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Response to immunosuppressive therapy in PLA₂R-associated and non-PLA₂R-associated idiopathic membranous nephropathy: a retrospective, multicenter cohort study

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Abstract

Background: According to renal M type phospholipase A₂ receptor (PLA₂R) immunohistochemistry, idiopathic membranous nephropathy (IMN) could be categorized into PLA₂R-associated and non-PLA₂R-associated IMN. We conducted a retrospective, multicenter cohort study with 91 patients to compare the effect of immunosuppressive therapy between PLA₂R-associated and non-PLA₂R-associated IMN patients.

Methods: A total of 91 biopsy-proven IMN patients from Huashan hospital and People's Hospital of Wuxi in past 5 years were collected into this study. IMN with positive PLA₂R immunohistochemistry in kidney biopsies were designated as PLA₂R-associated IMN. Seventy-eight of the 91 IMN patients was PLA₂R-associated IMN and 13 were non-PLA₂R-associated IMN. Forty-five patients were treated with prednisone plus cyclophosphamide (CTX), and 46 with prednisone plus calcineurin inhibitors (CNIs). The follow-up duration was 15 months.

Results: The total remission rate (76.9% versus 44.9%, $p = 0.032$) and complete remission rate (30.8% versus 2.6%, $p = 0.003$) were both significantly higher in the non-PLA₂R-associated group than in the PLA₂R-associated group at the 3rd month visit point, and at the 6th month time point, the complete remission rate was still significantly higher in the non-PLA₂R-associated group (46.2% versus 11.5%, $p = 0.007$). But similar remission rates were found after the 9th month. Relapses were observed in 8 patients in PLA₂R-associated group and none in non-PLA₂R-associated group, although there was no significant difference between these two groups.

Conclusion: Compared with the PLA₂R-associated IMN, the non-PLA₂R-associated IMN responded quicker to the immunosuppressive therapy.

Keywords: Calcineurin inhibitors, Cyclophosphamide, Idiopathic membranous nephropathy, M type phospholipase A₂ receptor, Immunosuppressive therapy, Remission

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Background

Idiopathic membranous nephropathy (IMN) is one of the most common causes of adult nephrotic syndrome and its incidence is increasing dramatically in China [1]. The clinical course of IMN is heterogeneous: about 30% of patients have spontaneous remission; 20–40% of patients progress to renal failure; and the remaining patients maintain their proteinuria with normal renal function [2–4]. Among the patients without spontaneous remission, 70–80% was reported to respond to cyclophosphamide (CTX) or calcineurin inhibitors (CNIs) and have a better outcome than those who failed to respond to immunosuppressive therapies [5–9].

In 2009, Beck et al. identified an antibody against M-type phospholipase A₂ receptor (PLA₂R) in about 70% of IMN patients [10]. Recently another autoantibody that recognizes thrombospondin type 1 domain containing 7A (THSD7A) has been identified and is responsible for about 3% of IMN [11]. Studies showed that serum anti-PLA₂R autoantibody (PLA₂R-Ab) is a potential biomarker that is associated with disease activity, response to treatment and relapse [12–14]. Our previous study as well as others has shown that most of the patients who have positive serum PLA₂R-Ab also have detectable PLA₂R expression in the kidney along capillary loops; while those who have a positive PLA₂R in the kidney tissue do not necessarily have positive serum PLA₂R-Ab [15]. These data may suggest that kidney PLA₂R expression is a more reliable marker for PLA₂R-associated IMN, since serum PLA₂R-Ab may turn negative in situations such as automatic remission or following treatment [15–18]. Accordingly, renal PLA₂R can be used to classify PLA₂R-associated IMN or non-PLA₂R-associated IMN. Although strong evidence supports that subepithelial immunocomplex formation is a common mechanism responsible for the pathogenesis of MN, different autoantibodies may reflect different disease initiation and/or may lead to different target-antigen-associated changes, which may contribute to the heterogeneity of IMN. In the present study, we used renal PLA₂R to classify IMN into PLA₂R-associated and non-PLA₂R-associated IMN and examine whether there was any difference in response to immunosuppressive therapy between these two groups.

