immunofluorescence microscopy. Secondary MN was diagnosed in patients who had a suggestive cause of secondary MN including hepatitis B virus, hepatitis C virus, lupus, and malignancy with pathognomonic histologic findings.

2. Clinical data

The clinical information about severity of the disease, treatment, remission, and relapse was collected through the review of medical records. Study subjects were categorized into three groups according to risk for progression
based on clinical characteristics. Low risk was defined as urine protein-creatinine ratio (uPCR) remained less than 4.0 g/g and renal function was normal. Moderate risk and high risk patients were those with uPCR between 4.0-8.0 g/g and normal or near normal renal function, and those with uPCR exceeding 8 g/g and impaired renal function (estimated glomerular filtration rate <60 mL/min/1.73m²), respectively. Remission was defined as proteinuria reduction of 50% or greater from baseline and uPCR less than 3.5 g/g was achieved. Patients with relapsed idiopathic MN were those with uPCR >3.5g/g after some period of remission.

3. Human glomerular extracts

Normal renal cortexes which were part of the radical nephrectomy specimens from patients with renal cell cancer were obtained, with institutional review board approval. Human glomerular protein was extracted as previously described. Briefly, glomeruli were isolated from the kidney with graded sieving using 100, 125, 150 μm sized meshes. The glomerular pellet was homogenized in equal volume of RIPA buffer (150mM NaCl, 1% Triton- X-100, 1% Deoxicholic acid sodium salt, 0.1% SDS, 50mM Tris-HCl, pH 7.5, 2mM EDTA;GenDEPOT, Barker, TX, USA) with complete protease inhibitor cockatil (Roche Applied Science, Indianapolis, IN, USA) on ice. The glomerular homogenate was centrifuged at 12,000 rpm for 20 minutes at 4°C twice and the supernatant was isolated. To remove the contaminated human
IgG, human glomerular extracts (HGEs) were incubated with Puredown Protein G-Agarose (GenDEPOT, Barker, TX, USA). I proved the removal of contaminated human IgG by confirming that there was no detectable band in the immunoblotting with anti-human IgG.

4. Western blotting

Equal amounts of HGEs were loaded into 6% SDS-polyacrylamide gels (PAGE) under nonreducing condition and transferred to Immobilon-FL 0.4 μM polyvinylidene difluoride membranes (Millipore, Bedford, MA, USA). Membranes were incubated in 5% blocking buffer containing 2% bovine serum albumin, and human serum was used as primary antibody at a dilution of 1:100 initially. For the samples with negative results at 1:100, I performed western blotting again at a dilution of 1:25 and confirmed non-reactivity based on the negative results in the decreased dilution of sample. The serum samples with positive results were retested at gradually increased dilutions up to 1:8000. Sheep anti-human IgG4 (The Binding Site, Birmingham, UK) antibodies were used as a secondary antibody, at a dilution of 1:3000. And then, I used peroxidase-conjugated donkey anti-sheep IgG (The Binding Site) antibodies as the detecting antibody, at a dilution of 1:10000. A goat polyclonal antibody against PLA₂R (Sigma-Aldrich, Saint Louis, MO, USA) was used to confirmed the location of the PLA₂R band, at a dilution of 1:400. The labeled proteins were detected by the enhanced chemiluminescence
system (ECLTM PRN 2106; Amersham Pharmacia Biotech, Buckinghamshire, UK). To explore the interrelation between severity of clinical status and anti-PLA2R levels, I categorized the patients into 4 groups according to anti-PLA2R levels. The group 1 (negative) included the patients without anti-PLA2R, and the group 2 (1:100, +) was for the patients whose serum samples were reacted with PLA2R at dilution up to 1:100. The patients whose serum were reacted with PLA2R at more increased dilution up to 1:2000 belonged to the group 3 (1:2000, ++), and the others with positive results at maximally increased dilution up to 1:8000 were categorized to the group 4 (1:8000, +++).

5. Statistical analysis

For the data description, continuous variables with a symmetric distribution were presented as the means (±SD), non-normally distributed variables were expressed as medians (25-75% interquartile range). Student’s t-test and analysis of variance (ANOVA) were used for parametric analysis. Mann-Whitney U test and Kruskall-Wallis test were used for nonparametric analysis. Categorical variables were described as frequencies or percentages, and the data were analyzed with chi-squared tests. All of the statistical analyses were conducted using SPSS, version 19.0 (Chicago, IL, USA).
II. RESULTS

1. Clinical characteristics of idiopathic MN

Table 1 demonstrates clinical characteristics of idiopathic MN patients at the time of kidney biopsy. Average age was 54.7±13.9 years. Mean serum creatinine and eGFR levels were 0.91±0.35 mg/dL and 90±28 mL/min/1.73m², respectively. Mean serum albumin level was 2.7±0.7 g/dL, and median value of uPCR was measured as 6.07 g/g.

