Received: 24 September 2019 | Revised: 4 December 2019 | Accepted: 11 December 2019

DOI: 10.1111/nep.13691

ORIGINAL ARTICLE

NEPHROLOGY WILEY

Clinical value of renal phospholipase A2 receptor deposit in the prognosis evaluation and treatment options of idiopathic membranous nephropathy: A retrospective cohort study

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Funding information
Guangdong Science and Technology Project, Grant/Award Numbers: No.2017A070709008, 2017A070709008; Guangzhou Science and Technology Project, Grant/Award Numbers: No.201604020037, 201604020037; National Natural Science Foundation of China, Grant/Award Number: No.81570609; National Clinical Key Specialty Construction Projects

Abstract

Aim: Phospholipase A2 receptor (PLA2R) is a target antigen for idiopathic membranous nephropathy (IMN). However, the association between renal PLA2R antigen and disease prognosis had not been fully investigated. In addition, there was a paucity of studies investigating the difference of therapeutic effects between cyclophosphamide and cyclosporine A in PLA2R-associated IMN.

Methods: This retrospective cohort study recruited 300 eligible patients diagnosed with biopsy-proven IMN between September 2015 and July 2018 in Guangdong Provincial People's Hospital. The remission of proteinuria was compared between PLA2R-associated and non-PLA2R-associated IMN. The difference of therapeutic effects between cyclophosphamide and cyclosporine A were also investigated in PLA2R-associated IMN.

Results: The positive rate of renal PLA2R antigen in recruited IMN patients was 82.3%. Non-PLA2R-associated IMN patients had a higher probability to achieve remission than PLA2R-associated IMN patients (Log–rank test, P = .013). Multivariate COX analysis showed that renal PLA2R antigen was an independent risk factor for not achieving remission in IMN patients (Hazard Ratio: 1.619; 95% confidence interval: 1.133 to 2.313; P = .008). In PLA2R-associated IMN, patients receiving cyclophosphamide had a higher probability to achieve remission compared with those receiving cyclosporine A (Log–rank test, P = .018) while there was no difference in renal survival. Multivariate COX regression analysis showed that compared with cyclosporine A, patients receiving cyclophosphamide had a higher probability to achieve remission.

Conclusion: Phospholipase A2 receptor -associated IMN patients had a lower probability to achieve remission compared with non-PLA2R-associated IMN. Compared

Zhiyong Xie and Wei Dong contributed equally to this study.

with cyclosporine A, cyclophosphamide exerted better therapeutic effects in remission of proteinuria and may be the preferred immunosuppressant for PLA2R-associated IMN.

KEYWORDS

idiopathic membranous nephropathy, phospholipase A2 receptor, prognosis evaluation, treatment options

Idiopathic membranous nephropathy (IMN) is a common autoimmune glomerular disease and the primary cause of nephrotic syndrome in adults. Recently, the prevalence of IMN had dramatically increased and IMN had remained the second most common primary glomerular diseases in China.[1] In IMN patients, disease prognoses vary from spontaneous remission to progressing to end stage renal disease (ESRD).[2] Finding potential prognostic biomarkers were conducive to assessing prognosis of IMN. Phospholipase A2 receptor (PLA2R)[3] and Thrombospondin type 1 domain-containing 7A (THSD7A)[4] were recently identified as target antigens in IMN with higher specificity and sensibility. Renal PLA2R antigen deposit was detected in approximately 70% to 90%[5,6] while the positive rate of THSD7A antigen deposition ranged from 0.7% to 9.1% in IMN patients.[4,7]

The anti-PLA2R antibody titre was found to be closely correlated with disease remission, treatment response and recrudescence.[8,9] However, antibody titre would be easily affected by individual immune response and immunosuppressant. In the early course of the disease, PLA2R antigen will be detectable in the immune deposits by immunofluorescence staining, prior to the detection of anti-PLA2R antibodies, enable early identification of PLA2R-associated IMN.[10] Therefore, investigation on the association between renal PLA2R antigen deposit and disease prognoses would be conductive to assess prognosis of IMN.

Despite multiple controlled trials of various immunosuppressive regimens had been conducted in IMN, the optimal immunosuppressant for IMN still remain controversial. The therapeutic options of IMN should base on risk stratification, which included proteinuria, serum creatinine, estimated glomerular filtration rate (eGFR), urine IgG and PLA2R antibody levels according to recent Kidney Disease: Improving Global Outcomes controversies conference.[11] For the IMN patients at high risk of progression, immunosuppressive agents should be initiated early. Alkylating agents are recommended to be initial therapy in IMN and remain the only agents proven effective in preventing renal function progression.[12] As the main alkylating agents, cyclophosphamide (CTX) could inhibit lymphocyte proliferation, rapidly depleting B and T cells and further suppressing humoral and cellular immunity.[13] Cyclosporine A (CsA) is an alternative regimen for the initial therapy of IMN, with a comparable remission rate compared with CTX.[14] CsA have been demonstrated to suppress T cell responses through calcineurin-phosphatase pathway and the nuclear translocation of nuclear factor of activated T-cells (NFAT).[15,16] However, in PLA2R-associated IMN, the difference of therapeutic effects between CTX and CsA had not been investigated. Therefore, the objective of this study was to investigate the prognostic value of renal PLA2R antigen deposit and to compare the difference of therapeutic effects between CTX and CsA in PLA2R-associated IMN.

SUMMARY AT A GLANCE

This article highlighted the prognostic value of intra-renal phospholipase A2 receptor deposition in idiopathic membranous nephropathy (IMN). Renal phospholipase A2 receptor (PLA2R)-associated IMN patients had a lower probability to achieve remission compared with non-PLA2R-associated IMN.

