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Serum phospholipase A2 receptor antibodies and immunoglobulin G subtypes in adult idiopathic membranous nephropathy: Clinical value assessment

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ABSTRACT

Background: Idiopathic membranous nephropathy (IMN) is widely considered as an organ-specific autoimmune disorder. Implicated in its pathogenesis are the phospholipase A2 receptors (PLA2R) expressed on glomerular podocytes against which serum antibodies are formed. In this study we quantified and assessed the clinical value of total serum PLA_2R antibodies and the subtype antibodies in IMN.

Methods: We measured serum levels of total PLA2R antibodies and IgG subtype antibodies by Enzyme Linked Immunosorbent Assay (ELISA) in 146 biopsy-proven IMN patients, 51 non-IMN patients and 62 healthy controls. We went ahead and determined the diagnostic potential of total serum PLA2R antibodies and assessed if a relationship exists between the dominat subtype antibody and the clinical parameters.

Results: The diagnostic sensitivity and specificity of total serum PLA2R antibody for IMN were found at 69.9% and 100% respectively. Significant differences in systolic blood pressure, serum Cystatin C, serum albumin and estimated glomerular filtration rate (eGFR) were found between the antibody-positive and antibody-negative groups of IMN patients. Subtype antibody 4 and 1 exhibited the highest positive rates of 94.4% and 91.6% respectively. The mean serum proportion of subtype antibodies was 65.4, 12.7, 7.6 and 4.6% for subtype 4, 1, 3 and 2 respectively. Serum levels of total protein and albumin were significantly decreased among patients with high serum titres of antibody subtype 4.

Conclusion: Our findings underscore the diagnostic potential of total serum PLA2R antibodies and highlight the importance of antibody subtype 4 over other subtype antibodies in IMN.

1. Introduction

Idiopathic membranous nephropathy (IMN) is a leading cause of adult nephrotic syndrome. Its pathogenesis is linked to various antigens including aldose reductase, superoxide dismutase, α-Enolase, Thrombospondin type-1 domain-containing 7A (THSD7A) and the M-type phospholipase A2 receptor (PLA2R) [1–4]. The discovery of PLA2R, the major glomerular target antigen illuminated the pathogenesis of IMN in a significant manner [1]. The antibodies formed against PLA2R are present in the majority of patients and are believed to be of importance in diagnosis and monitoring of IMN. As such, a number of methods including western blotting, indirect immunofluorescence assay (IFA), addressable laser bead immunoassay (ALBIA) and enzyme linked immunosorbent assay (ELISA) have recently been invented to facilitate their detection and measurement [1,5–7].

A renal biopsy is still held as a diagnostic standard despite the discovery of PLA2R antibodies. Unlike the PLA2R antibodies, however, renal biopsy does not distinguish IMN from secondary membranous nephropathy (SMN) hence necessitating the screening for SMN-associated conditions such as infections, autoimmune diseases, malignancies and drugs before the confirmation of IMN. On renal biopsy, IMN is characterized by immune complexes that deposit in

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subepithelium of the glomerular basement membrane (GBM). The immune complexes vary in IgG antibody composition. IgG4 is the most dominant IgG subtype found in IMN immune complexes followed by IgG1. Since IgG1, IgG2 and IgG3 subtype antibodies also exist in patients with SMN in general [8–12], they are of a limited value in distinguishing IMN from SMN. Although researchers have drawn a conclusion that IgG4 subclass antibodies participate in the formation of GBM immune complexes, they have failed to clarify which antigen stimulates the body to produce antibodies and the role that the antibodies play in the pathogenesis of IMN.

In this study we determined PLA2R antibody level in the serum by ELISA and further evaluated its diagnostic capability for IMN. We also quantified anti-PLA2R subtype antibodies using ELISA and determined their distribution in antibody positive IMN patients. We assessed their correlations with total anti-PLA2R on one hand and determined their correlations with clinical parameters on the other hand. We proved how IgG subtype of anti-PLA2R made an important impact on the pathogenesis and severity of the IMN among adults at the same time. Furthermore, we also evaluated the clinical significance of PLA2R antibodies and their subtypes in disease.