2. Prevalence of the anti-PLA₂R in idiopathic MN

I examined the anti-PLA₂R reactivity with western blotting, and confirmed the approximately 185 kD sized protein band using native human glomerular PLA₂R and serum samples from MN patients (Figure 1). A total of 69 out of 100 idiopathic MN (69.0%) patients had autoantibodies against PLA₂R at the time of kidney biopsy. The prevalence of anti-PLA₂R was 80.0% (60 of 75) in idiopathic MN patients who had nephrotic ranged proteinuria at diagnosis.

I compared the anti-PLA₂R reactivity between patients in diagnostic stage (n=100) and those in remission (n=19) after treatment. While large number of idiopathic MN patients had autoantibodies against PLA₂R in diagnosis, the prevalence of anti-PLA₂R reactivity was significantly lower (15.8%) in patients who achieved remission after treatment and remained steady state.
Table 1. Baseline clinical characteristics of idiopathic MN

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Anti- PLA₂R(-)</th>
<th>Anti- PLA₂R(+)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>100</td>
<td>31</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>54.7±13.9</td>
<td>53.8±16.5</td>
<td>55.1±12.7</td>
<td>0.697</td>
</tr>
<tr>
<td>Male gender (N/%)</td>
<td>51/53.0%</td>
<td>13/41.9%</td>
<td>40/58.0%</td>
<td>0.137</td>
</tr>
<tr>
<td>Serum creatinine (mg/dL)</td>
<td>0.91±0.35</td>
<td>0.90±0.35</td>
<td>0.91±0.35</td>
<td>0.979</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>90±28</td>
<td>91±35</td>
<td>91±25</td>
<td>0.921</td>
</tr>
<tr>
<td>Serum albumin (g/dL)</td>
<td>2.7±0.7</td>
<td>3.1±0.9</td>
<td>2.5±0.6</td>
<td>0.004</td>
</tr>
<tr>
<td>Proteinuria, uPCR (g/g)</td>
<td>6.07 (3.17-9.86)</td>
<td>2.95 (1.14-9.09)</td>
<td>6.85 (4.87-9.98)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Data are expressed as means±SD or median (25-75% interquartile range).
Abbreviations: eGFR, estimated glomerular filtration rate calculated using Modification of Diet in Renal Disease formula[^10]; uPCR, urine protein-creatinine ratio; Anti-PLA₂R, anti-phospholipase A2 receptor antibody; N, number of patients.
Figure 1. Detection of anti-PLA₂R in serum of idiopathic MN patients using western blotting. HGEs were electrophoresed and serum samples of idiopathic MN patients were used as primary antibody at 1:100. The band that was detected by immunoblotting with commercial anti-PLA₂R antibody was used as a reference to the position of PLA₂R. At the position of approximately 185Kd, serum samples from a number of idiopathic MN patients (except MN5, MN9, and MN10) as well as commercial anti-PLA₂R antibody showed positive bands.

3. Prevalence of the anti-PLA₂R in secondary MN

The causes of secondary MN were HBV (n=6), HCV (n=1), malignancy (n=2), and systemic lupus erythematosus (n=1). The anti-PLA₂R reactivity was considerably lower in secondary MN patients compared with idiopathic MN showing that only 2 of 10 (20%) patients had autoantibodies to PLA₂R. Anti-PLA₂R antibodies were detected in HBV-associated and malignancy-associated MN patients.

4. The relationship between clinical status and anti-PLA₂R reactivity/levels

There were significant correlations between anti-PLA₂R reactivity and clinical parameters including serum albumin and proteinuria in idiopathic MN patients (Table 1). Proteinuria was more severe in the patients with anti-PLA₂R
compared with those without anti-PLA2R (uPCR, 2.95 g/g vs. 6.85 g/g, \( P=0.003 \)). Initial serum albumin levels were significantly much lower in the patients with anti-PLA2R than in the patients without anti-PLA2R (3.1g/dL vs. 2.5 g/dL, \( P=0.004 \)). However, there was no significant difference in renal function between the groups.

