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Phospholipase-A2 receptor antibody, 24 hours proteinuria, and serum albumin as indicators of cyclophosphamide efficacy in idiopathic membranous nephropathy

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Abstract

Background: We aimed to evaluate cyclophosphamide efficacy in the treatment of idiopathic membranous nephropathy (IMN) and explore the efficacy of phospholipase-A2 receptor antibody (PLA2R-Ab), 24 hours proteinuria, and serum albumin in predicting 6- and 12-month treatment effects.

Methods: A retrospective analysis was performed on 135 patients with IMN who followed up after treatment. The observation points were before, and after 3, 6, and 12 months of treatment. We collected clinical indicator data at each observation point and measured PLA2R-Ab levels before and after 3-month treatment.

Results: The remission rates at 3, 6, and 12 months of cyclophosphamide therapy for patients with IMN were 41.4, 74.8, and 76.1%, respectively. Patients in whom PLA2R-Ab turned negative within 3 months had high remission rates at 3, 6, and 12 months after treatment ($P < .05$). PLA2R-Ab change at 3 months had a strong correlation with 24 hours proteinuria change at 6 months. The change in albumin concentration before and after 3-month treatment was an independent variable related to remission rate at 6 months, and 24 hours proteinuria change before and after 6-month treatment was an independent variable related to remission rate at 12 months after treatment.

Conclusion: Cyclophosphamide showed good efficacy at 3, 6, and 12 months for patients with IMN. Serum albumin change and PLA2R-Ab change at 3 months can be used as indicators to predict remission at 6 months, respectively. Moreover, 24 hours proteinuria change at 6 months can predict remission at 12 months.

KEYWORDS
24h proteinuria, cyclophosphamide, Idiopathic Membranous Nephropathy, PLA2R-Ab, prediction, remission

1 | INTRODUCTION

Idiopathic membranous nephropathy (IMN) is an organ-specific autoimmune disease of unknown etiology. The characteristic pathological feature of IMN is the deposition of immune complexes on the epithelial side of the glomerular basement membrane with glomerular basement membrane thickening.[1] IMN, with high morbidity and mortality, is a common cause of nephrotic syndrome, which easily leads to thromboembolic and cardiovascular events.[2]

The selection of an optimal therapeutic modality for IMN has become a focus of discussion in recent years. In 2012, Kidney Disease: Improving Global Outcomes (KDIGO) provided clinical practice guidelines for the therapy of adult IMN and recommended cyclophosphamide as the first-line drug for treatment of IMN.[3] However, individualized treatment of IMN has remained controversial owing to cyclophosphamide cytotoxicity. Cyclophosphamide is converted into aldophosphamide by microsomal functional oxidase in the liver. Its metabolites have a strong immunosuppressive effect, which can reduce the number of T lymphocytes and B lymphocytes available for inhibiting IMN progression.[4] Some Chinese scholars have improved the cyclophosphamide Ponticelli protocol recommended by KDIGO by using low-dose drugs to treat Han patients with IMN. A clinical report showed that a reduced dose is effective and causes fewer adverse reactions,[5] suggesting that the therapeutic dose of European and American populations is not suitable for Asian populations. In addition, many patients received intravenous cyclophosphamide instead of oral cyclophosphamide because of poor compliance.[6,7] A previous study showed that intravenous cyclophosphamide is safe and effective, with few obvious side effects.[8]

In recent years, several studies suggested combining indicators such as the titer of M-type phospholipase A2 receptor autoantibodies (PLA2R-Ab) and 24 hours proteinuria to guide cyclophosphamide drug regimen. Discovered by Beck in 2009, PLA2R-Ab is an autoantibody present at high levels in the serum of patients with IMN.[9] Many studies have shown that serum PLA2R-Ab levels are related to IMN activity.[10-13] Besides PLA2R antibodies, 24 hours urine protein, and serum albumin (Alb) are also important indicators for clinical condition evaluation.[10,12] Because the change in 24 hours proteinuria always lags behind the change in PLA2R-Ab titer,[13] immunosuppressive therapy should be continued until the antibody status turns negative. If the antibody status does not turn negative and 24 hours proteinuria is observed persistently, substitution of immunosuppressants should be considered.[14]

