Research Article

Anti-Phospholipase A2 Receptor Antibody Expression at Different Stages of Idiopathic Membranous Nephropathy

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The significance of blood anti-phospholipase A2 receptor (PLA2R) antibodies in the diagnosis of different stages of idiopathic membranous nephropathy (IMN) was investigated. The expression and distribution of anti-PLA2R antibodies in renal biopsy tissue of patients with different stages of IMN were examined by immunohistochemistry. In addition, blood anti-PLA2R antibodies were determined by indirect immunofluorescence for the same patients, and the results were compared with the anti-PLA2R antibody expression in renal biopsy tissue. The positive fluorescence intensities of IMN stages I, IV, and V were mostly ± or + (40/80). There was no significant difference in fluorescence titer between these stages (p > 0.05). These results were consistent with the immunohistochemistry results, and the kappa statistic was 0.95. The positive fluorescence intensities of IMN stages II and III were mostly ++ to ++++ (33/60). There was no significant difference in fluorescence intensities between these two stages (p > 0.05), but there was a significant difference in fluorescence intensities between stages II and III and stages I, IV, and V (p < 0.001). These results were consistent with the immunohistochemistry results, and the kappa statistic was 0.97 (p < 0.001). Therefore, blood anti-PLA2R levels were positively correlated with anti-PLA2R expression in renal biopsy tissue in patients with different stages of IMN. In addition, the fluorescence intensities of IMN stages II and III were significantly different from those of stages I, IV, and V. Therefore, blood anti-PLA2R levels can be used for in vitro differential diagnosis and the monitoring of treatment, as it can distinguish stage II; and III; from stage I, IV, and V IMN.

1. Introduction

Idiopathic membranous nephropathy (IMN) is a common primary glomerular disease characterized by massive proteinuria or nephrotic syndrome [1]. It is associated with long-term risk of renal failure, as well as thrombosis and cardiovascular complications. Typical pathological features are glomerular subepithelial deposition and intramembranous immune complex deposition, with segmental or global sclerosis. Immunopathological examination shows fine granular deposition of IgG and C3 along the basement membrane. Electron microscopy shows electron-dense deposits in different parts of the basement membrane. The basement membrane lesions are classified into five stages of severity.

(i) Stage I: the glomerular lesions are mild. PASM staining shows ribbon-shaped vacuolar degeneration of the basement membrane, similar to minimal change glomerulopathy, and sometimes Masson staining shows a small amount of subepithelial eosinophilic deposits.

(ii) Stage II: diffuse thickening of the basement membrane. Masson staining shows a large amount of subepithelial eosinophilic deposits, and PASM staining shows spikes projecting from the basement membrane.

(iii) Stage III: severe diffuse thickening of the basement membrane, stenosis of the capillary lumen, and mild to moderate diffuse proliferation of mesangial cells and stroma; segmental or global sclerosis is seen in severe cases. Masson staining shows a large number of eosinophilic deposits in the basement membrane, and PASM staining shows double-track or ring-like changes of the basement membrane.
(iv) Stage IV: there are two types of changes. (i) Immune complex deposition stops, and deposited immune complexes are gradually absorbed; most cases are advanced from stage I or II; only irregular thickening of the basement membrane is seen. Masson staining shows a small number of eosinophilic deposits, and PASM staining shows segmental thickening of the basement membrane. (ii) A deterioration from stage III, manifested as global and segmental sclerosis.

(v) Stage V: the glomeruli return to normal [2].

Before the identification of the autoantigen phospholipase A2 receptor (PLA2R) in 2009, IMN was mainly diagnosed by renal biopsy, histological examination, or electron microscopy [3]. However, histopathological examination alone cannot fully distinguish IMN from membranous nephropathy (MN) from other causes. In addition, it is often difficult to distinguish between the different stages due to objective factors such as material selection. Moreover, as histopathological examination is an invasive procedure, many patients do not receive timely diagnosis due to contraindications to biopsy, psychological disorders, or other reasons and consequently do not receive timely standard treatment. In the present study, we explored the significance of the correlation between anti-PLA2R in blood and anti-PLA2R expression in renal biopsy tissue for each stage. Indirect immunofluorescence was determined using EU90 cells coated with non-transfected and transfected PLA2R. The kit was purchased from EURO-IMMUN Medical Laboratory Diagnostics Stock Company (Germany) and was used as a semi-quantitative method according to the instructions given: titer 1:10 equals ±, titer 1:32 equals +, titer 1:100 equals ++, titer 1:320 equals ++, and titer 1:1000 equals ++++.

The sensitivity and specificity of the results for each group were calculated. Chi-square and kappa agreement tests were performed using SPSS 18.0. The positive, negative, and total accuracy were calculated.

3. Results

The expression of anti-PLA2R in renal biopsy tissue and the anti-PLA2R fluorescence intensity in blood are shown in Table 1.

The positive fluorescence intensities of stages I, IV, and V were mostly ± to + (40/80). There was no significant difference in fluorescence titer between these stages (p > 0.05). These results were consistent with immunohistochemistry, and the kappa statistic was 0.95 (p < 0.01) (Table 2).

