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Article

Calcineurin Inhibitor Combined with Rituximab Is Superior to Combined with Glucocorticoids or RTX Alone in the Treatment of IMN: A Retrospective Study

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Abstract: BACKGROUND Studies demonstrate that T cells secrete interleukin and other cytokines to promote the growth and activation of B cells, and B lymphocytes release antibodies that attach to the PLA2R and THSD7A surface antigens on podocytes to create immunological complexes that are deposited beneath the glomerular epithelium, which damage the filtration barrier and trigger proteinuria.The combination regimen of Calcineurin inhibitors (CNIs) and rituximab(RTX) could simultaneously inhibit the proliferation of T and B lymphocytes and reduced the production of immune complexes.This kind of therapeutic method for idiopathic membranous nephropathy may achieve better clinical results and less complications. **METHODS** Fifty-eight patients who were diagnosed as idiopathic membranous nephropathy (IMN) through a biopsy suffering from nephrotic syndrome (NS) were recruited, and were divided into RTX treatment group(RTX group), CNIs combined with RTX treatment group(CNIs+RTX group) and CNIs combined with glucocorticoids(GC) treatment group(CNIs+CG group) according to their treatment background. All patients in 3 groups were followed up for at least 12 months. Clinical composite remission(including complete or partial remission) at 12 months was the main result; safety and the incidence of adverse events were the secondary outcomes. **RESULTS** The PLA2R antibody level of RTX+CNIs group at the baseline much higher than RTX and CNIs+GC groups (240.30(84.56,386.99)RU/ml vs. 44.94(11.48,143.94)RU/ml, 7.72(2.00,102.68) RU/ml, respectively, p=0.006). The PLA2R antibody concentrations among the three groups become comparable at the end of 3 months(36.80(2.10,88.05) RU/ml vs.14.32(2.00,47.98)RU/ml, 2.60(2.00,24.50) RU/ml, respectively, p>0.05). At 12 months, all patients in the CNIs+GC group achieved immune remission, and the PLA2R-ab concentration was less than 14RU/ml. At 3 months, 33of 58 patients(56.90%) had a composite remission(complete or partial remission), including 4 of 14 patients (27.85%) in the RTX group, 11 of 17 patients(64.71%) in the CNIs+RTX group and 18 of 27 patients (66.67%) in the CNIs+GC group (p=0.048). At 12 months, 51 of 58 patients(87.93%) had a composite remission, including 12 of 14 patients (85.71%) in the RTX group, 15 of 17 patients(88.24%) in the CNIs+RTX group and 24 of 27 patients (88.89%) in the CNIs+GC group (p=0.957). During the 12-month study, 7 patients did not response for the treatment. 1 patient relapsed during the 12-month treatment with a re-elevation of antibodies. Adverse events occurred in 18(31.03%)patients in the whole study, including 3 patients(5.17%) in the RTX group, 2 patients(3.45%) in the CNIs+RTX and 13patients(22.41%) in the CNIs+GC group(p< 0.05). **CONCLUSIONS** The combination of CNIs and RTX can effectively alleviate idiopathic membranous nephropathy with nephrotic syndrome in the first 3 months, as well as the combination of CNIs and GC, and the efficacy of both treatment methods for IMN was superior to that of RTX alone at the first 3 months. Furthermore, the RTX+CNIs group had less side effects compared with GC+CNIs group. So CNIs combined with RTX

could be recommended as a superior therapeutic method for IMN treatment to shorten remission durion with less complications.

Keywords: membranous nephropathy; rituximab; calcineurin inhibitor

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Membranous nephropathy is one of the most important pathological types of nephrotic syndrome.About 30 percent patients may acquire spontaneous remission, however,40 to 50 percent patients proceed to end stage renal desease step by step within ten years [1]. The symptoms observed in patients with membranous nephropathy include nephrotic syndrome (characterized by macroalbuminuria, hypoalbuminemia, edema, hyperlipidemia, etc.) and proteinuria without any noticeable symptoms. Based on the presence or absence of a clear cause, this condition can be categorized into idiopathic membranous nephropathy (IMN) and secondary membranous nephropathy (SMN) [2]. In recent years, the identification of anti-phospholipase A2 receptor antibody (PLA2R-Ab) and anti-platelet reactive protein 7A-domain antibody (THSD7A-Ab) has led to the recognition of IMN as a distinct autoimmune disorder. The primary mechanism behind its development involves the secretion of interleukin and other cytokines by T cells, which then facilitate the growth and activation of B cells. These B cells are responsible for producing antibodies that bind to podocyte surface antigens PLA2R and THSD7A. Consequently, immune complexes are formed and deposited beneath glomerular epithelial cells, leading to damage of the filtration barrier and the onset of proteinuria [3].