2. Materials and methods

2.1. Subjects selection

This study was done at Shengjing hospital of China Medical University. A total of 197 patients and 62 healthy subjects from the hospital's nephrology and health screening departments respectively were enrolled in this study from December 2014 to November 2015. Among the patients considered for this study were the 146biopsy-proven IMN patients for whom the causes of SMN were excluded by routine screening and the 51 patients definitively diagnosed for non-IMN renal diseases. The cases and controls were matched for age and sex prior to their recruitment. The non-IMN cases included diabetic nephropathy (4), purpura nephritis (7), IgA nephropathy (12), minimal change nephropathy (13) and lupus nephritis (15). Upon their recruitment, the participants were divided into the IMN group and the non IMN group.

Patient serum samples and their laboratory results were collected before renal biopsy and before patients were administered immunosuppressant treatments on admission. Simultaneously, we also collected demographic and clinical data of these study participants. Blood was collected in both plain and EDTA-anticogulated vaccutainers while urine was collected in clean plastic containers. Upon collection, whole blood was left standing to clot thoroughly before centrifuging at 3000 rpm/min (1710 × g) for 4 min to obtain serum. Serum and urine were then stored at −80 °C pending analysis.

All laboratory tests were conducted in accordance with the standard operating procedures. The assay of serum electrolytes(K^+, Na^+ and Cl^-), total protein (TP), creatinine (Cr), Urea, total cholesterol (TC), triglycerides (TG), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), cystatin C (Cys C), albumin (Alb), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and 24-h urine protein were performed using Architect C16000 Analyzer (Abbott, US) while the analysis for C3, IgM, IgG, and IgA was accomplished with Immage 800 Immunology Analyzer (Beckman, US). The white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), platelet count (PLT) were determined using DxH 800 Hematology Analyzer (Beckman, US). This study was approved by the ethics committee of Shengjing hospital of China Medical University.

2.2. Determination of total serum phospholipase A2 receptor antibody concentration

The assay of serum total PLA2R antibodies was performed with ELISA kits (EUROIMMUN, Lübeck, Germany) according to the manufacturer's instructions. As recommended by the manufacturer, all samples with titer values above 20 relative light units (RU)/ml were considered antibody positive and those with less or undetectable values were considered antibody negative.

2.3. Quantification of serum phospholipase A2 receptor IgG subtype antibodies

The assay for PLA2R IgG subtype antibodies was done in a manner similar to the assay of total serum PLA2R antibodies. The same kits were used save for their constituent standards and anti-IgG HRP conjugate. To determine the serum concentration of PLA2R IgG1, IgG2, IgG3 and IgG4, samples were diluted to 1:101, 1:101, 1:101 and 1:4040 respectively using a buffer. The diluted sera were separately dispensed into the antigen-coated microplate wells bearing negative and positive control wells. For subtype antibody 1, 2, 3 and 4, anti-human IgG1, IgG2, IgG3 and IgG4 HRP conjugate (eBioscience, San Diego, CA) was diluted to 1:200, 1:100, 1:100 and 1:200 respectively using a buffer (innovation, Beijing, China) and used in the place of anti-IgG HRP conjugate supplied with the kits.

Due to unavailability of commercial standards for the subtype antibodies, serum samples of IMN patients with total PLA2R IgG antibody titres ≥800 RU/ml were pooled, serially diluted to 1:8192 and used as standards. The undiluted standard was arbitrarily assigned a value of 10,000 RLUs/ml while others were assigned values according to their levels of dilution. Optical densities (ODs) were measured on each plate using a curve fit calculation (four parameters with variable slope). Samples with calculated RLUs/ml greater than the highest standard were diluted two-fold and remeasured. Samples were considered positive for subtype antibodies if they went beyond mean +3 standard deviations(SD) from the healthy controls.

2.4. Statistical analyses

SPSS version 19.0 (SPSS Inc., Chicago, IL) and GraphPad Prism version 5.01 (GraphPad Software Inc., San Diego, CA) were used for statistical analyses. Quantitative variables were expressed as mean ± SD or median (interquartile range) as appropriate. The normality of variables was tested using Kolmogorov–Smirnov test. Unpaired *t*-test was used to compare variables between two groups, one-way ANOVA for multiple groups and LDS-*t*-test for comparison among groups. Proportions were compared using Chi-square test or Fisher's exact probability test. A receiver operating characteristic curve (ROC) was plotted to evaluate the diagnostic performance and correlations determined by Spearman's rank correlation. All tests were two-sided and P values < .05 were considered statistically significant.