In the past few years, cyclophosphamide had to be maintained for 6 months and substituted with cyclosporine when patients did not achieve remission after 12-month treatment. In this study, we combined the above-mentioned clinical laboratory indicators to predict the efficacy of cyclophosphamide after 6- or 12-month treatment for predicting whether patients need to upgrade to cyclosporine or rituximab[3] without delay and ensuring benefits to patients for selecting an optimal therapeutic modality economically.

2 | MATERIALS AND METHODS

2.1 | Specimens and clinical data collection

Data of 135 cases with pathological diagnosis of IMN treated with cyclophosphamide from 2014 to 2019 were collected at Shengjing Hospital of China Medical University using retrospective analysis. Follow-up was performed after treatment for 3, 6, and 12 months. The patients included in the study were between 18 and 90 years of age and received renal biopsy. The exclusion criteria were as follows: those with autoimmune diseases such as systemic lupus erythematosus and allergic purpura; Hepatitis B or C virus infections; thyroid and other site tumors; and history of exposure to other immunosuppressants, organic solvents and heavy metals. All the samples were centrifuged with 2214 g for 10 minutes within two hours after collection, aliquoted with screw-cap cryovials, and stored in a −80°C refrigerator for later use. This study was approved by the ethics committee of Shengjing Hospital.

2.2 | Detection of serum PLA2R-Ab and other indicators

The serum PLA2R-Ab levels were determined by enzyme-linked immunosorbent assay double-antibody sandwich method by using EUROIMMUN kits (EUROIMMUN Medizinische Labordiagnostika AG, Germany) according to the manufacturer's instructions. The anti-PLA2R antibody status was considered positive for antibody titer greater than 20 RU/mL. The serum PLA2R-Ab levels were determined at baseline and 3 months after treatment; the other parameters were determined at all time points. Twenty-four h proteinuria, Alb, and creatinine (Cr) were determined by bromocresol green (BCG) method, enzyme method, and colorimetric method, respectively, using the ARCHITECT C16000 Biochemical Analyzer (Abbott Company).

2.3 | Treatment programs

A combination therapy of prednisone and cyclophosphamide was administered to all patients. A 1 mg/kg oral dose of prednisone was administered and gradually reduced after 6-8 weeks. Based on the above treatment, a 0.4 or 0.2 g dose of cyclophosphamide was then added to the treatment plan once every two weeks until an accumulated value of 6-8 g.

2.4 | Definition of clinical remission

(1) Complete remission: 24 hours urine protein excretion of <0.3 g or urine protein–creatinine ratio (UPCr) of <300 mg/g, and normal levels of serum Alb and Cr (2) Partial remission: 24 hours urine protein excretion of <3.5 g or UPCr <3500 mg/g; urine protein reduction ≥50%; and normal serum Alb or elevated and stable serum Cr.

2.5 | Statistical analysis

The Frequencies, mean ± SD, and median (interquartile range, IQR) were used to describe the variables as appropriate. Comparison between categorical variables was performed by Chi-square test. Student's t test and Mann-Whitney U test were used to compare variables with normal and skewed distributions, respectively. Bivariate correlation analysis was used for detection of changes in serum Alb, 24 hours proteinuria, and PLA2R-Ab at 3 months. Correlation of clinical parameters and efficacy in patients with IMN was analyzed by logistic regression. A $P < .05$ indicates that the difference is statistically significant.