Methods

Patients

Patients with biopsy-proven IMN and treated with immune-suppressants for at least 6 months in Huashan Hospital and People's Hospital of Wuxi were included into this retrospective, multicenter cohort study from January 2008 to June 2014. Secondary causes of membranous nephropathy, such as lupus, hepatitis or

malignancy, were excluded. This study was approved by ethic committee of Huashan Hospital, Fudan University.

Renal PLA₂R staining

Renal PLA₂R of IMN patients was detected by indirect immunofluorescence in paraffin-embedded sections. Citrate buffer of pH 6.0 and microwaving at 100% power for 8 min were used for antigen retrieval and 3% bovine serum albumin was used for blocking. A commercial available anti-PLA₂R antibody (produced in rabbit, Sigma, HPA012657) was diluted at 1: 500 and incubated at 4 °C over-night. The secondary antibody was a fluorescein Cy3-conjugated donkey anti-rabbit IgG antibody (Chemicon, AP182C) and diluted at 1: 200. Each case was run with a positive and negative control (secondary antibody only) (Fig. 1).

Treatment protocols

The patients in CTX group received CTX intravenously with a dose of 0.5–0.75 g/m²(maximum of 1 g) monthly for six months and then once every 2 or 3 months. The initial dose of oral prednisone was 1 mg/kg/day (maximum of 70 mg/day) and tapered after 6–8 weeks. Ninety-six percent of the patients were treated with angiotensin converting enzyme inhibitors (ACEI)/angiotensin receptor blockers (ARB). The patients in CNIs group received an initial tacrolimus dose of 0.05–0.1 mg/kg/day or an initial cyclosporin A (CsA) dose of 3 mg/kg, which was then adjusted based on blood concentration. These patients visited the clinic every 2 weeks during the first 3 months of treatment. The blood trough concentration of tacrolimus was maintained in 5–10 ng/ml, and that of CsA was 100–150 ng/ml. The patients in CNIs group received oral prednisone at the dose of 0.5 mg/kg/day and tapered after 8–12 weeks. Ninety-four percent of the patients in CNI group were on ACEI/ARB.

Definition of remission

Complete remission (CR) was defined as urinary protein excretion <0.3 g/24 h, with a normal serum albumin (ALB) concentration and a normal serum creatinine (SCR). Partial remission (PR) was defined as urinary protein excretion <3.5 g/24 h or a ≥ 50% reduction from peak value, accompanied by an improving or normalization serum ALB concentration and stable SCR. Total remission (TR) was defined as patients achieved CR or PR. Non-remission (NR) was defined when a patient received neither CR nor PR. Relapse in patients who had achieved CR or PR was defined as urinary protein excretion >3.5 g/24 h or >50% of the peak value, with a reduction of serum ALB concentration.

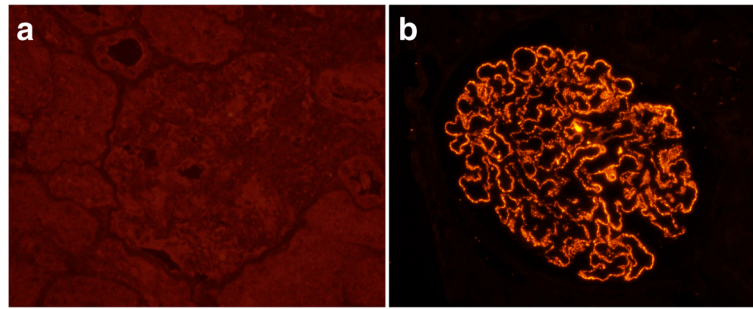


Fig. 1 Renal PLA₂R staining in IMN patients. (indirect immunofluorescence; original magnification ×400) **a** non-PLA₂R-associated IMN; **b** PLA₂R-associated IMN

Calculations and statistics

One-sample K-S testing was used to detect whether continuous variables were normal distribution. Normally distributed continuous variables were compared using the independent samples T test, and the results were expressed as mean values with standard deviations (mean ± SD). Abnormally distributed continuous variables were compared using two independent samples non-parametric test, and results were given as median (range interquartile). Categorical variables were compared using the χ^2 test. A p value <0.05 was considered significant. The statistical analysis was performed using SPSS 13.0 software.