To investigate whether clinical disease activities are correlated with anti-PLA2R levels quantitatively, I compared the clinical parameters among the groups categorized by anti-PLA2R levels. As indicated in Figure 2, proteinuria and hypoalbuminemia were more severe as the anti-PLA2R levels increased. Moreover, there was a significant difference in proportion of groups stratified by risk for progression (Figure 3). The proportion of high risk group was the most dominant in group with the highest anti-PLA2R levels, and the reverse was observed in the lowest group.
Figure 2. Levels of proteinuria and serum albumin according to anti-PLA₂R levels in idiopathic MN patients. Proteinuria and hypoalbuminemia were gradually more severe as the anti-PLA₂R levels increased.
**Figure 3.** Proportion of patients categorized by risk for progression according to the anti-PLA2R levels. The proportion of high risk group was gradually increased as the anti-PLA2R increased to a higher level.

5. **The relationship between clinical outcomes and anti-PLA2R reactivity/levels**

To address whether anti-PLA2R could predict the clinical outcomes of idiopathic MN patients, I investigated clinical course of patients. For this analysis, I included 77 out of 100 idiopathic MN patients who had been followed up after kidney biopsy and had available clinical information including treatment response and relapse. Median follow-up time was 30 months, and 67.5% of the patients received immunosuppressive treatment (Table 2). A total of 81.8% patients entered remission state and the median time
to remission was 2.0 months.

However, neither remission rate nor time to remission was significantly different between patients with anti-PLA₂R and those without autoantibody. Furthermore, these clinical outcomes were not different among the groups with different levels of anti-PLA₂R either (Table 3)
<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Anti-PLA₂R(-)</th>
<th>Anti-PLA₂R(+)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>77</td>
<td>21</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>Immunosuppressive treatment (N/%)</td>
<td>52/67.5%</td>
<td>14/66.7%</td>
<td>38/67.9%</td>
<td>0.921</td>
</tr>
<tr>
<td>Remission rate (N/%)</td>
<td>63/81.8%</td>
<td>18/85.7%</td>
<td>45/80.4%</td>
<td>0.746</td>
</tr>
<tr>
<td>Treatment-induced (N/%)</td>
<td>46/73.0%</td>
<td>13/72.2%</td>
<td>33/73.3%</td>
<td></td>
</tr>
<tr>
<td>Spontaneous (N/%)</td>
<td>17/27.0%</td>
<td>5/27.8%</td>
<td>12/26.7%</td>
<td></td>
</tr>
<tr>
<td>Time to remission (months)</td>
<td>2.0(1.0-4.0)</td>
<td>2.0(1.0-5.5)</td>
<td>2.0(1.0-4.0)</td>
<td>0.580</td>
</tr>
<tr>
<td>Relapse (N/%)</td>
<td>7/9.1%</td>
<td>2/9.5%</td>
<td>5/8.9%</td>
<td>1.000</td>
</tr>
</tbody>
</table>

Data are expressed as frequencies and percentages for categorical variables and median (25-75% interquartile range) for continuous variable.

Abbreviations: Anti-PLA₂R, anti-phospholipase A2 receptor antibody; N, number of patients
### Table 3. Clinical outcomes of idiopathic MN patients according to anti-PLA2R levels

<table>
<thead>
<tr>
<th>Anti-PLA2R levels</th>
<th>Remission</th>
<th>Spontaneous remission</th>
<th>Time to remission (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (Negative) (N/%)</td>
<td>18/85.7%</td>
<td>5/27.8%</td>
<td>2.0 (1.0-5.5)</td>
</tr>
<tr>
<td>1:100&lt;sup&gt;a&lt;/sup&gt; (N/%)</td>
<td>10/90.9%</td>
<td>5/50.0%</td>
<td>3.0 (1.3-4.0)</td>
</tr>
<tr>
<td>1:2000&lt;sup&gt;b&lt;/sup&gt; (N/%)</td>
<td>16/72.7%</td>
<td>3/18.8%</td>
<td>1.5 (1.0-4.8)</td>
</tr>
<tr>
<td>1:8000&lt;sup&gt;c&lt;/sup&gt; (N/%)</td>
<td>19/82.6%</td>
<td>4/21.1%</td>
<td>2.0 (1.0-5.3)</td>
</tr>
<tr>
<td>P value</td>
<td>0.540</td>
<td>0.380</td>
<td>0.895</td>
</tr>
</tbody>
</table>

Abbreviations: Anti-PLA2R, anti-phospholipase A2 receptor antibody; N, number of patients

<sup>a</sup>Group of patients whose serum samples showed anti-PLA2R reactivity at dilutions of up to 1:100 with negative results at over 1:100