The combination of CNIs and GC is a frequently method used for managing IMN [4]. CNIs has the ability to protect podocytes and stabilize the cytoskeleton, thereby reducing proteinuria. Research has demonstrated that this treatment approach effectively alleviates IMN, with an efficacy rate ranging from 50% to 75%, it has been a common clinical treatment strategy now [5]. Although the remission rate for this regimen is relatively high, it is important to note the potential side effects, including elevated blood sugar, blood pressure, and acute renal tubular injury. In fact, reported recurrence rates for patients treated with this approach can be as high as 40%-50% [6]. Additionally, patients may develop a dependency on this treatment and be at risk for chronic nephrotoxicity. Therefore, there is an urgent need to explore alternative treatment options that offer improved efficacy(lower recurrence rates)and enhanced safety.Anti-CD20 biologic therapies, especially RTX,have brought new hope for the treatment of membranous nephropathy [7]. The clinical efficacy and safety of RTX has been confirmed in the treatment of IMN with RTX compared with conventional regimens, and the 2021 KDIGO guideline   [8] also recommends the clinical application of RTX.The median duration for response is about several months [9], however, prolonged exposure to nephrotic syndrome increased the risk of complication such as renal failure, infection and thrombosis.

In the present study, we designed a clinical trial to explore the effect of CNIs combined with RTX on the treatment of idiopathic membranous nephropathy compared with traditional treatment projects, and aimed to investigate whether this kind of treatment method could shorten remission durion and lessen complication.

2. Methods

Study Patients

In the present study, 58 biopsy-proven idiopathic membranous nephropathy (IMN) patients were recruited in Weifang People's Hospitl from November in 2019 to June in 2023. The mean age was 52.50±11.72 years. Three differrent therapeutic methods were used on these patients, aimed to investigate which treatment project could achieve better clinical results and less complications. Patients who met the follow inclusion criteria were included: (1) aged between 18 and 80 years; (2) diagnosed as IMN through a biopsy;(3) met the diagnostic criteria of proteinuria >3.5 g/24h and plasma albumin concentrations <30g/L prior to treatment.Patients with one or more exclusion criteria were excluded:(1) plasma creatinine level above 1.7 milligram per deciliter (150umoL/L);(2) positive

hepatitis B virus antigen, hepatitis C virus antibodies or HIV carrier,and other infectious diseases;(3)Patients complicated with diabetes nephropathy;(4) pregnancy or breast feeding; (5)neoplasm, hemopathy or active autoimmune disease.

Patients were divided into three groups according to their treatment schemes:

RTX group, (n = 14), patients got an intravenous dose of 1000 mg of rituximab on days 1 and 15. About half a year later, according to the depletion of B cells, anti-PLA2R antibody levels, and the presence of nephrotic albuminuria, choose whether to proceed with the next treatment.

RTX+CNIs group, (n = 17), patients were given RTX as RTX gruop. simultaneously, patients were administered tacrolimus (TAC) of 0.05-0.1 mg/kg.d or cyclosporine (CsA) of 3.0 mg/kg.d at 12-hour intervals untill 6 months,then TAC or CsA were tapered off and ceased gradually within anthoer 3 months.

CNIs+ GC group,(n=27),patients were given glucocorticoid 10mg combined with CsA/TAC orally discribed as RTX+CNIs group every day. Cyclosporine target trough blood levels were 100–150 ng/mL; TAC target valley concentration was 5-8 ng/mL.

Baseline data were collected. Concentration of Alb, 24hUTP, Scr, Bun and PLA2R antibody levels were determined at 0, 3rd, 6th, 9th and 12th months. The clinical efficacy of the patients in the three groups was assessed, Additionally, the alterations in the clinical biochemical indexes and adverse responses of the patients in the three groups were examined and compared.