1 | METHODS

1.1 | Subjects and data collection

Four hundred and twenty-two consecutive patients with MN were diagnosed through renal biopsy in Guangdong Provincial People's Hospital from September 2015 to July 2018. Patients who fulfilled the following criteria were enrolled in this retrospective cohort study: patients without a past medication history of steroid and immunosuppressants within 6 months at the time of renal biopsy; the follow-up period was more than 6 months; patients were over 18 years old. Patients with secondary causes detected at onset or during follow up were excluded, including systemic lupus erythematosus, Sjogren's syndrome, malignancy, hepatitis B virus infections, medication and so on. All MN patients were performed serologic evaluation including the detection of anti-nuclear antibody, anti-dsDNA antibody, rheumatoid factor, anti-cyclic citrullinated peptide antibody, anti-neutrophil cytoplasmic antibody and complement to exclude autoimmune diseases. HBsAg and anti-Hepatitis C virus antibody were detected in all MN patients to exclude hepatitis B virus and hepatitis C virus infection. All MN patients were performed the detection of serum tumour markers, chest computed tomography, colour doppler ultrasound of thyroid gland, liver, gallbladder, pancreas, spleen and gynaecological organ (womb and appendix in female) to exclude malignancy. Gastrointestinal endoscopy was performed in 55.7% (246/442) of membranous nephropathy (MN) patients to exclude gastrointestinal tumours. Positron emission tomography was performed in 14.7% (65/442) of MN patients to exclude tumours. Totally, 300 IMN patients were enrolled in this retrospective cohort study (Figure 1). This study

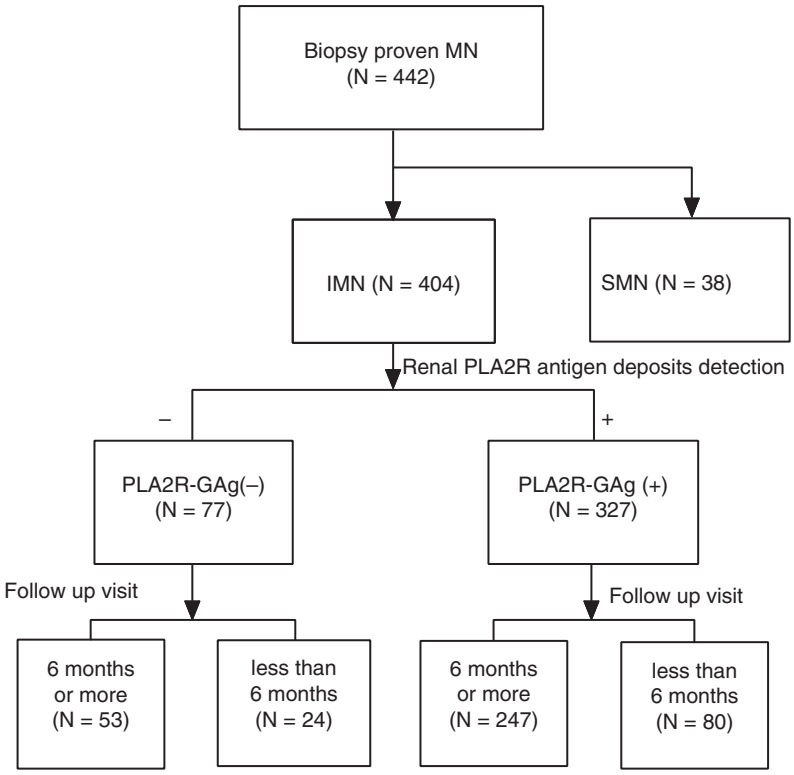

FIGURE 1 Flow chart of the patients enrolled in the study

complied with the declaration of Helsinki and was approved by the Ethics Committee of Guangdong Provincial People's Hospital (No. GDREC2017318H).

The clinical and pathological parameters were derived from electronic medical records and hospital's computerized database manually. The clinical parameters included age, sex, blood pressure, concomitant disease (hypertension, diabetes, anaemia and hyperuricaemia), urinary erythrocyte phase, serum creatinine (Scr), 24 hours proteinuria, 24 hours albuminuria, serum albumin, cholesterol, triglyceride, high density lipoprotein, low density lipoprotein, serum IgG, serum IgA, serum IgM, complement C3, complement C4 and serum anti-PLA2R antibody titre. Proteinuria and Scr were measured every 3 months. The pathological parameters included Ehrenreich-Churg stage, glomerulosclerosis, tubular atrophy/interstitial fibrosis, crescent formation, immunohistological staining (IgG subgroup, IgA, IgM, C3 and C1q). IMN was diagnosed based on the pathological parameters including light microscopy, immunofluorescence and electron microscopy. Detection of renal PLA2R antigen was performed in renal biopsy specimens of all participants. Due to the retrospective nature of the study, sequential serum samples were only available from 84 IMN patients and stored at −80°C until use. Anti-PLA2R antibody titre was measured by ELISA (Enzyme-linked immunosorbent assay) method (Euroimmun AG, Lubeck, Germany).

1.2 | Definitions

According to renal PLA2R immunohistochemistry, IMN was categorized into PLA2R-associated and non-PLA2R-associated IMN. We used the formula of the Chronic Kidney Disease Epidemiology Collaboration to calculate the eGFR. Glomerular haematuria was defined as urinary red blood cell (RBC) greater than 8000/mL and dysmorphic RBC accounted for greater than 75% of the total number of RBC.[17] Complete remission was defined as urinary protein excretion<0.3 g/day, accompanied by a normal serum albumin (ALB) and a normal Scr. Partial remission was defined as urinary protein excretion <3.5 g/day and a 50% or greater reduction from peak values, accompanied by an improvement or normalization of serum ALB and stable Scr.[18] The presence of complete remission or partial remission was all defined as remission. Spontaneous remission was defined as proteinuria<3.5 g/24 hours and at least 50% reduction from the time of initiating conservative treatment.[19] The end point of renal survival was defined as a 50% decline in eGFR or double of serum creatine or progression to ESRD with eGFR < 15 mL/min/1.73 m^2 compared with baseline. ELISA provided quantitative results for the determination of anti-PLA2R antibodies and a negative antibody titre was defined as an anti-PLA2R antibody titre <14 RU/mL. MN was graded into four stages according to the Ehrenreich and Churg's classification criteria.[20] Tubular atrophy/interstitial fibrosis was graded as absent (T0), mild (T1) <25%, moderate (T2) 25%-50% or severe (T3) >50%.[21] IgG4 dominant deposition was defined as stronger deposition of IgG4 subgroup than IgG1, IgG2 and IgG3 subgroup.