Results

A total of 231 adult patients were diagnosed as IMN by kidney biopsy from January 2008 to June 2014 in Huashan Hospital and People's Hospital of Wuxi. Among these patients, 189 were PLA₂R-associated IMN and 42 were non-PLA₂R-associated IMN. Ninety-one patients received immunosuppressive therapy for at least 6 months were included in the study by December 2014. Seventy-eight of them were PLA₂R-associated IMN and 13 were non-PLA₂R-associated IMN. Forty-five were treated with prednisone plus CTX, and 46 with prednisone plus CNIs. Follow-up was scheduled every 3 months. Nine patients in CTX group were switched to CNIs (8 NR patients, 1 PR patients who received transurethral resection of bladder neoplasm) and 2 patients lost to follow-up during the 15 months observation period. Six patients in CNIs group lost to follow-up and 5 NR patients were switched to CTX (Fig. 2).

Between the 78 PLA₂R-associated IMN and 13 non-PLA₂R-associated IMN patients, there were no significant differences in demographic or laboratory characteristic at baseline (Table 1). One third of the patients with PLA₂R-associated IMN had previously been treated with ACEIs/ARB for 1.34 ± 2.39 months, whereas 38.5% of non-PLA₂R-associated patients had been treated for 0.92 ± 1.26 months. No significant difference was

observed in previous non-immunosuppressive treatments between the two groups. None of the patients in both groups received previous immunosuppressive treatment. The average time for TR and CR in non-PLA₂R-associated IMN patients was 3.36 ± 1.91 and 5.50 ± 4.18 months, significantly shorter than TR (4.46 ± 2.39 months, $p = 0.041$) and CR (8.72 ± 3.31 months, $p = 0.020$) in PLA₂R-associated patients. At the 3rd month point, both TR rate (76.9% versus 44.9%, $p = 0.032$) and CR rate (30.8% versus 2.6%, $p = 0.003$) in non-PLA₂R-associated group were significantly higher than that in PLA₂R-associated group. Although TR rate was similar in the two groups at the 6th month point, the CR rate in non-PLA₂R-associated group was still higher (46.2% versus 11.5%, $p = 0.007$). All patients in non-PLA₂R-associated group got remission at the 12th month point except one patient who lost to follow-up, and 79.7% of the patients in the PLA₂R-associated group got remission. However, there was no significant difference between these 2 groups at this point (Fig. 3a). Relapse was found in 8/78 patients of PLA₂R-associated group and 0/13 of non-PLA₂R-associated group, but there was no statistical difference ($p > 0.1$). Similar results were found using intention-to-treat analysis at every follow-up point.

Among the 91 patients in present study, 45 were treated with prednisone plus CTX, and 46 with prednisone plus CNIs. There were no significant differences in demographic or laboratory features at baseline between the two groups, except higher SCR in CTX group ($p = 0.003$) (Table 2). Thirty-eight percent of the patients in CTX group had previously been treated with ACEIs/ARB for 1.42 ± 2.51 months, whereas 30.4% in CNIs group for 1.14 ± 2.00 months. Other patients received immunosuppressive therapy immediately when they diagnosed with IMN. No significant difference was observed in previous non-immunosuppressive treatments between the two groups. Remission and relapse in CTX and CNIs group during the observation period were illustrated in Fig. 4. There was no significant difference in TR and CR between CTX and CNIs group during the