The positive fluorescence intensities of stages II and III were mostly ++ to ++++ (33/60). There was no significant difference in fluorescence titer between the two stages (p > 0.05), but there was a significant difference with those of stages I, IV, and V (p < 0.01) (Table 3). These results were consistent with the immunohistochemistry, and the kappa statistic was 0.97 (Table 4).

4. Discussion

IMN is an autoimmune disease affecting specific organs. It is a common cause of nephrotic syndrome in adults and can occur at any age. It was previously thought that all cases of immune complex nephritis were due to passive deposition of circulating immune complexes in the glomerulus. However, research in recent years has changed this understanding. It has been demonstrated in an animal model of IMN that circulating antibodies binding to podocyte antigens led to in situ immune complex deposition on the epithelial side [4].

PLA2R is a type I transmembrane receptor and one of the four members of the mammalian mannose receptor family. The N-terminus of PLA2R is a cysteine-rich domain. Adjacent to the N-terminus is a fibronectin type II domain and eight tandem C-type lectin-like domains (CTLD). The C-terminus in the cytoplasm contains structures that interact with the endocytic apparatus, which may be involved in the transport of extracellular ligand molecules into the cell. PLA2R is mainly divided into two types: M-type and
N-type. It has been shown that M-type PLA2R is the main target antigen of autoantibodies [5].

In IMN patients, anti-PLA2R in blood binds to a conformation-dependent epitope of PLA2R to form in situ immune complexes deposited on the glomerular basement membrane, causing damage to podocytes, thickening of the glomerular basement membrane, and changes in permeability [6]. PLA2R is highly expressed in glomerular podocytes and co-localizes with IgG4 in the glomerular immune complex deposits of IMN patients. Anti-PLA2R antibodies in IMN patients are predominantly of the IgG4 subtype, which is the predominant immunoglobulin subclass in glomerular deposits. IgG eluted from deposits of IMN patients recognizes PLA2R. Thus, PLA2R is a major antigen leading to IMN. In 2009, Beck et al. [7] detected M-type PLA2R in 26 of 37 patients with IMN using Western blotting, suggesting that detection of anti-PLA2R in circulating blood is likely to play an important role in diagnosing and monitoring IMN activity. IgG4 is the most important subtype of anti-PLA2R in IMN, which is different from secondary MN (e.g. lupus MN or malignancy-associated MN), where the main subtypes are IgG2 and IgG3. It has been shown that the anti-PLA2R titer is related to IMN activity and can also be used as an indicator of therapeutic efficacy [8].

It has been reported that immunohistochemistry can be used to detect anti-PLA2R [9]. Qin et al. [10] suggested that anti-PLA2R has high specificity for IMN, and its antibody titer in blood can be used to measure the therapeutic efficacy and prognosis of patients. Zhou et al. [11] suggested that anti-PLA2R may be specific pathogenic antibodies of IMN. Guan et al. [12] found a high serum anti-PLA2R antibody positive rate in IMN patients and suggested that combined detection of serum anti-PLA2R antibodies and glomerular IgG4 subtype is helpful for the diagnosis and differential diagnosis of MN etiology. Indirect immunofluorescence using transfected cells has been reported to be a convenient and reliable method for hematological screening for anti-PLA2R [9]. It is suitable for the qualitative and quantitative analysis of anti-PLA2R [13–17].

Hofstra et al. [18] suggested that anti-PLA2R levels can be used to monitor IMN activity and treatment outcomes. Anti-PLA2R is a useful biomarker for the diagnosis of primary MN and can be used to monitor disease activity and predict remission or relapse. Anti-PLA2R positivity was observed in 50–80% of MN patients, depending on disease activity. The sensitivity of anti-PLA2R detection depends on the biological and population differences of the disease rather than the specificity of the analytical method. Hence, anti-PLA2R positivity is diagnostic for patients with contraindications to renal biopsy, such as those on anti-coagulation to treat thrombosis, or those with only one kidney (solitary kidney).

In the early stages of disease, when anti-PLA2R antibodies are produced and bind to antigens on podocytes, immune complex deposits containing anti-PLA2R antigen-antibody complexes are formed, which induces podocyte damage and proteinuria. At this time, serum anti-PLA2R cannot be detected by stable methods because the kidneys act as a sink to absorb all circulating antigen-antibody complexes. In these cases, definitive diagnosis can only be made by the detection of PLA2R deposits in immunofluorescent stained renal biopsy tissue. With the development of immune activity and disease, renal absorption of anti-PLA2R antibodies gradually ceases, and serum anti-PLA2R can be detected and used as a tool to diagnose and monitor disease activity. When a patient enters remission, anti-PLA2R disappears from the serum, although proteinuria is not yet resolved. At this time, immune complex deposits are still detected in renal biopsy tissue [19].