3. Results

3.1. Comparison of demographic and clinical characteristics of patients

The demographic and clinical information of IMN patients and non-IMN patients was compared. With an average age of 50.92 ± 12.50 years, the IMN patients were older than their non-IMN counterparts (P < .05).The levels of serum Cr (70.67 ± 25.60), Urea (5.48 ± 1.97), Cys C (1.13 ± 0.44), TP (43.29 ± 6.80), Alb (22.54 ± 4.61) and eGFR (99.35 ± 19.80) were significantly lower in IMN (P = .036, 0.002, 0.004, 0.003, 0.006 and 0.004 respectively). However, there was no significant difference in the levels of 24-h urine protein between the IMN and non-IMN patients (P = .538).

3.2. Assessing the diagnostic value of total serum phospholipase A2 receptor antibodies

Of the 146 patients with IMN, 102 tested positive for serum PLA2R antibody while 44 tested negatives. All the 51 non-IMN patients tested

Table 1
Results of PLA2R antibody assay between IMN group and Non-IMN group.

		Diagnosis of renal biopsy		Total
		IMN group	Non-IMN group	
Anti-PLA2R	+	102	0	102
	−	44	51	95
Total		146	51	197

Note: +: anti-PLA2R positive, −: anti-PLA2R negative.

negative for the antibody (Table 1). The overall diagnostic accuracy of total serum PLA2R antibodies was 77.7%. The sensitivity and specificity was 66.9% and 100% respectively and as shown in Fig. 1, the area under the ROC curve was 0.935 (95%CI = 0.901–0.969). The positive and negative predictive values were 100% and 53.7% respectively while the positive and negative likelihood ratio was infinity and 30.1% respectively.

3.3. Comparison of baseline clinical information between the antibody-positive and the antibody-negative groups of idiopathic membranous nephropathy

On establishing their serum antibody status, we grouped IMN patients into antibody positive and antibody negative groups and compared their baseline clinical characteristics. As shown in Table 2, statistically significant differences in systolic blood pressure, serum Cys C and serum Alb were found ($P < .05$). The systolic blood pressure and the serum Cys C were significantly higher in the antibody positive group while serum Alb was higher in the antibody negative group.

3.4. The concentration of phospholipase A2 receptor IgG subtype antibodies in the standard serum

Due to unavailability of PLA2R IgG subtype antibody commercial standards, we prepared a standard serum using seven patient samples with anti-PLA_2R titer above 800 RU/mL to determine the PLA_2R IgG subtype by ELISA [14]. Sera PLA2R IgG1, IgG2, IgG3, IgG4 subtype concentrations of the standard serum was 707 RLUs/ml, 257 RLUs/ml, 549 RLUs/ml and 8490 RLUs/ml respectively. On each ELISA plate, appropriate dilution series were made and sample concentrations calculated from the standard curves of the IgG subtypes.

Table 2
Comparison of baseline information of the total serum antibody-positive and antibody-negative patients with idiopathic membranous nephropathy.