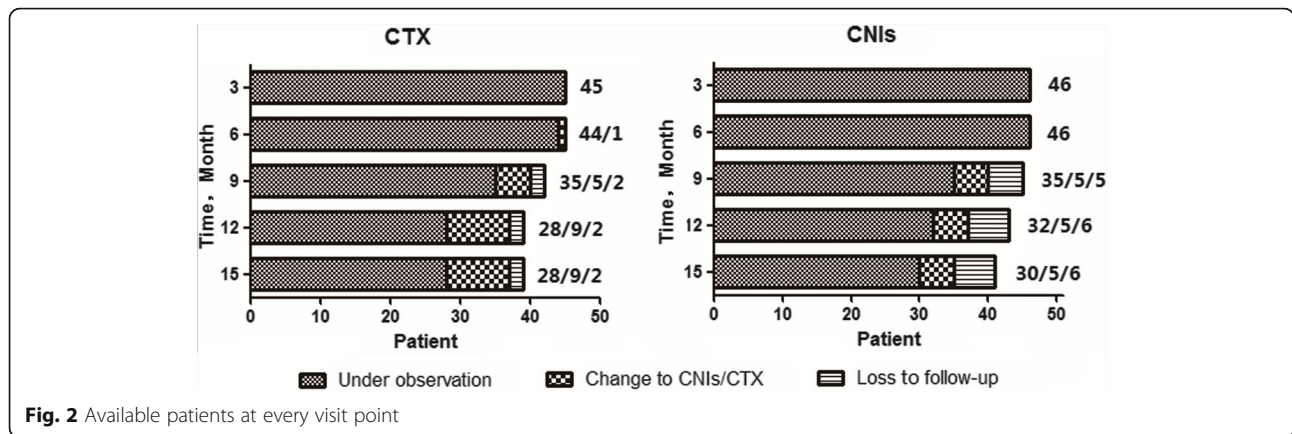


Fig. 2 Available patients at every visit point

15 months of follow-up. One of the relapses in CNIs group occurred at the 12th month point, and 5 occurred at the 15th month point. The only relapse in CTX group occurred at the 15th month. The relapse rate was 4.2% in CTX group and 20.7% in CNIs group during the 15-month study period ($p = 0.174$). Side effects were observed in 7 patients in CTX group and 5 in CNIs group ($p > 0.05$, Table 2).

To remove the confounding of immunosuppressant, stratified analysis was processed according to CTX or CNIs therapy. Fifty-three percent (41/78, tacrolimus 32 and cyclosporine 9) of the patients in PLA₂R-associated group and 38% (5/13, tacrolimus 3 and cyclosporine 2) in non-PLA₂R-associated group were treated with CNIs, which was not significantly different between these 2 groups. In the patients treated with CNIs, CR rate in non-PLA₂R-associated patients was higher than that in PLA₂R-associated patients at the 3rd ($p = 0.028$) and 6th month point ($p = 0.020$), whereas no significant difference in TR rate between the two groups (Fig. 3b). In the patients treated with CTX, there was no statistical difference