1.3 | Detection of renal PLA2R antigen deposits

Renal PLA2R antigen deposits in IMN patients were detected by indirect immunofluorescence in frozen sections. Renal biopsy tissues were cut

TABLE 1 Comparison of clinicopathological characteristics in patients with positive and negative PLA2R antigen deposit

	Total (N = 300)	PLA2R-GAg (−) (N = 53)	PLA2R-GAg (+) (N = 247)	*P*-value
Clinical parameters				
Age (years)	53 (43, 63)	52 (42, 63)	52 (43, 63)	.890
Men (n, %)	176 (58.7)	30 (56.6)	146 (59.1)	.737
Systolic pressure (mmHg)	134 (122, 148)	136 (126, 150)	134 (122, 148)	.370
Diastolic pressure (mmHg)	81 (73, 89)	82 (76, 89)	81 (72, 89)	.381
Hypertension (n, %)	133 (44.3)	24 (45.3)	109 (44.1)	.878
Diabetes (n, %)	65 (21.7)	9 (17.0)	56 (22.7)	.361
Anaemia (n, %)	63 (21.0)	9 (17.0)	54 (21.9)	.429
Hyperuricaemia (n, %)	174 (58.0)	28 (52.8)	146 (59.1)	.401
Glomerular haematuria (n, %)	23 (7.7)	3 (5.7)	20 (8.1)	.777
eGFR (mL/min/1.73 m^2)	89.1 (67.1, 106.6)	89.3 (62.2, 107.9)	89.1 (67.9, 105.9)	.951
Serum creatine (μmol/L)	77.1 (61.9, 101.9)	77.0 (62.0, 104.6)	77.2 (61.9, 102.0)	.949
Proteinuria (g/24 hours)	5.1 (2.7, 8.4)	4.9 (2.4, 8.4)	5.3 (2.7, 8.4)	.571
Albuminuria (g/24 hours)	2.7 (1.8, 4.3)	2.7 (1.8, 4.8)	2.7 (1.8, 4.2)	.986
Serum albumin (g/L)	21.9 (17.9, 27.6)	22.4 (18.4, 28.0)	19.6 (14.7, 24.1)	.003
Cholesterol (mmol/L)	8.2 (6.5, 10.1)	9.0 (6.9, 11.3)	8.0 (6.4, 9.9)	.091
Triglyceride (mmol/L)	2.6(1.7, 4.1)	2.6 (1.7, 3.7)	2.6 (1.7, 4.2)	.841
High density lipoprotein (mmol/L)	1.4 (1.1, 1.8)	1.4 (1.0, 1.9)	1.4 (1.1, 1.8)	.485
Low density lipoprotein (mmol/L)	5.0 (3.9, 6.7)	5.4 (4.2, 7.2)	5.0 (3.9, 6.4)	.287
IgG (g/L)	6.0 (4.6, 8.1)	5.5 (4.5, 8.0)	6.2 (4.6, 8.3)	.342
IgA (g/L)	1.9 (1.4, 2.7)	2.0 (1.4, 2.8)	1.9 (1.4, 2.7)	.946
IgM (g/L)	1.1 (0.8, 1.6)	1.2 (0.8, 1.6)	1.1(0.8,1.6)	.558
Complement C3 (mg/L)	975.5 (851.0, 1120.0)	973.0 (855.5, 1185.0)	978.0 (846.0, 1110.0)	.205
Complement C4 (mg/L)	247.5(200.3, 325.0)	238.0 (186.5, 341.0)	250.0 (204.0, 314.0)	.767
[a]Serum anti-PLA2R antibodies (RU/mL)	34.0 (10.7, 93.1)	4.0 (2.1, 66.5)	38.5 (12.5, 94.3)	.026
Pathological parameters (n, %)				
Ehrenreich-Churg stage				.860
Stage I or II	229 (76.3)	40 (75.5)	189 (76.5)	
Stage III or IV	71 (23.7)	13 (24.5)	58 (23.5)	
Glomerulosclerosis				
Global sclerosis	40 (13.3)	8 (15.1)	32 (13.0)	.678
Segmental sclerosis	62 (20.7)	10 (18.9)	52 (21.1)	.722
Tubular atrophy/Interstitial fibrosis				
T0	88 (29.3)	15 (28.3)	73 (29.6)	.856
T1	119 (39.7)	25 (47.2)	94 (38.1)	.218
T2	75 (25.0)	8 (15.1)	67 (27.1)	.066
T3	18 (6.0)	5 (9.4)	13 (5.3)	.333
Crescent formation	8 (2.7)	1 (1.9)	7 (2.8)	.698
Immunohistological staining				
IgG4 dominant deposition	243 (81.0)	27 (50.9)	216 (87.4)	<.001
IgA deposition	7 (2.3)	4 (7.5)	3 (1.2)	.020
IgM deposition	255 (85.0)	43 (81.1)	212 (85.8)	.398
C3 deposition	289 (96.3)	49 (92.5)	240 (97.2)	.110
C1q deposition	8 (2.7)	7 (13.2)	1 (0.4)	<.001