Parameters	PLA2R+	PLA2R−	*P* value
Number (male/female)	102 (66/36)	44 (29/15)	0.901
Age	51.6 ± 11.9	50.0 ± 13.3	0.473
SBP (mmHg)	137.7 ± 17.4*	130.8 ± 17.5	0.027
DBP (mmHg)	87.0 ± 10.6	83.5 ± 10.2	0.060
Cr (μmol/L)	77.1 ± 44.1	66.2 ± 23.5	0.054
Urea (mmol/L)	5.8 ± 2.5	5.7 ± 1.8	0.804
Cys C(mg/L)	1.2 ± 0.4*	1.0 ± 0.3	0.006
eGFR [mL/(min·1.73m^2)]	94.9 ± 21.8*	103.8 ± 18.5	0.013
24 h urine Protein(g/d)	7.7 ± 5.0	6.9 ± 5.2	0.370
K^+ (mmol/L)	3.8 ± 0.4	3.8 ± 0.5	0.444
Na^+ (mmol/L)	142.0 ± 2.7	139.0 ± 21.1	0.340
CL^- (mmol/L)	109.5 ± 2.9*	108.5 ± 2.6	0.028
TP (g/L)	42.6 ± 7.3	43.4 ± 6.4	0.488
Alb (g/L)	21.6 ± 4.6*	23.6 ± 4.6	0.019
ALT (U/L)	22.1 ± 20.7	21.7 ± 14.7	0.908
AST (U/L)	24.6 ± 20.4	24.8 ± 20.5	0.965
TC (mmol/L)	7.9 ± 2.0	7.6 ± 2.0	0.387
TG (mmol/L)	3.2 ± 3.0	2.6 ± 1.9	0.256
LDL-C (mmol/L)	5.6 ± 1.8	5.5 ± 1.8	0.670
HDL-C(mmol/L)	1.4 ± 0.6	1.7 ± 1.2	0.103
C3 (g/L)	1.0 ± 0.3	1.0 ± 0.2	0.508
IgM (g/L)	1.1 ± 0.6	1.1 ± 0.6	0.954
IgG (g/L)	5.0 ± 2.1	4.6 ± 1.6	0.267
IgA (g/L)	1.9 ± 0.9	2.0 ± 0.9	0.389
WBC	6.6 ± 1.9	7.9 ± 3.5	0.019
RBC	4.3 ± 0.6	4.3 ± 0.6	0.984
HGB	129.7 ± 20.4	126 ± 23.4	0.425
PLT	227.6 ± 69.46	203.5 ± 68.9	0.054
Pathological stage (number, %)			0.189
I	13 (52%)	12 (48%)	
II	76 (72.3%)	28 (27.7%)	
III	13 (76.5%)	4 (23.5%)	

Note: *Compared with PLA2R- group, $P < .05$. SBP, systolic blood pressure; DBP, diastolic blood pressure; Cr, creatinine; Cys C, cystatin C; eGFR. estimated glomerular filtration rate [CKD-EPI(Cr)] [13]; TP, total protein; Alb, albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TC, total cholesterol; TG, triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; C3, complement 3; IgM, immunoglobulin M; IgG, immunoglobulin G; IgA, immunoglobulin A; WBC, white blood cell; RBC, red blood cell; HGB, hemoglobin; PLT, platelet.

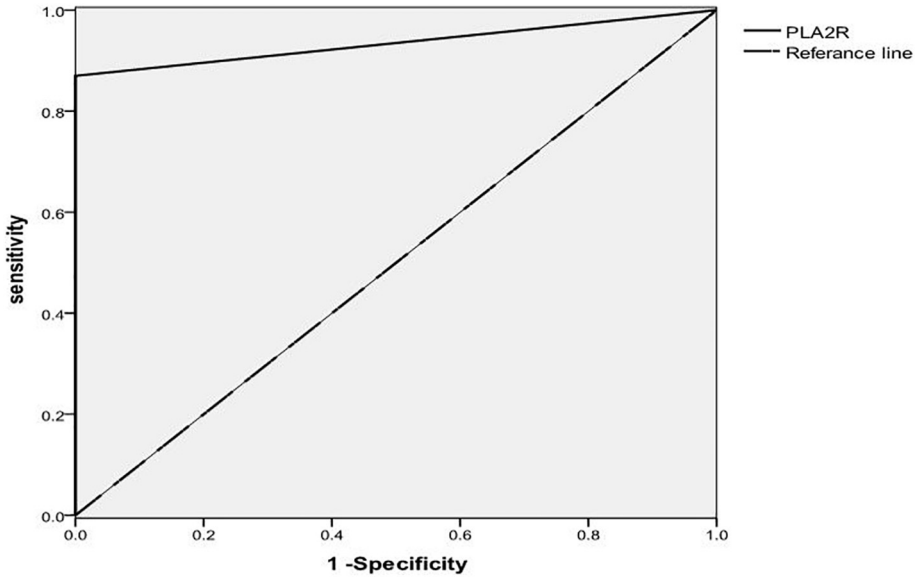

Fig. 1. ROC curve of phospholipase A2 receptor antibody (ELISA) in the diagnosis of idiopathic membranous nephropathy.

Table 3
The cutoff values for anti-PLA2R IgG subtype from the healthy participants (n = 62).