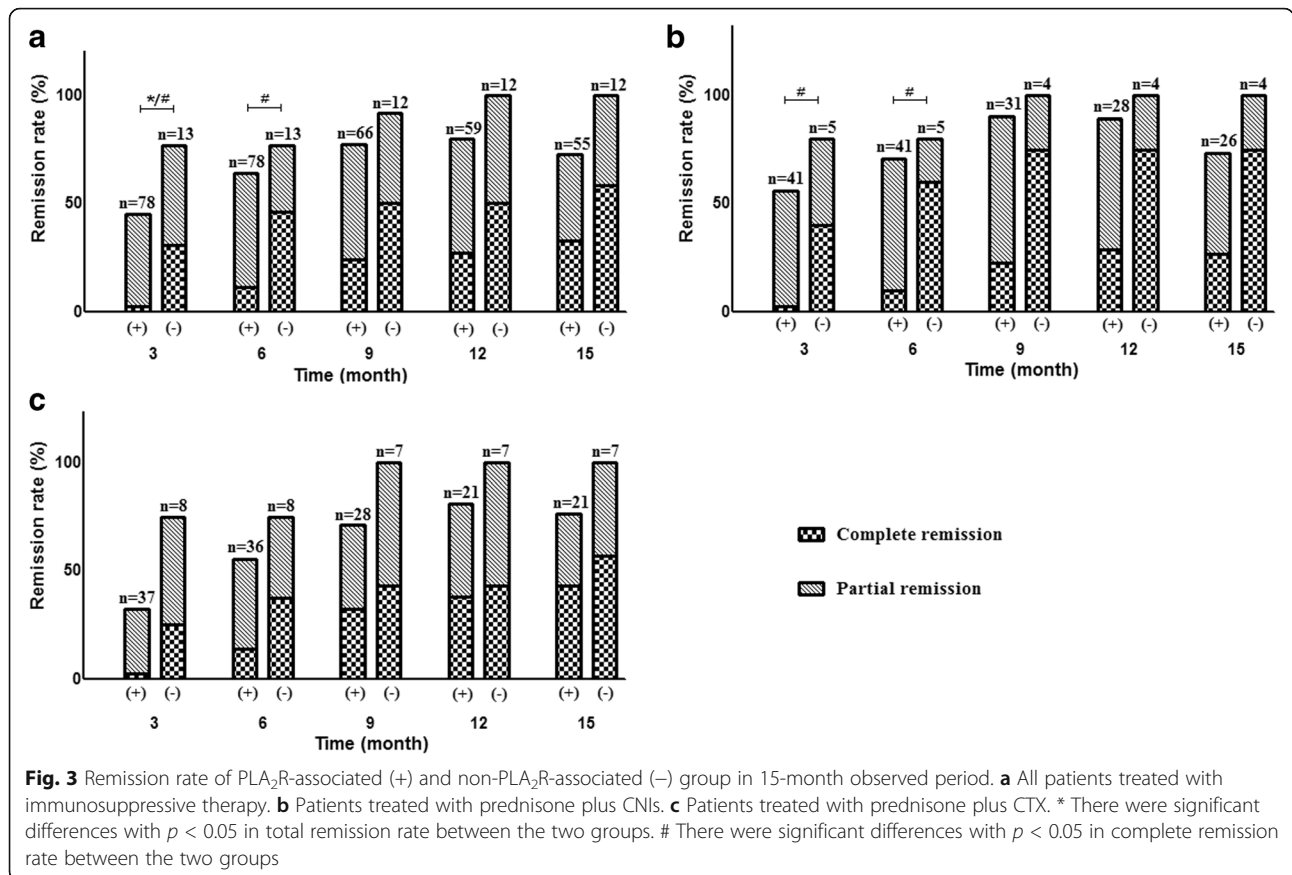
in both the TR and CR rate between non-PLA₂R-associated and PLA₂R-associated groups throughout the whole study period.

Discussion

Although spontaneous remission occurred in about 30% of the untreated IMN patients, worsen renal function had been observed in another 20–40% patients, and they were recommended to receive immunosuppressive therapy [2–4]. Previous studies showed that the remission rates of prednisone plus CTX and prednisone plus CNIs were similar, so both of them were recommended as first line therapy for IMN patients. Since the finding of the autoantibody to podocyte antigen PLA₂R in membranous nephropathy patients in 2009, accumulating evidences have shown that there was no significant difference in some relevant clinical parameters, such as age, gender, proteinuria or serum creatinine, between PLA₂R-associated and non-PLA₂R-associated IMN [16, 18], but there is a paucity of data regarding the treatment response between the PLA₂R-associated

Table 1 Baseline characteristics between PLA₂R-associated and non-PLA₂R-associated groups