(Continues)

TABLE 1 (Continued)

	Total (N = 300)	PLA2R-GAg (−) (N = 53)	PLA2R-GAg (+) (N = 247)	*P*-value
Therapeutic regimen (n, %)				
Supportive Therapy	82 (27.3)	13 (24.5)	69 (27.9)	.614
Immunosuppressive	218 (72.7)	40 (75.5)	178 (72.1)	
Steroid+ CTX	40 (18.3)	6 (15.0)	33 (18.5)	.689
Steroid+ CsA	128 (58.7)	25 (62.5)	103 (57.9)	.465
Steroid+ FK506	21 (9.6)	4 (7.5)	17 (9.5)	.773
Steroid+ MMF	7 (3.2)	1 (1.9)	6 (3.4)	.812
Follow-up time (months, M P25, P75])	12 (9, 15)	12 (7, 18)	12 (9, 15)	.309

Abbreviations: CsA, cyclosporine A; CTX, cyclophosphamide; eGFR, estimated Glomerular filtration rate; FK506, tacrolimus; MMF, mycophenolate mofetil; PLA2R-GAg, glomerular PLA2R antigen.

[a]Due to the retrospective nature of the study, the measurements of serum anti-PLA2R antibody were only available for 84 consecutive IMN patients, including six non-PLA2R-associated IMN patients and 78 PLA2R-associated IMN patients.

into 3 μm-thick sections and fixed in acetone for 10 minutes, followed by washing with phosphate buffered saline (PBS) buffer solution twice. Rabbit anti-human PLA2R polyclonal antibody (Abcam, 1:100 dilution) was used as the primary antibody and incubated for 1 hours. After the slides were rinsed with PBS for twice, fluorescein isothiocyanate labelled mouse anti-rabbit IgG antibody (Dako, 1:100 dilution) was used as secondary antibody. Incubation was carried out at 37°C for 40 minutes. The slides were dried and sealed with glycerin for observation under fluorescence microscope. PLA2R staining was defined as positive with granular staining along the glomerular capillary wall in the glomeruli.

1.4 | Statistical analyses

All data were analyzed using statistical product and service solutions (SPSS) statistical software for Windows, version 23.0 (SPSS, Inc., Chicago, Illinois). The measurement data accorded with normal distribution were expressed as mean ± SD and differences between two groups were compared using student *t* test. The non-normally distributed data were expressed as medians (25th, 75th percentiles) and differences between two groups were compared using non-parametric Mann-Whitney *U* test. Categorical variables were compared using the χ^2 test or Fisher exact test. Spearman correlation analysis was used to analyze the clinical correlation between glomerular PLAR2 deposition and anti-PLA2R antibodies titres. Kaplan-Meier survival analysis was performed to compare remission rate and renal survival between two groups, and the log–rank test was used to evaluate the significance of differences. COX regression analysis was performed to analyze factors associated with remission and renal survival and hazard ratios (HR) and 95% confidential intervals (95% CI) were calculated. The adjusted variables were selected into the multivariate COX regression model (Enter selection; $P < .05$ criterion for variable retention) based on the univariate COX regression analysis and clinical judgements. Two-tailed tests were used for all comparisons and a $P < .05$ was considered to be statistically significant.

2 | RESULTS

2.1 | Comparison of clinicopathological characteristics in PLA2R-associated and non-PLA2R-associated IMN

Among 300 IMN patients enrolled, 200 and 47 (82.3%) were PLA2R-associated IMN and 53(17.7%) were non-PLA2R-associated IMN. Two hundred and eighteen IMN patients (72.7%) received immunosuppressive therapy for at least 6 months. Forty patients (18.3%) were treated with prednisone plus CTX, 128 patients (58.7%) with prednisone plus CsA, 21 patients (9.6%) with prednisone plus tacrolimus and 7(3.2%) with prednisone plus mycophenolate mofetil. No patients were treated with anti-CD20 monoclonal antibody. There was no significant difference in the proportion of therapeutic regimen between PLA2R-associated and non-PLA2R-associated IMN. The median follow-up time of PLA2R-associated and non-PLA2R-associated IMN patients were both 12 months and had no statistical difference between two groups (Table 1). Two hundred and fifty-seven (85.7%) enrolled patients were still on active following up at the end of study, including 215 (87.0%) PLA2R-associated IMN and 42 (79.2%) non-PLA2R-associated IMN. There was no statistical difference in the proportion of loss to follow up between PLA2R-associated and non-PLA2R-associated IMN (13.0% vs 20.8%, $\chi^2 = 2.162$, $P = .141$).

In our centre, serum anti-PLA2R antibody was detected in IMN patients since December 2017. Due to the retrospective nature of the study, the measurements of serum anti-PLA2R antibody were only available in 84 consecutive IMN patients. PLA2R-associated IMN presented with a higher level of serum anti-PLA2R antibody and glomerular PLAR2 deposition were positively correlated with anti-PLA2R antibodies titres ($r_S = 0.244$, $P = .025$). Serum albumin was lower in PLA2R-associated IMN than in non-PLA2R-associated IMN. No significant differences were observed in other clinical parameters between two groups. In terms of pathological parameters, compared with

non-PLA2R-associated IMN, PLA2R-associated IMN presented with a higher proportion of IgG4 dominant deposition, a lower proportion of IgA and C1q deposition (Table 1).