3 | RESULTS

3.1 | Overview of cyclophosphamide treatment effect

There were 135 patients at baseline, 135 patients at 3-month follow-up, 132 patients at 6-month follow-up, and 74 patients at 12-month follow-up. Except for two patients in whom 24 hours proteinuria was not measured at 3 months, the complete remission, partial remission, and non-remission rates were 2.3% (3/133), 39.1% (52/133), and 58.6% (78/133), respectively, at 3-month follow-up. At 6 months after treatment, the remission rate of only 123 patients was evaluated because the serum Cr or 24 hours proteinuria data of 9 patients were missing. The complete remission, partial remission, and non-remission rates were 13.0% (17/123), 61.8% (81/123), and 25.2% (33/123), respectively, with a total remission rate of 74.8%. In three of 74 patients who were followed up at 12 months, 24 hours proteinuria was not measured. The complete remission, partial remission, and non-remission rates were 38.0% (27/71), 38.0% (27/71), and 23.9% (17/71), respectively, with a total remission rate of 76.1%.

3.2 | Efficacy of cyclophosphamide treatment in different PLA2R-Ab groups

PLA2R-Ab at baseline was not determined in three of 135 patients. There were 51 PLA2R-Ab-negative patients and 81 PLA2R-Ab-positive patients. Excluding one patient for whom 24 hours proteinuria result was not available, there were 80 patients (61.36%) in the PLA2R-Ab-positive group. Serum Alb was lower in the PLA2R-Ab-positive group than in the PLA2R-Ab-negative group. However, 24 hours proteinuria was not different between the two groups (Table 1). At 3, 6, and 12 months after medication, the overall remission rate between the two groups was not significantly different.

Patients in the PLA2R-Ab-positive group were divided into two groups (A and B) according to whether PLA2R-Ab titer turned negative within 3 months. Group A included 63 patients in whom PLA2R-Ab turned negative and group B included those patients in whom PLA2R-Ab did not turn negative (n = 17). There was no significant difference between the two groups before medication. Alb level and total remission rate in group A were significantly higher than those in group B at 3, 6, and 12 months after medication, while 24 hours proteinuria was significantly lower than that in group B. At 12 months, the Cr level was lower and complete remission was higher in group A than in group B (Table 2).

The difference between PLA2R-Ab concentration at baseline and 3-month follow-up was the change in 3-month PLA2R-Ab. Serum PLA2R-Ab level before and after 3-month treatment was determined in 127 patients. In 17 patients, PLA2R-Ab levels were less than the detection limit both before and after 3-month treatment. The remaining 110 patients were grouped according to their quartile of absolute value of PLA2R-Ab change. Among those patients, 27 cases were in the low-value group (0-10.840 RU/mL), 28 cases in the median-value group (10.840-56.848 RU/mL), 28 cases in the median-high-value group (56.848-171.016 RU/mL), and 27 cases in the high-value group (>171.016 RU/mL). The 3-month PLA2R-Ab change in low-value, median-value, and median-high-value groups was associated with the 6-month 24 hours proteinuria change (see

TABLE 1 Comparison of baseline indicators and monthly remission rates in different PLA2R-Ab groups

Indicators	PLA2R-Ab-negative(n = 51)	PLA2R-Ab-positive(n = 80)	*P*
Alb(g/L)	23.173 ± 4.470	20.450 (18.050-22.850)	**.003**
Cr(μmol/L)	69.512 ± 20.178	67.490 ± 15.231	.516
24 h proteinuria(g/d)	6.150 (4.200-8.610)	6.735 (4.810-11.030)	.105
3-mo complete remission rate	2 (9.80%)	1 (1.28%)	.331
3-mo total remission	18 (35.29%)	36 (46.15%)	.222
6-mo complete remission rate	7 (13.73%)	9 (11.84%)	.754
6-mo total remission	42 (82.35%)	54 (71.05%)	.146
12-mo complete remission rate	15 (55.56%)	12 (27.91%)	**.021**
12-mo total remission	23 (85.19%)	31 (72.09%)	.204

TABLE 2 Comparison of baseline indicators and monthly remission rates in groups with different PLA2R Ab status