All patients were provided with optimal supportive care, which encompassed the administration of renin-angiotensin system blockers, meticulous management of blood pressure to achieve a target value below 130/80 mmHg, adherence to a diet limiting sodium intake to less than 3 gram per day, and restriction of dietary protein to a daily intake of 0.8 to 1.0 gram per kilogram of body weight for a minimum of 3 months prior to randomization [10].

This study was approved by the Ethics Committee of Clinical Research, Weifang People's Hospital. And written informed consents were obtained from all patients.

Outcomes

The main clinical outcome was composite remission(including complete and partial remission) at 3 and 12 months.The secondary outcomes were the safety, the occurrence of adverse events,change of anti-PLA2 receptor levels, treatment failure, and end-stage renal disease during the time of therapy. To estimate the treatment response, proteinuria <0.3g per 24 hours and serum albumin ≥3.5g per deciliter were defined as CR. A reduction in proteinuria ≥50% from baseline or final proteinuria between 0.3-3.5g per 24 hours, an increase in serum albumin ≥30% or normalization, and an increase in serum creatinine <30% or stable were defined as PR. Patients who did not achieve the above definitions were defined as treatment failure. Creatinine clearance≤ 15ml per minute, the inception of dialysis, or renal transplantation were defined as end-stage renal disease. the occurrence of a serious pulmonary infection or pulmonary embolism, cerebral infarction or myocardial infarction, clinical death, and the prolonged hospitalization were defined as serious adverse events.

Statistical Methods

The statistical analysis was performed by SPSS 26.0. Normally distributed values, such as age, blood urea nitrogen,albumin, serum creatinine and hemoglobin, were presented as mean ± SD. Non-normally distributed variables, such as anti-PLA2 receptor antibody and 24h-urinary protein levels in GC+CNIs groups, were expressed as median and IQR. Levels of age, blood urea nitrogen, albumin, serum creatinine and hemoglobin among groups were compared using one-way analysis of variance (one-way-ANOVA). Categorical variables among groups were compared using χ^2 test. Levels of anti-PLA2 receptor antibody and 24h-urinary protein levels among groups were compared using Kruskal Wallis Test. P value<0.05 was regarded as statistically significant.

Result

Seventy IMN adult patients were recruited in the retrospective study in Weifang People's Hospital. Twelve patients who had followed-up shorter than a year were excluded. fifty-eight patients with a mean age of (52.50±11.72) years completed the study. Fourteen patients were assigned to the RTX treatment group (RTX group), seventeen patients were assigned to the RTX combined with CNIs treatment group(RTX+CNIs group),and twenty-seven patients were assigned to the CNIs combined with GC treatment group(CNIs+GC group).Among the three groups, compared with RTX group and CNIs+GC group, RTX+CNIs group had higher level of anti-PLA2 receptor antibody and lower level of serum albumin; compared with RTX+CNIs group and CNIs+GC group, RTX group had higher level of blood urea nitrogen. The baseline characteristics of the 3 groups were showed as follow(Table 1).

Table 1. Baseline characteristics of the three groups.