Anti-PLA2R IgG subtype	Concentration range (RLUs/ml)	Cutoff value (mean ± 3SD, RLUs/ml)
IgG1	0.18–5.90	3.14
IgG2	5.01–103.62	90.56
IgG3	0.30–10.33	5.00
IgG4	0.15–1.18	0.93

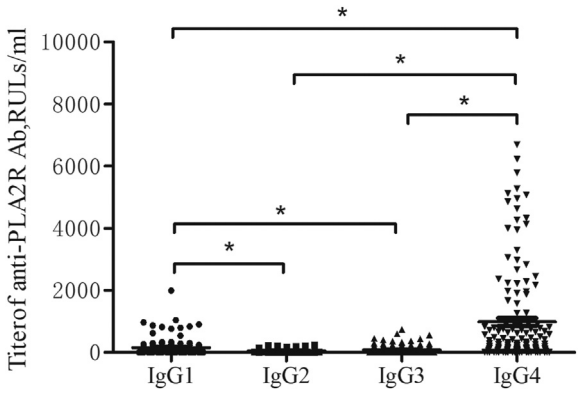

Fig. 2. The concentration of serum PLA2R IgG subtype antibody in IMN group. Note: The horizontal lines in the figure indicate the median concentration of subtype antibodies. * indicates a statistically significant difference between the groups being compared, $P < .05$.

3.5. Concentration of PLA2R IgG subtypes among the PLA2R-antibody positive IMN patients

We first established the cutoff value for each subtype antibody using the health subjects as shown in Table 3. We went ahead and determined the concentration of PLA2R IgG subtypes in the 102 PLA2R-antibody positive IMN patients as shown in Fig. 2. The levels of PLA2R IgG1 subtype was considerably higher than IgG2 and IgG3 subtypes, while the levels of PLA2R IgG4 subtype antibody was significantly higher than that of any other subtype antibody. The positive rates of IgG4 and IgG1 PLA2R subtype antibodies in PLA2R-Ab positive patients with IMN were 94.4% and 91.6% respectively. Using the concentration of each IgG subtype, we calculated the total serum anti-PLA2R IgG concentration for each antibody positive patient (n = 102) (Table 4). We further calculated the mean proportion of each IgG subtype (%IgG subclass) and as shown in Table 5, the mean proportion IgG4 was highest at 65.4%. Second to IgG4 was IgG1 followed by IgG2 and IgG3. However, the mean proportions of IgG2 and IgG3 were significantly decreased ($P < .05$) (Table 4).

3.6. The correlation analysis between serum PLA2R IgG subtype antibodies and total serum anti-PLA2R

In the IMN group, we detected total serum anti-PLA2R concentration directly by ELISA test kit. There was also a strong correlation between directly measured concentration of total serum anti-PLA2R and the concentration of individual IgG1, IgG2, IgG3 and IgG4 subtype antibodies (Fig. 3A, B, C and D). The correlation between directly measured total antibody concentration and IgG4 subtype antibody concentration was the highest (r = 0.882, $P < .01$). 102 IMN patients were further subdivided into a negative group (< 0.93 RLUs/ml) and a positive group (> 0.93 RLUs/ml) according to the concentration of IgG4. The positive group was further divided into three categories based on the concentration of IgG4: lowest category (low level group), middle category (middle level group) and highest category (high level group). There was statistically significant difference in TP and Alb among the above four groups (P = .02, < 0.01, respectively) (Table 5). Hypoalbuminemia occurred more frequently in patients in the highest category of PLA2R IgG4 subtype titer (Fig. 4A, B).

4. Discussion

Various antigens have already been discovered in connection with the pathogenesis of IMN. The discovery of PLA2R, the major target antigen contributed to the understanding of IMN's pathophysiology in many ways. Due to their specificity, the antibodies against PLA2R have become a biomarker candidate for the diagnosis and monitoring the activity of IMN. The prevalence of these antibodies in IMN ranges from 52% to 82% in different countries, regions and races. In our single-center retrospective study, we quantified total serum PLA2R antibodies and the subtype antibodies, and assessed their clinical value in IMN. Total serum PLA2R antibodies yielded a high sensitivity and specificity for IMN indicated by an area of 0.935 under the ROC (Fig. 1).