### Table 1: Anti-PLA2R expression in tissue and anti-PLA2R fluorescence intensity in blood.

| Group              | Anti-PLA2R expression in tissue | Anti-PLA2R fluorescence intensity in blood |
|--------------------|--------------------------------|------------------------------------------|
|                    | −  ±  +  ++  +++  −  ±  +  ++  +++ |                                          |
| Stage I (20)       | 6  6  8  0  0  5  6  9  0  0     |                                          |
| Stage II (20)      | 2  1  1  8  8  1  0  2  9  8     |                                          |
| Stage III (20)     | 2  0  1  8  9  2  0  1  9  8     |                                          |
| Stage IV (20)      | 6  6  8  0  0  5  6  9  0  0     |                                          |
| Stage V (20)       | 8  8  4  0  0  8  9  3  0  0     |                                          |
| Control group (20) | 19  0  1  0  0  19  0  1  0  0    |                                          |
| Normal group (20)  | —  —  —  —  —  20  0  0  0  0     |                                          |
| Total              | 43  21  21  21  21  60  21  25  25  16 |                                          |

### Table 2: Correlation of blood anti-PLA2R levels with renal biopsy levels of stages I, IV, and V.

| Blood          | Renal biopsy | Positive (± to +) | Negative | Total |
|----------------|--------------|------------------|----------|-------|
| Positive (± to +) | 41           | 0                 | 41       |       |
| Negative        | 2            | 37                | 39       |       |
| Total           | 43           | 37                | 80       |       |

$x^2 = 0.50, p > 0.05; \kappa = 0.95, p < 0.001.$

### Table 3: Comparison of blood anti-PLA2R fluorescence intensity between stages II and III and stages I, IV, and V.

| Group              | ± to +  | ++ to +++ | Total |
|--------------------|---------|-----------|-------|
| Stages I, IV, and V| 42      | 0         | 42    |
| Stages II and III  | 3       | 34        | 37    |
| Total              | 45      | 34        | 79    |

$x^2 = 68.76, p < 0.001.$

### Table 4: Correlation of blood anti-PLA2R levels with renal biopsy levels of stages II and III.

| Blood          | Renal biopsy | Positive (++) to (+++) | Negative | Total |
|----------------|--------------|------------------------|----------|-------|
| Positive (++) to (+++) | 33           | 0                      | 33       |       |
| Negative        | 1            | 26                     | 27       |       |
| Total           | 34           | 26                     | 60       |       |

$x^2 = 0.00, p > 0.05; \kappa = 0.97, p < 0.001.$
Numerous studies conducted over the past 3 years have shown that anti-PLA2R levels correlate with urinary protein excretion and disease activity. Antibody levels are undetectable during spontaneous remission or treatment-induced remission and reappear or increase during relapses. Antibody levels also predict prognosis, as high antibody titers are associated with a reduced risk of spontaneous remission or immunosuppression-induced remission and an increased risk of nephrotic syndrome and worsening renal function in non-nephrotic patients. The interval between initiation of immunosuppressive therapy and remission was significantly increased in patients in the group with the highest antibody titers. In response to this issue, a research team led by Zhihong Liu at the General Hospital of Eastern Theater Command conducted a study in 572 patients with IMN confirmed by renal biopsy [20]. The results suggested that glomerular PLA2R antigen is more reliable than serum anti-PLA2R for the diagnosis of IMN, whereas serum anti-PLA2R may be more reliable than glomerular PLA2R antigen for measuring disease activity and renal function. Therefore, combined detection of serum anti-PLA2R and glomerular PLA2R antigen deposits may provide more empirical evidence for diagnosis, treatment response, and relapses in patients with IMN. In the study, the positive fluorescence intensities of stages I, IV, and V were mostly ± to + (40/80), with no significant difference in fluorescence titer between these stages. These results were consistent with the immunohistochemistry, and the kappa statistic was 0.95. The positive fluorescence intensities of stages II and III were mostly ++ to ++++. There was no significant difference in fluorescence intensity between these two stages, but there was a significant difference with the fluorescence intensity of stages I, IV, and V (p < 0.001). These results were consistent with the immunohistochemistry, and the kappa statistic was 0.97. These findings also support the above conclusion. Hence, blood anti-PLA2R measurements are useful for in vitro differential diagnosis and the monitoring of treatment, as it can distinguish stage II and III from stage I, IV, and V IMN. Anti-PLA2R can reflect the progress of IMN, judge the treatment effect and prognosis of patients to a certain extent, and provide help for the individualized treatment strategy of IMN patients with different stages. Also, it will pay more attention to the stage II and III IMN patients with high titer of anti-PLA2R.

In addition, the present study showed that MN occurs mostly (65%) at ages 30–60 years, and that the proportion of MN in kidney disease is on the rise, which is consistent with the findings of other studies [21,22]. Therefore, patients in this age group should receive sufficient attention.

5. Conclusion

Blood anti-PLA2R detected by indirect immunofluorescence using EU90 cells coated with non-transfected and transfected PLA2R can be used as a specific indicator for in vitro diagnosis of IMN. The method facilitates the differential diagnosis of IMN and secondary MN and, more importantly, the differential diagnosis and treatment monitoring of different stages of IMN.

Data Availability

The underlying data supporting the results of our study can be found in Hospital Information System of Hainan General Hospital.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Keying Fu and Pei Zhang contributed equally to this work.

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