Characteristic	RTX group (N=14)	RTX+CNIs group (N=17)	CNIs+GC group (N=27)	P value
Male sex, n (%)	7 (50.00)	10 (58.82)	21 (77.78)	0.667
Age (years)	57.42±10.15	50.59±10.94	51.15±12.60	0.195
Urine RBC/ml	17.34±16.71	14.84±22.98	6.60 (2.65,18.00)	0.870
Proteinuria (g/24 h)	4.13±1.84	5.54±3.73	4.10±2.71	0.236
Hemoglobin (g/L)	130.92±27.25	136.35±17.41	143.18±18.20	0.184
TG(mmol/L)	2.70 ±1.88	2.39±1.30	2.25±1.65	0.704
LDL(mmol/L)	4.43±1.60	5.49±2.24	5.50±2.12	0.242
Platelet (×10^9/L)	234.07±55.91	247.29±55.54	244.63±43.09	0.743
ALT (u/L)	16.71±8.67	27.69(8.51)	20.67±15.51	0.335
AST (u/L)	19.84(6.85)	25.59±11.54	25.04±19.02	0.515
Total protein (g/L)	52.30±6.52	48.64±5.02	50.68±8.81	0.385
Albumin (g/L)	29.17±3.78	25.02±2.84[a]	26.50±6.08	0.063
blood urea nitrogen (mmol/L)	7.41±3.59	5.66±1.96 [a]	5.21±1.53 [a]	**0.018**
Serum creatinine(umol/L)	71.36±34.53	60.47±13.78	63.56±13.83	0.333
eGFR (mL/min/1.73 m²) [a]	104.09±50.96	132.40±44.70	130.70±33.18	0.109
Cholesterol (mmol/L)	7.60±1.71	8.65±2.85	8.16±2.35	0.478
Calcium(mmol/L)	2.10±0.15	1.97±0.11	2.07±0.16	0.335
Phosphorus(mmol/L)	1.17±0.38	1.19±0.21	1.20±0.19	0.923
Absolute values of CD19(/ml)	13.15(2.57)	18.31(5.68)	-	0.608
Anti-PLA2R antibody positivity, n (%)	9/12 (75.00)	16/17 (94.12)	11/25 (44.00)	0.056
Anti-PLA2R antibodies(>150U/mL)	3 (23.08)	11 (68.75)	3 (12.00)	**0.040**
Anti-PLA2R antibodies (U/mL)[b]	(44.94(11.48,143.94)	240.30(84.56,386.99)	7.72(2.00,102.68)	**0.003**
low-risk, n	3 (3/14)	3 (3/17)	7(7/27)	0.212

| intermediate-risk, n | 4 (4/14) | 9(9/17) | 13(13/27) | 0.315 |
| high-risk, n | 7 (7/14) | 5 (5/17) | 7(7/27) | 0.436 |

Notes: a:p<0.05 compared with RTX group; b:p<0.05 compared with RTX+CNIs group. The data in the table are expressed as mean ± standard deviation if they are normally distributed, and as quartile distance if they are skewed. Red blood cell (RBC), estimated glomerular filtration rate (eGFR), glutamic oxaloacetic transaminase (AST), glutamic pyruvic transaminase (ALT), blood urea nitrogen (BUN). eGFR was calculated according to Modification of diet in Renal Disease(MDRD) equation. The threshold for anti-PLA2R positive was set at >14 U/ml. Bold values denote P<0.05.

Clinical Outcomes

In the present study, the remission rate of the three groups gradually increased with the extension of the follow-up time, and the overall remission rate was 87.93% (51/58). 7 patients did not respond to treatment at 12 months, including 1 patient with diabetes, negative anti-PLA2R antibody, serum creatinine increased at 12 months, 4 patients were positive for PLA2R antibody at baseline but negative for PLA2R antibody at 12 months, and 2 patients were still had positive antibody at 12 months.

The complete remission rates were comparable among the three groups during the whole observation period(p>0.05), and there were also no significant difference of complete or partial remission rate in the three groups at 6,9,12 months (p>0.05). However, RTX+CNIs group and CNIs+GC group had higher composite remission (complete or partial) rates than RTX group at 3 months (64.71% vs. 28.57%, P=0.045; 66.67% vs. 28.57%, P=0.020, respectively) (Table 2, Figures 1 and 2).

Table 2. Remission at 3,6,9,12 months in three groups.

Item	Patients with Remission No. (%)				
	total	Group 1	Group 2	Group 3	p value
Complete remission					
3months	10/58(17.24%)	1/14(7.14%)	2/17 (11.76%)	7/27(25.93%)	0.248
6months	15/58(25.86%)	2/14(14.29%)	3/17(17.65%)	10/27(37.04%)	0.189
9months	20/58(34.48%)	3/14(21.43%)	5/17(29.41%)	12/27(44.44%)	0.296
12months	30/58(51.72%)	5/14(35.71%)	9/17(52.94%)	16/27(59.26%)	0.357
Complete or partial remission					
3months	33/58(56.90%)	4/14(28.57%)	11/17(64.71%)	18/27(66.67%)	0.048
6months	40/58(68.97%)	8/14(57.14%)	12/17(70.59%)	20/27(74.07%)	0.531
9months	45/58(77.59%)	11/14(78.57%)	12/17(70.59%)	22/27(81.48%)	0.704
12months	51/58(87.93%)	12/14(85.71%)	15/17(88.24%)	24/27(88.89%)	0.957

Notes: a:p<0.05 compared with RTX group.