2.2 | Association between renal PLA2R antigen and proteinuria remission

Among 300 IMN patients enrolled for prognostic analysis, Kaplan-Meier analysis showed that non-PLA2R-associated IMN patients had a higher probability to achieve remission than PLA2R-associated IMN patients (Log–rank test, P = .013) (Figure 2). Univariate COX regression analysis showed that renal PLA2R antigen was risk factor for not achieving remission in patients with IMN (HR: 1.533; 95% CI: 1.083 to 2.170; P = .016). After adjusting for positive renal PLA2R antigen, eGFR, serum albumin, proteinuria and immunosuppressive therapy, multivariate COX regression analysis showed that positive renal PLA2R antigen (HR: 1.619; 95% CI: 1.133 to 2.313; P = .008) and

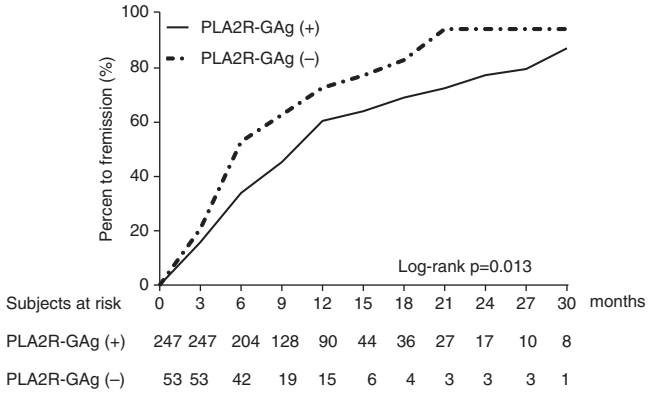

FIGURE 2 Kaplan-Meier analysis of the remission of proteinuria in patients with positive and negative phospholipase A2 receptor (PLA2R) antigen deposit. The numbers of at-risk patients at selected time points (3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 months) were indicated below the plot. Log–rank method was used to evaluate the significance of differences

TABLE 2 The risk factors for no reaching remission in univariate and multivariate COX regression analysis

	Univariate COX analysis		Multivariate COX analysis	
Variables	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
PLA2R-GAg (+)	1.533 (1.083, 2.170)	.016	1.619 (1.133, 2.313)	.008
Age	0.995 (0.985, 1.005)	.318	—	—
Male	1.128 (0.843, 1.510)	.417	—	—
eGFR		.305		.288
≥90 mL/min/1.73 m^2	Reference	Reference	Reference	Reference
60-89 mL/min/1.73 m^2	0.727 (0.522, 1.011)	.058	0.750 (0.537, 1.048)	.092
30-59 mL/min/1.73 m^2	0.855 (0.564, 1.298)	.463	0.730 (0.471, 1.132)	.159
<30 mL/min/1.7 3m^2	0.848 (0.345, 2.084)	.720	0.811 (0.324, 2.031)	.655
Serum creatinine	0.999 (0.996, 1.002)	.628	—	—
Serum albumin		.703		.153
<20 g/L	Reference	Reference	Reference	Reference
20-30 g/L	0.909 (0.659, 1.254)	.560	1.079 (0.774, 1.504)	.655
>30 g/L	1.062 (0.716, 1.576)	.764	1.543 (0.980, 2.427)	.061
Proteinuria		.049		.041
<4.0 g/24 hours	Reference	Reference	Reference	Reference
4.0-8.0 g/24 hours	1.199 (0.845, 1.701)	.309	1.281 (0.878, 1.868)	.199
>8.0 g/24 hours	1.547 (1.091, 2.192)	.014	1.680 (1.123, 2.511)	.011
Cholesterol	1.015 (0.969, 1.063)	.534	—	—
Triglyceride	1.029 (0.972, 1.089)	.329	—	—
High density lipoprotein	1.161 (0.896, 1.504)	.260	—	—
Low density lipoprotein	1.016 (0.943, 1.094)	.680	—	—
Hypertension	0.842 (0.629, 1.128)	.249	—	—
Diabetes	0.948 (0.664, 1.354)	.768	—	—
Anaemia	0.901 (0.632, 1.284)	.564	—	—
Hyperuricaemia	1.158 (0.866, 1.550)	.322	—	—
Immunosuppressive therapy	0.689 (0.487, 0.974)	.035	0.694 (0.478, 1.006)	.054

Note: HR was adjusted for positive renal PLA2R antigen, eGFR, serum albumin, proteinuria and immunosuppressive therapy. The variables were selected into the multivariate COX analysis in an "Enter" manner.

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; PLA2R-GAg, glomerular PLA2R antigen.

higher level of proteinuria (HR: 1.680; 95% CI: 1.123 to 2.511; *P* = .011) were independent risk factors for not achieving remission in IMN patients (Table 2).

2.3 | Association between renal PLA2R antigen and spontaneous remission

Among 300 IMN patients, 82 (27.3%) patients received conservative therapy without using immunosuppressive agents or corticosteroids during follow up. Kaplan-Meier analysis showed that non-PLA2R-associated IMN patients had a higher probability to achieve spontaneous remission than PLA2R-associated IMN patients (Log–rank test, *P* = .012) (Figure 3). Univariate COX regression analysis showed that positive renal PLA2R antigen was risk factor for not achieving spontaneous remission in IMN patients receiving conservative therapy (HR: 2.233; 95% CI: 1.089 to 4.580; *P* = .028). After adjusting for eGFR,

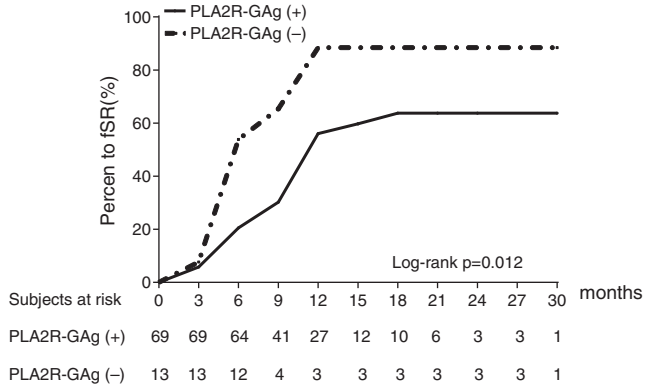

FIGURE 3 Kaplan-Meier analysis of spontaneous remission in patients with positive and negative phospholipase A2 receptor (PLA2R) antigen deposit. The numbers of at-risk patients at selected time points (3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 months) were indicated below the plot. Log–rank method was used to evaluate the significance of differences