	PLA ₂ R-associated	non-PLA ₂ R-associated	P value
Patients	78	13	
CTX %	47.4% (37/78)	61.5% (8/13)	0.346
Gender (M:F)	52:26	5:8	0.102
Age (year)	53.81 ± 14.64	53.54 ± 17.52	0.953
Urine Protein (g/24 h)	5.51 (4.02, 7.72)	5.23 (2.34, 12.51)	0.829
Albumin (g/L)	19.45 ± 5.05	21.58 ± 9.07	0.423
Creatinine (μmol/L)	78 (61.65, 93.75)	68.6 (60, 132.5)	0.875
Cholesterol (mmol/L)	7.86 ± 2.69	6.93 ± 1.88	0.238
Triglyceride (mmol/L)	2.1 (1.77, 3.21)	1.82 (1.57, 2.98)	0.434
Systolic Pressure (mmHg)	131.82 ± 17.99	121.54 ± 12.91	0.052
Diastolic pressure (mmHg)	80 (72, 90)	80 (66, 81)	0.180



and non-PLA₂R-associated IMN. A recent study compared PLA₂R-associated IMN patients who were serum PLA₂R-Ab(-) with patients who were serum PLA₂R-Ab(+), and found that patients who were serum PLA₂R-Ab(+) exhibited higher levels of proteinuria and a lower chance of proteinuria remission [19]. However, serum PLA₂R-Ab has been generally considered as a marker of disease severity and could be disappeared after immunosuppressive therapy or remission. Therefore, our study focused on the relationship between renal PLA₂R and the treatment response to immunosuppressive therapy, and found that the TR and CR rates were both significantly higher in non-PLA₂R-associated group than in PLA₂R-associated group at the 3rd month visit point, and the CR rate was still significantly higher in the non-PLA₂R-associated group at the 6th month time point. We also observed that relapses occurred in 8 out of 48 patients in PLA₂R-associated group and none in non-PLA₂R-associated group, although there was no significant difference.

The reason of faster response to immunosuppressive treatment in non-PLA₂R-associated IMN patients than in PLA₂R-associated IMN is unclear. Renal PLA₂R has been identified as a major target antigen in about 80% IMN patients [15, 20]. In renal PLA₂R negative IMN

patients (non-PLA₂R associated), alternative target antigens in podocyte other than PLA₂R could be responsible for the disease, such as recently identified thrombospondin type-1 domain-containing 7A (THSD7A) in about 10% of non-PLA₂R-associated IMN patients [11]; Cationic bovine serum albumin could cause childhood IMN, and children who had high levels of both circulating cationic BSA and BSA-specific antibodies were negative for anti-PLA₂R antibodies [21]; Antibodies against the superoxide dismutase 2 (SOD2) was found in serum and glomeruli of IMN patients but not in patients with other glomerular diseases or normal kidney and might cause IMN [22, 23]. Different auto-antigen targeted by an antibody may give rise to a different response to treatment. We then analyzed the remission stratified by suppressants CTX or CNIs and also found that the patients treated with CNIs had higher CR rate in non-PLA₂R-associated group than in PLA₂R-associated group at the 3rd and 6th month. In patients treated with CTX, the total remission rate (75% versus 32.4%, $p = 0.067$) and complete remission rate (25% versus 2.7%, $p = 0.077$) tended to be higher in non-PLA₂R-associated group at the 3rd point, but unfortunately the difference was not statistical significant. This may be attributed to the small sample size after stratification.