Indicators	Month	Group A(n = 63)	Group B(n = 17)	*P*
Alb (g/L)	Baseline	20.400 (18.000-22.000)	21.912 ± 3.989	.277
	3-mo	28.814 ± 4.8498	22.800 ± 6.062	**.000**
	6-mo	33.600 (30.975-36.000)	24.107 ± 8.309	**.001**
	12-mo	36.600 (35.000-39.200)	27.109 ± 7.216	**.000**
Cr(μmol/L)	Baseline	67.062 ± 14.076	69.076 ± 19.346	.631
	3-mo	62.416 ± 12.204	67.106 ± 11.9973	.162
	6-mo	63.740 ± 14.213	64.650 (56.150-79.750)	.319
	12-mo	63.000 (54.500-74.725)	91.400 (68.600-111.800)	**.003**
24 h proteinuria (g/d) (g/d)	Baseline	6.940 (4.840-11.380)	5.650 (4.335-9.880)	.332
	3-mo	2.730 (1.330-4.400)	7.759 ± 6.012	**.002**
	6-mo	1.305(0.478-2.488)	8.880 ± 6.385	**.000**
	12-mo	0.530 (0.160-1.813)	5.232 ± 3.268	**.000**
Complete remission	3-mo	1 (6.25%)	0	.595
	6-mo	9 (15%)	0	.099
	12-mo	12 (37.5%)	0	**.017**
Total remission	3-mo	34 (55.74%)	2 (11.76%)	**.000**
	6-mo	51 (85%)	3 (18.75%)	**.000**
	12-mo	27 (84.38%)	4 (36.36%)	**.002**

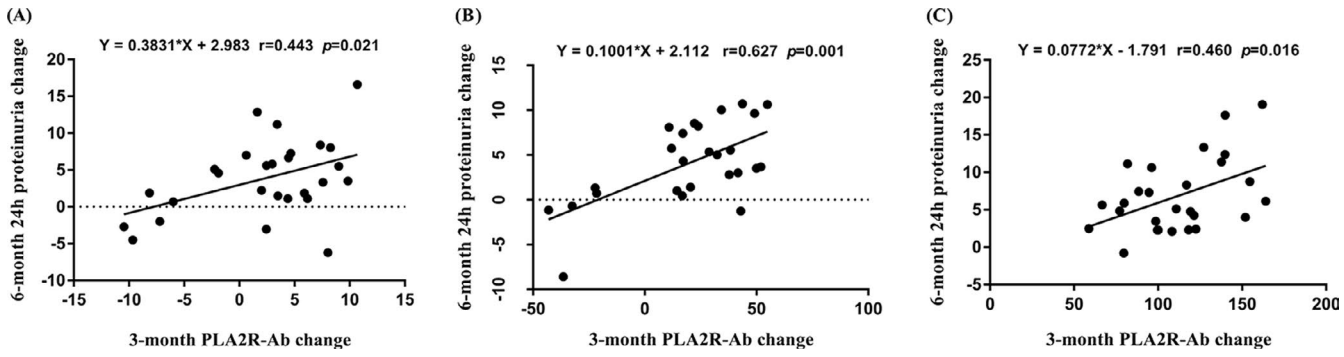

FIGURE 1 A-C, Correlation analysis between the 3-mo PLA2R-Ab changes and the 6-month 24h proteinuria changes in the low-, median-, and median-high-value groups

Figure 1A-C for details). These three groups were combined to form the non-high-value group. In the non-high-value group, the 3-month PLA2R-Ab changes were positively correlated with the 6-month 24 hours proteinuria changes (r = 0.437, *P* = .000 (Figure 2)).

3.3 | Logistic regression analysis of clinical indicators and effect of cyclophosphamide in patients with IMN

To explore the relationship between clinical parameters and the curative effect of cyclophosphamide on IMN, we performed a logistic regression analysis; the dependent variable was whether remission occurred after 6 or 12 months of cyclophosphamide treatment. The clinical parameters were independent variables.