Changes of PLA2R- Antibody Levels in 3 Groups

PLA2R antibody level was detected in 54 patients, including 36 patients with positive PLA2R antibody. All of the 36 patients showed a decrease trend in PLA2R antibody levels during the study. At the end of the study, 31 of the 36 patients achieved antibody negativity(<14ru/ml). The positive rate of PLA2R antibody at 0,3,6,9,12 months in the three groups were showed in Table 3. At the initial stage, there was a significant difference in PLA2R antibody concentration among the three groups(44.94(11.48,143.94)RU/ml,240.30(84.56,386.99) RU/ml,7.72(2.00,102.68) RU/ml, respectively, p=0.001), and the level of PLA2R antibody in RTX+CNIs group was the highest. After treatment, the PLA2R antibody concentrations among the three groups become comparable at the end of 3

months(14.32(2.00,47.98)RU/ml,36.80(2.10,88.05) RU/ml,2.60(2.00,24.50) RU/ml, respectively, p>0.05). At 12 months, all patients in the CNIs+GC group achieved immune remission, and the PLA2R-ab concentration was less than 14RU/ml.

Table 3. Changes of PLA2R- antibody levels in 3 groups during the study.

Item	Patients with positive PLA2R-ab No. (%)				
	total	RTX group	RTX+CNIs group	CNIs+GC group	p value
pre-treatment	36/54(66.67%)	9/12(75.00%)	16/17(94.12%) [a]	11/25(44.00%) [a]	0.001
3months	27/54(50.00%)	6/12(50.00%)	11/17 (64.70%)	10/25(40.00%)	0.291
6months	17/54(31.48%)	4/12(33.33%)	7/17(41.18%)	6/25(24.00%)	0.495
9months	10/54(18.52%)	1/12(8.33%)	4/17(23.53%)	5/25(20.00%)	0.523
12months	5/54(9.26%)	1/12(8.33%)	0/17(0%)	4/25(16.00%)	0.108

Notes: a:p<0.05 compared with RTX group.

Changes of Serum Albumin in 3 Groups

During a 12-month observation period, the serum albumin levels exhibited an increased trend in all the patients. The serum albumin were significantly increased at the end of the first 3 months compared with that at the baseline in the three groups(p<0.05), and then was maintain a state of continuous growth throughout the whole study (Table 3), and no statistical difference in serum albumin levels were found at the end of the 3,6,9,12 months among the three groups(p>0.05,Fig 3).

Change of Renal Function

In both RTX and RTX+CNIs groups, Renal functions were stable before and after treatment, there were no significant difference in serum creatinine and blood urea nitrogen levels at 3,6,9,12 months compared with the baseline (p>0.05). Hovever, in CNIs+GC group, compared with the baseline, serum creatinine and blood urea nitrogen levels were significantly increased at the end of 3 months, and then remained unchange until the end of 12 months(P<0.05).

Compared with RTX+CNIs group and CNIs+GC group, RTX group had higher blood urea nitrogen concentration at pre-treatment(p<0.05),but the difference was dispeared among three groups after 3 months' treatment. Compared with RTX group and RTX+CNIs group, CNIs+GC group had significantly increased serum creatinine level at the end of 12 months,although the serum creatinine level were similar at the pre-treatment(Table 4). Figures 4 and 5

Table 4. Changes of serum albumin levels in 3 groups during the study.

Groups	Alb (g/l)			
	pre-treatment	3months	6months	9months
RTX	29.17±3.78	**33.32**±5.84 [a]	36.86±5.38 [a]	37.90±5.40 [ab]
CNIs+RTX	25.02±2.84	**34.09**±5.06 [a]	36.52±6.39 [a]	37.28±5.89 [a]
CNIs+GC	26.50±6.08	**36.00**±7.44 [a]	38.77±8.52 [a]	39.64±8.35 [a]
P value	0.063	0.398	0.549	0.529

Notes: a:p<0.05 compared with baseline; b: p<0.05 compared with the end of the 3 months; c: p<0.05 compared with the end of the 6 months; d: p<0.05 compared with the end of the 9 months.

Changes of the 24h-Urinary Protein Levels

During the 12-month observational phase, the 24h-urinary protein levels showed a gradually decreased trend in the three groups. 3 groups showed a slightly decrease trend in 24h-urinary protein level at month 3,and showed a significant changes at month 6 compared with their initial levels(Table 5).