Table 2 Baseline characteristics between CTX and CNIs group

	CTX	CNIs	P value
Patients	45	46	
Gender (M:F)	28:17	29:17	0.935
Age (year)	55.18 ± 13.48	52.39 ± 16.34	0.377
PLA2R-associated %	82.2% (37/45)	89.1% (41/46)	0.346
Previously used ACEI/ARB %	37.8% (17/45)	30.4% (14/46)	0.460
Urine Protein (g/24 h)	5.87 (4.28, 9.53)	4.93 (3.66, 7.33)	0.096
Albumin (g/L)	19.48 ± 6.13	20.03 ± 5.45	0.650
Creatinine (μmol/L)	94.95 ± 37.48	74.37 ± 23.93	0.003*
Cholesterol (mmol/L)	7.80 ± 2.30	7.64 ± 2.90	0.769
Triglyceride (mmol/L)	2.02 (1.78, 3.53)	2.09 (1.68, 3.04)	0.504
Systolic Pressure (mmHg)	132.05 ± 19.03	128.62 ± 16.24	0.364
Diastolic pressure (mmHg)	80.73 ± 9.33	80.82 ± 10.82	0.965
Side effects	7	5	0.509
Pneumonia	1	3	0.625
Hepatic dysfunction	2	1	0.985
Myelosuppression	1	0	0.495
Neoplasm of bladder	1	0	0.495
Intracranial hemorrhage	1	0	0.495
Gastrointestinal hemorrhage	1	0	0.495
Renal function deterioration	0	1	0.495

In the present study, the treatment protocol of CTX was pulsed intravenous CTX once every month for 6 months and then once every 2 or 3 months, plus oral prednisone daily. Intravenous CTX was used because oral CTX is not available in our area. The remission rate was 82.1% after 15 months of treatment, similar to the rate in another study from China (87.5% at the 12th month point, 92.9% at the 18th month point) [24], and consistent with other studies used Ponticelli or Dutch method. According to Ponticelli protocol, which is a 6-month regimen consisting of daily oral CTX alternating monthly with corticosteroids, the remission rates are 72–93% [5, 6]; In Dutch protocol, which used oral CTX daily for 12 months plus oral prednisone daily or every other day for 6 months, the remission rates are 84–93% [9, 25]. So the remission rate of intravenous CTX in the present is similar to oral CTX protocol in the literature, but total CTX accumulation in intravenous CTX protocol is much less compared to oral protocol. In the CNIs group, the remission rate was 76.7%, also consistent with the literature that shows the remission rate of 75–85% in the patients with CsA plus prednisone treatment [26, 27], and 79–87.5% with tacrolimus plus prednisone treatment [24, 28].

There are some studies compared CTX and CNIs in the treatment of IMN. Two trials from China both drew a conclusion that response was quicker in CNIs group compared with CTX group [24, 28]. Whereas, a study from India, which included non-immunosuppressive therapy resistant IMN patients, observed a similar remission rate at the end of 6 and 12 months between CTX and CNIs groups, and the difference with these studies may be explained by genetic differences and patient selection criteria [29]. In our study, TR rate was 58.7% in CNIs group whereas 40% in CTX group at the 3rd month visit point, but difference was not significant

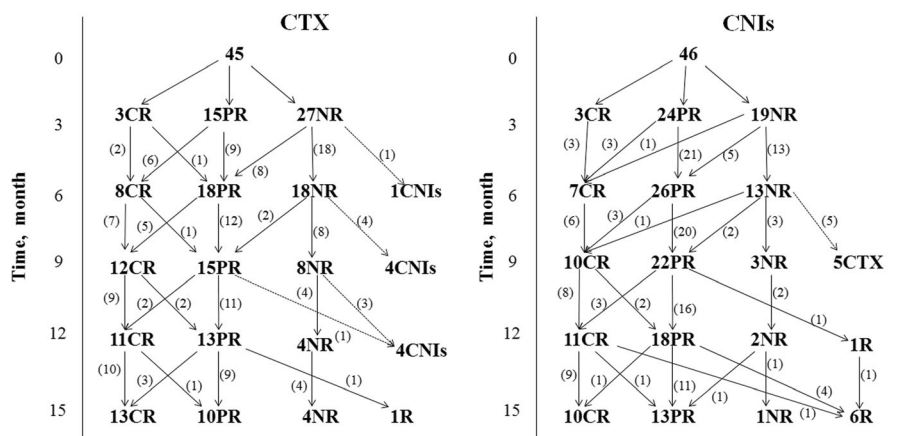


Fig. 4 Remission and relapses in the CTX and CNIs group in 15-month observed period. (Abbreviations are: CR, complete remission; PR, partial remission; NR, non-remission; R, relapse)