<sup>b</sup>Group of patients whose serum samples showed anti-PLA2R reactivity at dilutions of up to 1:2000 with negative results at over 1:2000

<sup>c</sup>Group of patients whose serum samples showed anti-PLA2R reactivity at dilutions up to 1:8000
6. Changes in anti-PLA₂R reactivity in follow-up samples

To investigate whether anti-PLA₂R reactivity might be changed in accordance with the disease activity, I performed western blotting with follow-up serum samples and compared the results with the ones from the test that had been conducted with the serum specimens obtained at diagnosis. Four of 6 patients who had had autoantibodies in diagnosis entered remission state, and the autoantibodies were disappeared in 3 of 4 patients in remission (Table 4). However, anti-PLA₂R antibodies were still persistent in patients who did not achieved remission. The anti-PLA₂R reactivity was continued to be native irrespective of remission in all of 4 patients who had no autoantibodies at the beginning.
Table 4. Changes in anti-PLA₂R reactivity in follow-up samples (n=10)

<table>
<thead>
<tr>
<th>Patient</th>
<th>Presentation</th>
<th>Remission</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proteinuria (g/g)</td>
<td>Anti-PLA₂R reactivity</td>
<td>Proteinuria (g/g)</td>
</tr>
<tr>
<td>1</td>
<td>5.48</td>
<td>Positive</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>9.86</td>
<td>Positive</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>13.17</td>
<td>Positive</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>12.22</td>
<td>Positive</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>4.25</td>
<td>Positive</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>7.11</td>
<td>Positive</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>5.60</td>
<td>Negative</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>5.38</td>
<td>Negative</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>5.85</td>
<td>Negative</td>
<td>No</td>
</tr>
<tr>
<td>10</td>
<td>13.20</td>
<td>Negative</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Abbreviations: Anti-PLA₂R, anti-phospholipase A2 receptor antibody
IV. DISCUSSION

I explored the presence of anti-PLA$_2$R in Korean idiopathic MN patients using western blotting, and showed that 69% patients had anti-PLA$_2$R in their serum. This finding is comparable to previous reports performed in idiopathic MN patients of different races and ethnicities$^{5-7, 10}$. In particular, the reactivity of 80.0% in idiopathic MN patients with nephrotic syndrome was similar with that in Chinese idiopathic MN patients$^6$. These results confirm that PLA$_2$R is an important target antigen of idiopathic MN also in Korean ethnic group as in other groups. Although anti-PLA$_2$R has been known as specific marker for idiopathic MN, the autoantibodies were detected in 2 of 10 secondary MN patients in this study. However, both of the patients showed pathognomonic findings such as subepithelial deposits and IgG4 staining in their kidney biopsy specimens, suggesting the possibility that idiopathic MN and hepatitis B or malignancy were occurred concurrently in these cases.

In addition, the present study demonstrated that anti-PLA$_2$R reflects disease activity in idiopathic MN. Indeed, the prevalence of anti-PLA$_2$R in patients who were just diagnosed with idiopathic MN and had not yet received any treatment was higher than in those who had achieved remission already. Moreover, anti-PLA$_2$R which had been detected at the time of initial diagnosis disappeared when the patient entered remission, but the autoantibodies were still persistent in case of non-remission state. Furthermore, anti-PLA$_2$R levels are in direct proportion to initial clinical parameters of serum albumin levels and proteinuria.
Taken together, these findings support that the presence of anti-PLA2R is essential to trigger the disease and reflects the disease activity. Though these findings have been reported in a few previous studies\(^7,9\), they were from small number of patients, non-homogenous group composed of patients with time interval between renal biopsy and sampling, and all of them were conducted in Caucasians. Being distinctive from previous studies, I conducted the study in comparatively large number of patients and they were almost in similar phase of disease course of the diagnostic stage. Therefore, these results might make sure the conclusion with more rationale and extend it to Asian MN patients.

Unfortunately, however, this study did not show significant difference in remission rate or time to remission according to the anti-PLA2R levels. There could be several limitations to interpret this data considering the following flaws. First, overall remission rate was high as 67.5\% and median time to remission was short as 2 months. In such circumstance, it is hard to expect a significant difference between groups. Second, the rate of immunosuppressive treatment was also high (67.5\%), which made it difficult to observe a clinical course of the disease according to the anti-PLA2R levels excluding an effect of immunosuppressive treatment. Therefore, I cannot evaluate the effect of solely anti-PLA2R on the outcomes. Nevertheless, I could notice an important finding from the results. This data showed that most of the patients who had immunosuppressive treatment achieved remission in a short time even though they had different severities with different levels of autoantibodies initially.
From the results, I could infer that an effect of intervention such as immunosuppressive treatment is so enormous, anti-PLA₂R levels cannot influence the outcomes in spite of it determines the disease activity.