The outcome after 6- or 12-month treatment with cyclophosphamide was taken as the dependent variable (1 is remission, 0 is no remission), and a univariate analysis was performed for each clinical indicator. The 3-month PLA2R-Ab change was divided according to the interquartile range. △L was < 7.40 RU/mL (at 6 months) and <0.61 RU/mL (at 12 months), △M was 7.40-50.93 RU/mL (at 6 months) and 0.61-38.02 RU/mL (at 12 months), △M-H was 50.93-154.00 RU/mL (at 6 months) and 38.02-122.21 RU/mL (at 12 months), and △H was >154.00 RU/mL (at 6 months) and >122.21 RU/mL (at 12 months). Univariate analysis showed that the following variables affected 6-month remission: 3-month PLA2R-Ab

△L; 3-month PLA2R-Ab △M; 3-month Alb change; and 3-month 24 hours proteinuria change. The detailed results are presented in Table 3. The variables affecting 12-month remission were as follows: 6-month 24 hours proteinuria change and baseline Alb (Table 4).

A multivariate analysis was performed in which outcome after 6 or 12 months of treatment with cyclophosphamide was taken as the dependent variable (1 is remission, 0 is no remission). The significant variable in the univariate analysis was used as the independent variable. The results obtained at 6 months after treatment showed that the 3-month Alb change was an independent relevant variable for 6-month remission of IMN (odds ratio [OR], 0778, Table 5)). The results obtained at 12 months after treatment showed that the 6-month 24 hours proteinuria change was an independent variable related to 12-month remission of IMN (OR,1.199, Table 6).

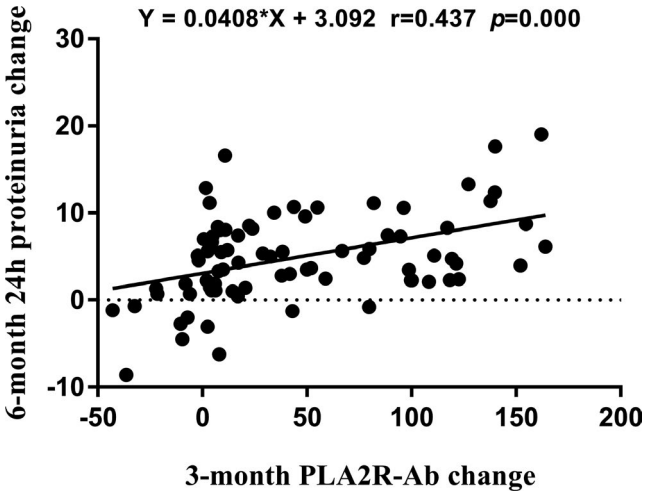

FIGURE 2 Correlation analysis between the 3-mo PLA2R-Ab changes and the 6-month 24h proteinuria changes in the non-high-value group

4 | DISCUSSION

The pathogenesis of IMN, a primary glomerular disease, has not been fully clarified. The selection of an appropriate treatment plan and prediction of treatment effect are hot topics of IMN research. In this study, we found that cyclophosphamide had a good therapeutic effect on IMN. There was no difference in the effect between PLA2R-Ab-positive or -negative patients before treatment. The patients whose PLA2R-Ab titer turned negative within 3 months had better prognosis than patients who maintained PLA2R-Ab-positive titer. In addition, when the absolute value was between 0-171.016, PLA2R-Ab change at 3 months had a strong correlation with 24 hours proteinuria change at 6 months, which could accurately predict the change in 24 hours proteinuria at 6 months. Moreover, Alb at 3 months was an independent variable related to 6-month remission of IMN, and 6-month 24 hours proteinuria change was an independent variable related to 12-month remission of IMN.

The remission rates of cyclophosphamide treatment in this study were similar to the findings of Qiu.[15] Our results showed that cyclophosphamide was effective for achieving remission in patients with IMN at 6 and 12 months. The complete remission rate after 1-year treatment greatly improved compared with that after 3- and 6-month treatment. This finding indicated that cyclophosphamide treatment should be maintained for at least 6 months and discontinuation of treatment prematurely was not conducive for patients. The total remission rate at 12 months was similar to that at 6 months, which may be related to the excessive number of patients who had dropped out during the follow-up and the small number of samples

TABLE 3 Univariate analysis for curative effect of IMN patients at 6-mo (n = 108)