($p = 0.075$). And there were no significant differences in TR and CR rate between the two groups after 6 months of follow-up. In previous studies, relapse was found in 13–50% patients treated by CNIs within 1 year of drug withdrawal [7, 28], but there was no significant difference when compared to the CTX plus corticosteroids treatment [24, 27, 28]. In our study, relapses tended to occur more often in the CNIs group but the difference was not significant (20.7% versus 4.2%, $p = 0.174$). If the sample enlarged, more difference between the two groups might be found.

There were several limitations in our study. Firstly, it's a non-randomized retrospective study. However, in our study, clinical doctors were not informed whether a patient was PLA₂R-associated or non-PLA₂R-associated IMN when they decided which immunosuppressive drug would be used, and there was no significant differences in baseline characteristic between PLA₂R-associated and non-PLA₂R-associated group. Baseline serum creatinine of CTX group was higher than that of CNI group, because patients with high baseline serum creatinine were tend to be treated with CTX, considering the effect of CNIs on renal function. Secondly, in the present study, only 1/3 of patients received ACEI or ARB before immunosuppressive therapy, since the non-immunosuppressive therapy using ACEI/ARB for at least 6 months before immunosuppressive therapy was not a standard practice in China before KDIGO guideline was released, particularly for patients with heavy proteinuria. However, according to our experience, in patients with severe nephrotic syndrome, spontaneous remission will not usually occur until one year after supportive therapy, mostly one and half years later. This experience is consistent with the literature [2]. Thirdly, the offending antibody in non-PLA₂R-associated IMN is not known, and could be heterogeneous. Finally, the sample size of the present study was small, particularly the number of patients with non-PLA₂R-associated IMN. PLA₂R-associated-IMN patients account for 85.7% in our study, higher than 74% in the literature [17], which also made the inclusion of non-PLA₂R-associated IMN patients more difficult. Therefore, large sample size, prospective controlled and randomized trials are needed to draw a more definitive conclusion.

Conclusion

Renal PLA₂R could be an important factor to predict the treatment response of IMN. Compared with PLA₂R-associated-IMN, patients with non-PLA₂R-associated IMN responded faster to the immunosuppressive therapy.

Abbreviations

ACEI: Angiotensin converting enzyme inhibitors; ALB: Albumin; ARB: Angiotensin receptor blockers; CNIs: Calcineurin inhibitors; CR: Complete remission; CsA: Cyclosporin A; CTX: Cyclophosphamide; IMN: Idiopathic membranous nephropathy; NR: Non-remission; PLA₂R: Phospholipase A₂ receptor; PLA₂R-Ab: Anti-phospholipase A₂ receptor autoantibody; PR: Partial remission; SCR: Serum creatinine; TR: Total remission

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Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

Conceived and designed the study: JW, QHX, ZXS, JX and CMH. Acquired the data: JW, QHX, ZXS, NXX, YL, LW and SJL. Analyzed and interpreted the data: JW, QHX, JX and CMH. Drafted the article: JW, QHX and CMH. Revised the manuscript critically for important intellectual content: JW, QHX, ZXS, NXX, YL, LW, SJL, JX and CMH. Agree with the manuscript's results and conclusions: JW, QHX, ZXS, NXX, YL, LW, SJL, JX and CMH. All authors read and approved the final manuscript.

Ethics approval and consent to participate

This study was approved by ethic committee of Huashan Hospital, Fudan University. The study was performed in accordance with the Declaration of Helsinki. Written consent was obtained from all participants in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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