TABLE 3 The risk factors for not reaching spontaneous remission in univariate and multivariate COX regression analysis

	Univariate COX analysis		Multivariate COX analysis	
Variables	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
PLA2R-GAg (+)	2.233 (1.089, 4.580)	.028	2.927(1.270, 6.743)	.012
Age	0.986 (0.965, 1.007)	.185	—	—
Male	0.779 (0.413, 1.472)	.443	—	—
eGFR		.586		.614
≥90 mL/min/1.73 m^2	Reference	Reference	Reference	Reference
60–89 mL/min/1.73 m^2	0.810 (0.423, 1.550)	.525	0.948(0.481,1.868)	.877
30–59 mL/min/1.73 m^2	0.269 (0.036, 1.999)	.199	0.248(0.032,1.952)	.186
<30 mL/min/1.73 m^2	0.683 (0.092, 5.084)	.710	1.119(0.087,14.361)	.931
Serum creatinine	0.994 (0.984, 1.004)	.235	—	—
Serum albumin		.168		.138
<20 g/L	Reference	Reference	Reference	Reference
20-30 g/L	0.657 (0.272, 1.590)	.351	0.850(0.306,2.358)	.754
>30 g/L	1.299 (0.568, 2.969)	.535	1.749(0.640,4.775)	.275
Proteinuria		.694		.884
<4.0 g/24 hours	Reference	Reference	Reference	Reference
4.0-8.0 g/24 hours	0.718 (0.315, 1.639)	.432	0.811(0.345,1.908)	.631
>8.0 g/24 hours	0.787 (0.276, 2.238)	.653	1.022(0.204,5.132)	.979
Cholesterol	0.959 (0.829, 1.108)	.567	—	—
Triglyceride	0.996 (0.818, 1.213)	.969	—	—
High density lipoprotein	1.109 (0.684, 1.797)	.674	—	—
Low density lipoprotein	0.894 (0.728, 1.098)	.286	—	—
Hypertension	0.612 (0.328, 1.144)	.124	—	—
Diabetes	0.522 (0.205, 1.332)	.174	—	—
Anaemia	0.480 (0.200, 1.154)	.101	—	—
Hyperuricaemia	1.093 (0.584, 2.046)	.781	—	—

Note: HR was adjusted for positive renal PLA2R antigen, eGFR, serum albumin and proteinuria. The variables were selected into the multivariate COX analysis in an "Enter" manner.

Abbreviations: CI, confidence interval; eGFR, estimated glomerular filtration rate; HR, hazard ratio; PLA2R-GAg, glomerular PLA2R antigen.

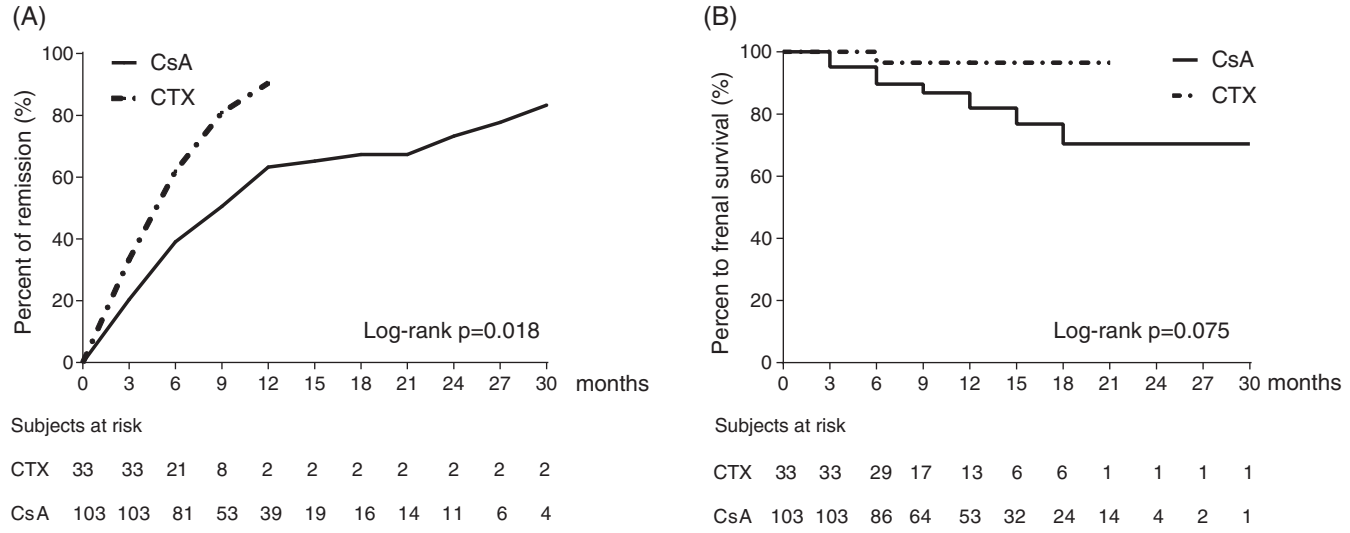

FIGURE 4 Kaplan-Meier analysis of the remission of proteinuria and renal survival in phospholipase A2 receptor (PLA2R)-associated idiopathic membranous nephropathy (IMN) patients receiving cyclophosphamide (CTX) or cyclosporine A (CsA). The numbers of at-risk patients at selected time points (3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 months) were indicated below the plot. Log–rank method was used to evaluate the significance of differences