In former studies, anti-PLA₂Rs were not detected in all patients with idiopathic MN. One of the possible explanations was that autoantibodies might be measured in immunologically inactive stage after spontaneous remission or immunosuppressive treatment, because serum samples had not been collected in conjunction with renal biopsy. To avoid this problem, I measured anti-PLA₂R using the serum samples that had been collected at the time of renal biopsy. Nevertheless, anti-PLA₂R was not detected in approximately 30% of idiopathic MN patients in this study. This finding suggests that alternative target antigens other than PLA₂R may contribute to the pathogenesis of idiopathic MN in part. In addition, the data derived from the tests using follow-up serum samples showed that anti-PLA₂R were continued to be negative in idiopathic MN patients who had no autoantibodies at the beginning, irrespective of remission status. Such result could be an evidence of above-mentioned hypothesis as well.

Moreover, up to date, several investigators have reported other possible target antigens involved in the development of idiopathic MN²⁰⁻²³. For an additional possibility, we cannot rule out the case that, in a quantitative respect, the anti-PLA₂Rs were present sufficiently to trigger the disease, but not enough to be detectable under current method. In other words, technically the current method might not be as sensitive as enough to detect the trace amount of autoantibodies.
V. CONCLUSION

In conclusion, I confirmed anti-PLA2R is a specific marker of idiopathic MN in Korean patients as well, and the autoantibodies are important to initiate a development of the disease. Additionally, anti-PLA2R levels reflect disease activity showing the worsening trend in clinical symptoms according the antibodies levels. Consequently anti-PLA2R could be a useful biomarker to diagnose the disease and to monitor a disease activity. However, the present study does not show a significant difference of clinical outcomes such as remission rate and time to remission in accordance with anti-PLA2R levels, indicating the autoantibodies could not be a practical marker to predict clinical outcomes or determine therapeutic strategies yet in current clinical practice.
REFERENCES


ABSTRACT(IN KOREAN)

막성사구체신염 환자에서 Antiphospholipase A₂ receptor antibodies와 임상상과의 관계

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Phospholipase A₂ receptor (PLA₂R)에 대한 자가항체는 성인막성사구체신염의 주요 병인으로 알려져 있다. 이전의 여러 연구들에서 항 PLA₂R 항체와 질병의 활성도 사이의 관계에 대하여 보고한 바가 있으나, 서로 일치되지 않는 결과를 보여줌으로 여전히 논란의 여지가 남아있다. 이러한 일관되지 못한 결과는 이전 연구들이 가지고 있는 제한점들에서 기인하였을 것으로 생각되며, 이전의 문제점을 최소화 하고자, 본 연구자는 막성사구체신염환자의 신장 조직검사 당시의 혈청 검체를 이용하여 western blotting을 시행함으로써 혈증의 항 PLA₂R 항체를 측정하였다. 총 100명의 일차성 막성사구체신염 환자 중 69명 환자의 진단 시 혈액에서 항PLA₂R 항체가 검출되었다. 항 PLA₂R 항체의 양성률은 치료 후 안정적으로 관해기를 유지하고 있는 환자에서 15.8%로 치료 전 진단단계에 있는 환자들에 비해 유의하게 낮았다. 자가항체를 가지고
있는 환자에서 단백뇨 (2.95 g/g vs. 6.85 g/g, \( P=0.003 \))와 저혈소단백증 (3.1 g/dL vs. 2.5 g/dL, \( P=0.004 \))이 유의하게 심하였으며, 이러한 임상증상은 자가항체 level이 높을수록 더욱 심해지는 양상을 보였다. 그러나, 관해율과 관해기까지의 기간과 같은 임상경과와 항 PLA\(_2\)R 항체의 발현 및 항체역가 사이에는 통계적으로 유의한 관련성이 없었다. 결론적으로, 항 PLA\(_2\)R 항체는 일차성 막성사구체신염의 특징적인 표지자로 이는 한국인이 막성사구체신염 환자에서 질환발생의 주요 요인으로 작용함과 본 질환의 임상양상과 유의한 연관성을 가지는 것으로 확인되었다. 그러나 항 PLA\(_2\)R 항체는 본 질환의 향후 환자의 임상경과를 예측 할 수는 없음으로 사료된다.

핵심되는 말 : phospholipase A2, 수용체, 사구체신염, 막성, 자가항체