Accordingly, our findings underscored the diagnostic value held by these antibodies. Furthermore, total serum PLA2R antibodies were found not only specific for IMN but also related with clinical parameters of the disease. For patients with severe clinical symptoms, the clinicians use immunosuppressive agents for empiric treatment before confirmation by renal biopsy which may cause a decline in serum antibody levels [15–19]. Although we did not screen the patients for the minor MN-related antibodies such as THSD7A, we found significant differences in serum Alb, Cys C, SBP and eGFR between the PLA2R antibody-positive and the antibody-negative patients (Table 2). These observations could have been attributed to the binding of serum antibodies to the renal PLA2R antigens triggering severe glomerular damage that eventually resulted into the increase of serum Cys C, SBP and urinary loss of Alb.

These findings indicate that antibody positive IMN patients suffered a more severe deterioration of renal function due to the glomerular basement membrane anti-PLA2R-related immune complex formation.

There are contradictory perspectives regarding the prognostic potential of serum PLA2R antibodies in IMN. While some studies reported that serum antibody concentration are closely related to the disease's clinical manifestations and could predict patient outcomes [20,21], the study by Oh et al. [22] suggested that the antibodies are incapable of predicting patient outcomes. This notwithstanding, total PLA2R antibodies reflect the activity of IMN and are potentially useful for monitoring condition of patients. The significant increase of serum Alb, Urea and the decrease of eGFR observed in the antibody-positive patients is evidence that PLA2R antibodies are directly related to the deterioration of renal functions in IMN.

At the same time, this study has shown that the concentration of antibodies especially PLA2R IgG4 was closely related to the degree of deterioration of renal function. As such the variation in the titres of this particular subclass antibody (as shown in Fig. 2) could be reflective of

Table 4
Concentration and proportion of IgG subtypes in sera of anti-PLA2R positive patients (n = 102).

	IgG1	IgG2	IgG3	IgG4
Median (RULs/ml) ($P_{2.5}$–$P_{97.5}$)	119.9 (21.8–1447.1)	52.1 (9.1–2753.9)	64.4 (4.0–921.6)	699.8 (49.5–6424.5)
Percentage of concentration, % (Median, P2.5~P97.5)	12.7 (1.5–52.1)	4.6 (1.3–45.0)*	7.6 (0.4–57.5)*	65.9 (19.9–93.9)

Note: *Compared with proportion of IgG4 subtypes in sera of anti-PLA2R positive patients, $P < .05$.

Table 5
Relationship between serum PLA2R IgG4 subtype antibody levels and the related clinical data.

Parameters	Concentration of serum PLA2R IgG4 subtype antibody				*P* value
	−	+	+ +	+ + +	
Cr (μmol/L)	79.2 ± 50.8	66.5 ± 16.2	73.8 ± 22.8	80.0 ± 60.3	0.407
Urea (mmol/L)	5.8 ± 2.6	5.8 ± 2.0	5.9 ± 2.0	5.6 ± 2.8	0.971
Cys C (mg/L)	1.0 ± 0.4	1.1 ± 0.3	1.2 ± 0.4	1.2 ± 0.5	0.530
eGFR [mL/(min·1.73m^2)]	94.1 ± 27.1	99.720.4	99.7 ± 20.8	94.9 ± 21.5	0.556
24 h urine Protein(g/d)	6.9 ± 3.1	6.5 ± 5.0	8.2 ± 5.8	7.9 ± 4.6	0.400
TP (g/L)	46.4 ± 3.5	44.8 ± 6.8	42.4 ± 6.8	40.8 ± 7.4	0.020*
Alb (g/L)	25.4 ± 3.0	24.0 ± 4.6	21.9 ± 4.5	20.2 ± 4.2	< 0.001*
TC (mmol/L)	7.4 ± 1.5	7.4 ± 1.8	7.9 ± 1.8	8.2 ± 2.2	0.317
TG (mmol/L)	2.8 ± 1.7	2.6 ± 1.9	3.4 ± 3.2	6.0 ± 2.6	0.596

Note: * indicates a statistically significant difference between the two groups, $P < .05$. Cr, creatinine; Cys C, cystatin C; eGFR. estimated glomerular filtration rate [CKD-EPI(Cr)] [13]; TP, total protein; Alb, albumin; TC, total cholesterol; TG, triglycerides.