	B	SE	Wald	OR	*P*
3-month PLA2R-Ab△L			7.901		**.048**
3-month PLA2R-Ab△M	−1.407	0.628	5.031	0.245 (0.072-0.837)	**.025**
3-month PLA2R-Ab△M-H	0.000	0.701	0.000	1.000 (0.253-3.948)	1.000
3-month PLA2R-Ab△H	−0.229	0.678	0.114	0.795 (0.211-3.004)	.736
Different baseline PLA2R-Ab	−0.316	0.498	0.402	0.729 (0.275-1.936)	.526
Baseline Alb (g/L)	−0.013	0.052	0.058	0.987 (0.891-1.094)	.810
Baseline Cr(μmol/L)	−0.008	0.013	0.370	0.992 (0.968-1.017)	.543
3-month albumin change	−0.215	0.053	16.802	0.806 (0.727-0.894)	**.000**
3-month 24 h proteinuria change	−0.139	0.058	5.705	1.149 (1.025-1.289)	.017

Note: PLA2R-Ab changes are divided into four groups according to the interquartile range. △L is a low change, △M is a medium change, △M-H is a medium-high change, and △H is a high change.
Abbreviations: B, Coefficient value; SE, Standard error; Wald, Chi-square value.

TABLE 4 Univariate analysis for curative effect of IMN patients at 12-mo (n = 68)

	B	SE	Wald	OR	*P*
6-month albumin change	−0.035	0.028	1.632	0.965 (0.915-1.019)	.201
6-month 24 h proteinuria change	0.173	0.080	4.692	1.189 (1.017-1.391)	**.030**
3-month albumin change	−0.054	0.046	1.39	0.947 (0.866-0.866)	.238
3-month 24 h proteinuria change	0.076	0.065	1.357	1.079 (0.949-1.227)	.244
Different baseline PLA2R-Ab	−1.264	0.696	3.297	0.282 (0.072-1.106)	.069
Baseline Alb (g/L)	0.163	0.082	3.904	1.177 (1.001-1.383)	**.048**
Baseline Cr (μmol/L)	0.017	0.017	1.008	1.018 (0.983-1.053)	.315

Abbreviations: B, Coefficient value; SE, Standard error; Wald, Chi-square value.

TABLE 5 Multivariate analysis for curative effect of IMN patients at 6-month (n = 108)

	B	SE	Wald	OR	*P*
3-mo PLA2R-Ab△L			1.258		.733
3-mo PLA2R-Ab△M	−0.694	0.761	0.831	0.500 (0.112-2.221)	.362
3-mo PLA2R-Ab△M-H	−0.276	0.856	0.104	0.759 (0.142-4.059)	.747
3-mo PLA2R-Ab△H	0.030	0.853	0.001	1.031 (0.194-5.487)	.972
3-mo albumin change	−0.251	0.071	12.473	0.778 (0.676-0.894)	**.000**
3-mo 24 h proteinuria change	0.013	0.061	0.048	1.013 (0.899-1.143)	.827

Note: PLA2R-Ab changes are divided into four groups according to the interquartile range. △L is a low change, △M is a medium change, △M-H is a medium-high change, and △H is a high change.
Abbreviations: B, Coefficient value; SE, Standard error; Wald, Chi-square value.

TABLE 6 Multivariate analysis for curative effect of IMN patients at 12-mo (n = 68)

	B	SE	Wald	OR	*P*
Baseline Alb (g/L)	0.157	0.089	3.080	1.17 (0.982-1.394)	.079
6-mo 24 h proteinuria change	0.182	0.078	5.397	1.199 (1.029-1.398)	**.020**

Abbreviations: B, Coefficient value; SE, Standard error; Wald, Chi-square value.

at 12 months. The treatment effect at 3 months was not as good as that at 6 and 12 months.