TABLE 4 COX regression analysis for proteinuria remission in PLA2R-associated IMN patients treated with CTX or CsA

	Univariate COX analysis		Multivariate COX analysis	
Variables	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
CsA (vs CTX)	1.851 (1.148, 2.982)	.011	1.933 (1.123, 3.325)	.017
Age	0.999 (0.986, 1.012)	.874	—	—
Male	1.279 (0.818, 1.999)	.281	—	—
eGFR		.359		.151
≥90 mL/min/1.73 m^2	Reference	Reference	Reference	Reference
60–89 mL/min/1.73 m^2	0.647 (0.396, 1.056)	.081	0.628 (0.380, 1.036)	.068
30–59 mL/min/1.73 m^2	0.927 (0.542, 1.586)	.783	0.769 (0.431, 1.373)	.375
<30 mL/min/1.73 m^2	0.732 (0.227, 2.356)	.600	0.354 (0.101, 1.240)	.104
Serum creatinine	1.000 (0.995,1.004)	.862	—	—
Serum albumin		.886		.478
<20 g/L	Reference	Reference	Reference	Reference
20-30 g/L	1.113 (0.713, 1.736)	.638	1.208 (0.746, 1.956)	.441
>30 g/L	1.102 (0.603, 2.014)	.752	1.507 (0.766, 2.965)	.235
Proteinuria		.242		.211
<4.0 g/24 hours	Reference	Reference	Reference	Reference
4.0–8.0 g/24 hours	1.383 (0.807, 2.370)	.238	1.616 (0.889, 2.935)	.115
>8.0 g/24 hours	1.552 (0.925, 2.603)	.096	1.661 (0.904, 3.053)	.102
Cholesterol	1.012 (0.947, 1.082)	.718	—	—
Triglyceride	0.988 (0.914, 1.067)	.753	—	—
High density lipoprotein	1.066 (0.683, 1.662)	.779	—	—
Low density lipoprotein	1.052 (0.946, 1.169)	.353	—	—
Hypertension	0.896 (0.592, 1.356)	.604	—	—
Diabetes	1.133 (0.719, 1.784)	.591	—	—
Anaemia	0.961 (0.597, 1.546)	.869	—	—
Hyperuricaemia	1.081 (0.717,1.631)	.709	—	—

Note: HR was adjusted for therapeutic protocol (CTX and CsA), eGFR, serum albumin and proteinuria. The variables were selected into the multivariate COX analysis in an "Enter" manner.

Abbreviations: CI, confidence interval; CsA, cyclosporine A; CTX, cyclophosphamide; eGFR, estimated Glomerular filtration rate; HR, hazard ratio.

serum albumin and proteinuria, positive renal PLA2R antigen was still an independent risk factor for not achieving spontaneous remission in IMN patients (HR: 2.927; 95% CI: 1.270 to 6.743; P = .012) (Table 3).

2.4 | Therapeutic difference between CTX and CsA in PLA2R-associated IMN

Among 247 patients with positive renal PLA2R antigen deposit, 33 patients received steroid and CTX, while 103 patients received steroid and CsA. The patients in CTX group received intravenous CTX with a dosage of 0.5-0.75 g/m^2 (maximum of 1 g) monthly for 6 months plus oral prednisone 0.5 mg/kg daily while the patients in CsA group received an initial CsA dose of 3.5 mg/kg/day plus oral prednisone 0.15 mg/kg/day. The dosage of CsA was adjusted according to its serum concentration. Patients treated with CTX experienced a higher level of proteinuria, albuminuria and a lower level of eGFR. Kaplan-Meier analysis showed that in PLA2R-associated IMN, patients in CTX group with a mean treatment duration of 10 months had a higher probability to achieve remission compared with CsA group with a mean treatment duration of 13 months (Log–rank test, P = .018) (Figure 4A). However, there was no significant difference in renal survival between CTX and CsA groups (Log–rank test, P = .075) (Figure 4B). Univariate COX regression analysis showed that compared with CTX, CsA had a lower probability to achieve remission (HR: 1.851; 95% CI: 1.148 to 2.982; P = .011). After adjusting for proteinuria, serum albumin and eGFR at baseline, patients receiving CTX still had a higher probability to achieve remission compared with those receiving CsA (HR: 1.933; 95% CI: 1.123 to 3.325; P = .017) (Table 4).

3 | DISCUSSION

Idiopathic membranous nephropathy is autoantigen-mediated autoimmune glomerular disease. Renal PLA2R antigen had been identified to be target antigen with higher specificity and sensitivity in IMN.[3] Several studies had found that serum anti-PLA2R antibodies were correlated with IMN prognosis.[8,9] Early in the course of the disease, circulating anti-PLA2R antibodies might not be detectable despite their presence in the glomerular deposits, because the kidney acts as a "sink" absorbing all detectable circulating anti-PLA2R antibodies.[10] Therefore, the detection of renal PLA2R antigen deposit would provide early evidence of PLA2R-associated IMN. In addition, along with the remission of proteinuria, anti-PLA2R antibodies would disappear from the circulation while renal PLA2R antigen, with a slow attenuation, would be detectable continuously. Therefore, renal PLA2R antigen deposit is more stable than circulating anti-PLA2R antibodies in different course of disease. We conducted this retrospective cohort to explore the clinicopathological characteristics of PLA2R-associated IMN and the association between renal PLA2R antigen deposit and remission of proteinuria.