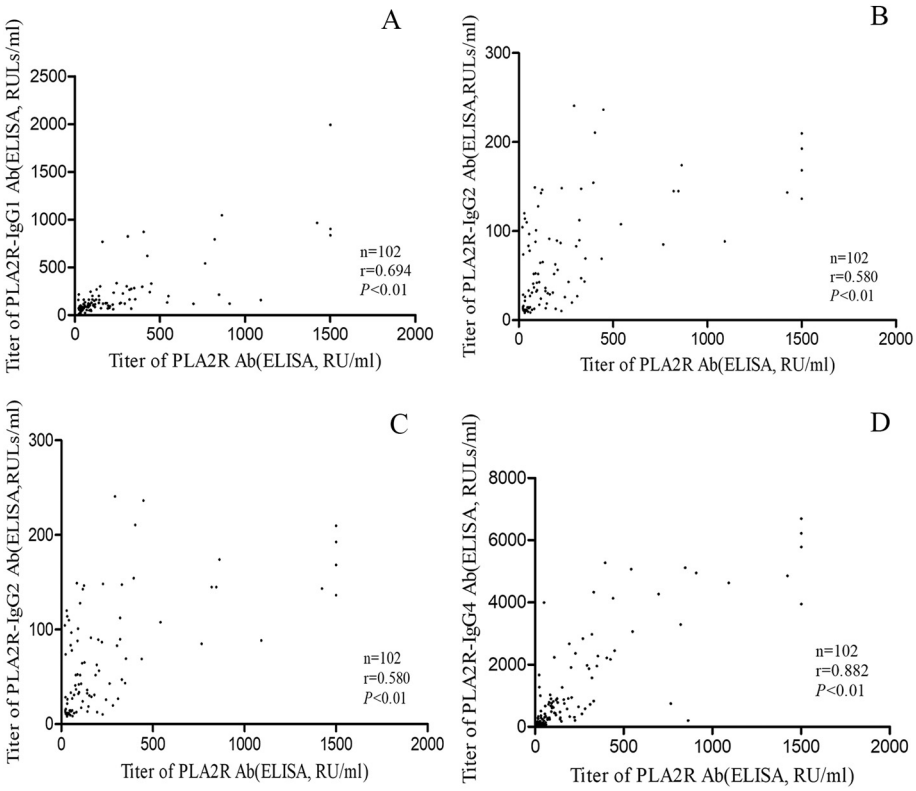

Fig. 3. A. B, C and D show the correlation analysis between total serum PLA2R antibodies and the concentration of subtype antibodies of IgG1, IgG2, IgG3 and IgG4 respectively.

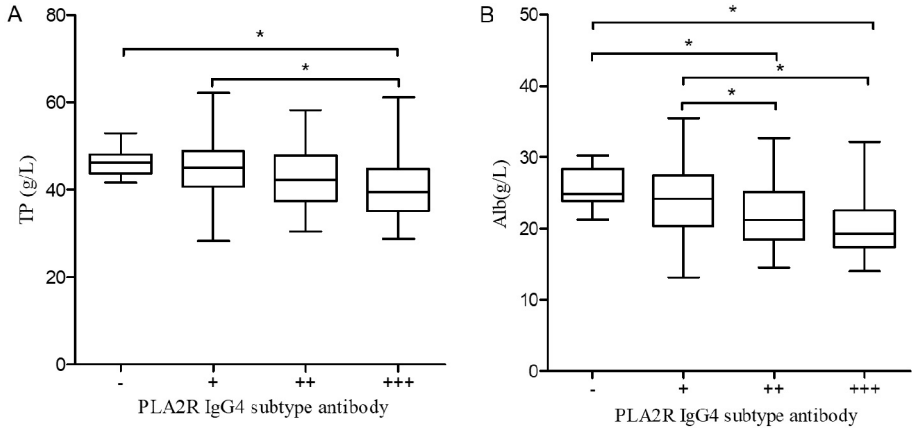

Fig. 4. Comparison of total serum protein and serum albumin among patient categories of different concentrations of antibody subtype 4.
Note: TP, total protein; Alb, albumin; * represent significant differences between categories under comparison; (−), (+), (+ +) and (+ + +) represent the negative, low, middle and high categories of subtype 4 antibody.

various degrees of renal damage among the study participants.