In the present study, the proportion of PLA2R-Ab-positive patients with IMN was 61.36%, which was similar to the results reported by Li[16] et al In addition, our results showed that the negative or positive titer of PLA2R-Ab before treatment was not associated with the total remission rate after treatment (Table 1), which is consistent with the results reported by Bech et al[17] and Medrano et al.[18] This finding suggested that the baseline PLA2R-Ab level may not be a predictor of late-stage efficacy. However, the results of meta-analysis by Liang[19] indicated that the clinical remission rate of PLA2R-Ab-positive patients is poor. The higher the PLA2R-Ab titer, the lower the chance of clinical remission. The results of Kim[20] et al and Xu[21] also support this view. The difference between our results and the meta-analysis above may be due to the following two reasons: (a) PLA2R-Ab is released in the circulatory system at the early stage of disease; it binds to the target antigen on podocytes and can be quickly cleared from blood. PLA2R-Ab shows seropositivity only when the production rate exceeds the clearance power of the kidneys. Thus, PLA2R-Ab may be false-negative in some patients at the early stage of the disease, resulting in no difference in late-stage efficacy between the two groups.[22] (b) The presence of autoantibodies other than PLA2R-Ab, such as autoantibody against THSD7A,[23] in patients

could be another reason for the difference. The incidence of IMN in these patients is not related to PLA2R-Ab.

As negative or positive titer of PLA2R-Ab at baseline is not correlated with the follow-up effect, we further explored whether remission rate was different between patients in whom PLA2R-Ab-positive titer turned negative and those in whom PLA2R-Ab remained positive within 3 months. We found that group A had significantly better treatment results and remission rates (Table 2) than group B. This shows that a decrease in PLA2R-Ab is a predictor of improvement and monitoring of PLA2R-Ab can effectively predict efficacy.[13,24] A previous study suggested that a positive PLA2R-Ab transfusion may indicate a recurrence of the disease.[25]

This is the first study that shows correlation between the 3-month PLA2R-Ab titer change and 6-month 24 hours proteinuria change. We conducted a series of analyses and found that there was a good correlation between the 3-month PLA2R-Ab change and 6-month 24 hours proteinuria change in the median-value group (Figure 1B). Thus, 3-month PLA2R-Ab change may predict the treatment effect after 6-month treatment. The 3-month PLA2R-Ab change in the low-value and middle-high-value groups (Figure 1A, C) was also correlated with the 6-month 24 hours proteinuria change; however, the correlation was not as strong as that in the median-value group. The median follow-up period in the study by Radice[13] et al was 14.9 (1-38) months. They reported that PLA2R-Ab change at the last observation point correlated with serum Alb change and 24 hours albuminuria change at the last observation point.[13] Nevertheless, we did not find a correlation between 3-month PLA2R-Ab change, Alb change, and 24 hours proteinuria change. This may be because of the short follow-up of only 3 months in our study. Another reason could be the long observation time in the study by Radice et al, which may include some recurrence cases.

Logistic regression analysis was performed to identify the factors that can predict the efficacy of cyclophosphamide treatment in patients with IMN after 6 or 12 months (Tables 3-6). The results showed that 3-month albumin change can predict remission at 6 months. In addition, a high 6-month 24 hours proteinuria change was one of the independent risk factors for patients with IMN achieving remission after 12-month treatment. The results of univariate analysis showed that baseline Alb can predict the 12-month efficacy. However, when multivariate analysis was performed, baseline Alb was not an independent risk factor for 12-month IMN remission. This result was different from a previous study by Chen,[26] wherein 3-month albumin change and 6-month 24 hours proteinuria change were not included in the equation. These findings suggested that the clinical monitoring of changes of various indicators should be adopted to predict the treatment effect more accurately in patients with IMN. The limitation of this study included a relatively small sample size and a 45% loss to 12-month follow-up.

In summary, cyclophosphamide has been shown to be a potent therapeutic agent for remission of IMN. The presence or absence of PLA2R-Ab before treatment is not correlated with the response rate after treatment. However, the combination of changes in serum Alb, 24 hours proteinuria, and PLA2R-Ab titer can effectively assist in predicting the efficacy during treatment. Therefore, it is important to monitor these parameters dynamically during IMN treatment period which may help the physicians to adjust the treatment plan timely.

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