In this retrospective cohort study, 82.3% of patients with IMN had positive renal PLA2R deposits, which was comparable to previous researches.[22,23] By comparing the clinical and pathological characteristics between PLA2R-associated and non-PLA2R-associated IMN patients, we found that patients with positive renal PLA2R antigen showed a lower level of serum albumin and a higher level of anti-PLA2R antibody. Previous study[24] has detected a significant lower level of serum albumin and higher level of proteinuria in PLA2R-associated IMN patients. In our study, PLA2R-associated IMN patients had higher level of proteinuria than non-PLA2R-associated IMN patients at baseline. Although the difference does not reach statistically significance, it could have been due to lack of power to detect the significance. The limited sample size may also be responsible for the lack of statistical significance for proteinuria at baseline. In addition, PLA2R-associated IMN patients experienced a higher proportion of IgG4 dominant deposition, and a lower proportion of IgA and C1q deposition. PLA2R had been founded before to co-localize with IgG4 in immune deposits of glomeruli,[3] which indicated that anti-PLA2R antibodies were IgG4 subtype. Therefore, IgG4 was the predominant glomerulus deposition in PLA2R-associated IMN instead of other immunoglobulins or IgG subtypes. Furthermore, IgG4 subclass could not activate classical complement pathway, which would lead to the lower proportion of C1q deposit in PLA2R-associated IMN.

We further explored the association between renal PLA2R antigen and the remission of proteinuria in IMN. Our findings suggested that non-PLA2R-associated IMN patients had a higher probability to achieve remission than PLA2R-associated IMN patients after adjusting for confounders. This result was consistent with previous reports. A retrospective study included 572 IMN patients had found that patients who did not achieve remission had a higher positive rate of renal PLA2R antigen than those achieved remission.[25] Another retrospective cohort study had also identified a higher remission rate in non-PLA2R-associated IMN.[26] The reason for lower probability to achieve remission in PLA2R-associated IMN patients still remained unclear. Autoantibody may target a specific conformational region of PLA2R protein on the podocyte surface and trigger the formation of immune complexes, which may lead to the activation of complement pathway and the enhancement of glomerular permeability to plasma proteins. Moreover, for those received conservative therapy alone without using immunosuppressive agents, patients with negative renal PLA2R antigen deposit at diagnosis had a higher probability of progressing towards spontaneous remission. Therefore, renal PLA2R antigen could be identified as evaluation indicator for initiating immunosuppressive therapy. Non-PLA2R-associated IMN patients were recommended to prolong the duration of conservative therapy on account of its high probability to achieve spontaneous remission compared with PLA2R-associated IMN.

The second point of our study was to investigate the therapeutic prognosis between two immunosuppressant regimens in PLA2R-associated IMN. Both CTX and CsA were the first line initial immunosuppressive therapy protocol for IMN, with similar remission rate according to previous findings.[14] However, there is a paucity of studies regarding the difference of therapeutic prognosis between CTX and CsA in PLA2R-associated IMN. In our study, CTX group had a higher level of proteinuria, albuminuria and a lower level of eGFR

compared with CsA group at baseline. After adjusting for baseline parameters, patients in CTX group had a higher probability to achieve remission than those in CsA group. However, there was no difference in renal survival between CTX and CsA in PLA2R-associated IMN. The disparity in immunosuppressive mechanisms may contribute to the difference of therapeutic prognosis between CTX and CsA. Rosenzwajg[27] et al had found that B and T lymphocyte were all involved in the pathogenesis of MN. B cell would develop into plasma cells, which could produce anti-PLA2R antibodies against corresponding antigen expressed on podocytes. The formation of immune complexes would lead to podocyte injury and persistent proteinuria. Additionally, PLA2R-specific T-cell[28] and B-cell epitope[29] in PLA2R were also associated with the pathogenesis of PLA2R-associated IMN. CTX, as one of cytotoxic drugs, could exert its immunosuppression effect through nonspecific inhibition of T and B lymphocyte proliferation. Nevertheless, CsA exerted its immunosuppression effect on T cell responses through calcineurin-phosphatase pathway and the nuclear translocation of NFAT. Therefore, the better therapeutic prognosis of CTX may be attributed to the co-suppression of T and B lymphocyte, which involved in the pathogenesis of PLA2R-associated IMN. In addition, Faul[30] et al had indicated that the beneficial effect of CsA on proteinuria is not only dependent on NFAT inhibition in T cells, but also results from the stabilization of the actin cytoskeleton in kidney podocytes. Thus, the anti-proteinuric mechanism of CsA may result in its inferiority in therapeutic prognosis compared with CTX in PLA2R-associated IMN.

This study investigated the clinical value of renal PLA2R antigen in the prognostic evaluation of IMN and provided guidance for therapeutic options in PLA2R-associated IMN. However, certain limitations still exist in this study. First, limited by the retrospective nature of our study, serum anti-PLA2R antibodies were unavailable to be detected in all enrolled patients. Second, selection bias could not be avoided due to a retrospective design and there were only 74.3% of patients with a follow-up time more than half a year. Although the mean duration of follow up for 300 consecutive IMN patients was 12 months, it was long enough to identify the difference of short-term therapeutic effects between CsA and CTX.

In conclusion, PLA2R-associated IMN patients had a lower probability to achieve remission compared with non-PLA2R-associated IMN. Compared with CsA, CTX exerted better therapeutic effects in the remission of proteinuria in PLA2R-associated IMN. CTX may be the preferred immunosuppressant for PLA2R-associated IMN.

ACKNOWLEDGEMENTS

This work was financially supported by National Clinical Key Specialty Construction Projects, National Natural Science Foundation of China (Grant No. 81570609), Guangdong Science and Technology Project (Grant No. 2017A070709008) and Guangzhou Science and Technology Project (Grant No. 201604020037).

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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How to cite this article: Xie Z, Dong W, Li Z, et al. Clinical value of renal phospholipase A2 receptor deposit in the prognosis evaluation and treatment options of idiopathic membranous nephropathy: A retrospective cohort study. *Nephrology*. 2020;25:219–229. https://doi.org/10.1111/nep.13691