It is well known that IgG is the most abundant of all human immunoglobulins (80%) and that it plays an important role in the body's humoral immune process [23]. IgG is classified into four subtypes according to the number and type of amino acids in the hinge region and the number of disulfide bonds in the region, namely IgG1, IgG2, IgG3, IgG4 [23]. IgG4 is the least abundant in plasma (typically 5%). Previous studies confirmed that regulation of IgG4 production by B cells is dependent on T-helper type 2 (Th2) cells [24]. In our study, the positive rate of IgG4 subtype of PLA2R antibody was higher than other special PLA2R antibody subtypes, this finding implied that the increase of special PLA2R antibody IgG4 may be produced by Th2-induced immunologic reaction, which might be more meaningful in the pathogenesis of IMN. The above findings contribute to further understand the pathogenesis of the disease. The positive rate of IgG4 subtype antibody was higher than in the previous study, but the dominant and pathogenic subtypes were the same [1]. The difference in this result may be related to the use of immunosuppressive therapy in IMN patients included in study, however, the previous study did not explain whether the subjects had received treatment [1,25]. Usually, the first-time patients with nephrotic syndrome are given supportive treatment, that is, physicians will help patients to control proteinuria, edema and other symptoms. In routine clinical practice, IMN is confirmed by a renal biopsy before initiation of treatment. After a period of treatment, the concentration and distribution of IgG subtypes in IMN patients may change in a manner reflecting a decrease in disease activity. There is a disagreement regarding relative serum concentrations of the subtype antibodies. Whereas the Kanigicherla et al. [25] reported that subtype 4 antibody is the most abundant followed by subtypes 2, 3 and 1 in the early course of the disease, Huang et al. [26] suggested otherwise. The study by Huang et al. suggested that antibody subtype 1 is dominant in the early stages and that subtype 4 becomes dominant in only in later stages.

In this study all the subtype antibodies were significantly correlated with total serum PLA2R antibodies (Fig. 3). However, PLA2R-IgG4 antibody exhibited the strongest correlation ($r = 0.882$, $P < .01$), further underscoring its importance over the other subtypes in the management of IMN. It thus plausible to speculate that the variations in the serum levels this particular antibody as observed in Fig. 2 is a reflection of various degrees in the severity of IMN among the patients we recruited. Regarding the clinical value of serum PLA2R subtype antibodies, our study demonstrated PLA2R-IgG4 as the most dominant antibody capable of reflecting the clinical status of patients. Out of the four, PLA2R-IgG4 antibody exhibited the highest positive rate and its serum level was negatively associated with serum total protein and albumin (Table 5). Patients with high titres of PLA2R-IgG4 antibody suffered a severe form of IMN as their serum total protein and albumin levels were significantly lower compared to their antibody-negative counterparts and those with lower titres of the same antibody. This observation is consistent with the previous study in which IgG4 was the dominant antibody and significantly correlated with patient outcomes [25,27]. Our study results were similar to previous studies. Not only do they indicate that the circulating antibodies produced against PLA2R-antigens are mainly IgG4 subclass, they also indicate that IgG4 subtype antibody can be found in immune complexes of renal tissue [1,27]. Moreover, the studies about specific antibody subtypes contributed to further discovering the mechanism of immune complex formation on glomerular basement membrane in IMN disease.

Taken together, our findings have underscored the diagnostic potential of total serum PLA2R antibodies for IMN suggested by the high specificity and sensitivity observed in this study. The high positivity and strong associations of PLA2R-IgG4 antibody clinical parameters of IMN also suggests its importance in evaluating the clinical status patients.

This study suffered shortcomings including the lack of information regarding the minor antibodies of IMN in patients who tested negative for PLA2R antibodies. Patients were therefore classified as antibody-negative only on the basis of serum PLA2R antibodies disregarding the possibility of being positive for the minor pathogenic antibodies such as THSD7A. Accordingly, future studies should investigate the importance of importance of total serum anti-PLA2R and subtype antibodies with making reference to patients screened for all IMN-related antibodies.

Additional information

Competing financial interests: The authors declared no competing financial interests.

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