Table 5. Changes of the BUN and SCr levels during the study.

Groups	pre-treatment	3months	6months	9months	12months	p value
Bun						
RTX group						0.984
CNIs+RTX group	7.41±3.59	6.98±3.51	6.98±3.90	7.51±5.04	6.75±3.19	0.389
	5.66±1.96 [b]	7.20±2.69	7.34±3.32	6.76±2.24	6.83±2.88	
CNIs+ GC group	5.21±1.53 [b]	8.33±3.76[a]	7.86±3.52 [a]	7.51±5.04 [a]	8.71±5.51 [a]	0.006
P value	**0.018**	0.401	0.741	0.560	0.268	
Scr						
RTX group	71.36±34.53	67.54±27.54	71.57±34.89	67.93±34.99	62.15±19.33 [c]	0.936
CNIs+RTX group	60.47±13.77	68.47±14.08	65.65±18.85	69.59±18.46	64.00±11.85[c]	0.494
CNIs +GC group	63.56±13.83	78.07±22.13 [a]	77.15±19.64 [a]	79.44±24.92 [a]	81.37±27.01 [a]	0.028
P value	0.333	0.223	0.305	0.307	**0.011**	

Note: a:p<0.05 compared with pre-treatment in the same treatment group; b:p<0.05 compared with RTX group among 3 groups(RTX group, CNIs +RTX group and CNIs +GC group); c:p<0.05 compared with CNIs +GC group among 3 groups (RTX group, CNIs +RTX group and CNIs +GC group).

Adverse Events

During the course of the study, 18 participants (31.03%) experienced adverse events, including 3 patients(5.17%) in the RTX group, 2 patients(3.45%) in the CNIs+RTX and 13 patients(22.41%) in the CNIs+GC group(p< 0.05). The incidence of adverse events between RTX group and CNIs+RTX group were comparable(p>0.05). However, the incidence of adverse events in CNIs+GC group was higher than the other two groups(p<0.05, p<0.05, respectively).

Three patients(5.17%) experienced serious adverse events (hospitalized for lung infection),including one in RTX group and two in CNIs+GC group. Infusion responses, such as rash, erythema, and pruritus, were the most frequent adverse reactions in the RTX and CNIs+ RTX groups, but these side effects relieved gradually before the infusions were finished. 3 patients suffered from acute kidney injury whose renal function returned to baseline 3 weeks after supportive treatment. All patients returned to normal after symptomatic treatment or adjustment of drug dosage. No patient had complications of pulmonary embolism, end-stage renal disease, cerebral infarction, myocardial infarction or clinical death (Table 6).

Table 6. Changes of the 24h-urinary protein levels during the study Figure 6.

Groups	pre-treatment	3months	6months	9months	12months	p
RTX(g/24h)	4.71(3.15, 5.87)	2.91(0.79,4.99)	**1.84**(0.55,3.29)[ab]	1.18(0.35,4.14)[ab]	0.96(0.17,2.55)[ab]	**<0.05**
CNIs+RTX(g/24h)	4.36(2.50, 8.66)	4.07(1.50,4.74)	**1.61**(0.47, 3.89)[ab]	2.18(0.57,5.01)[ab]	0.81(0.26, 3.32)[abcd]	**<0.05**
GC+CNIs(g/24h)	3.80(1.94,5.92)	2.00(0.22,3.47)	**1.15**(0.20,3.08)[a]	0.80(0.15,3.58)[ab]	0.34(0.12,1.27)[abcd]	**<0.05**
P	0.413	0.095	0.586	0.331	0.308	

Notes: a:$p<0.05$ compared with baseline; b: $p<0.05$ compared with the end of the 3 months; c: $p<0.05$ compared with the end of the 6 months; d: $p<0.05$ compared with the end of the 9 months.

Table 7. Adverse events in the three groups during the study.

adverse events	number of patients		
	RTX	CNIs+RTX	CNIs+GC
rash/erythema/pruritus:3	1	2	
lung infection:3	1		2
Tremor: 1			1
blurred vision1			1
new-onset hypertension requiring therapy: 2			2
worsening of hypertension requiring additional therapy: 1			1
Hyperglycemia: 2			2
Increased hair growth or coarser hair: 1			1
acute kidney injury:3	1		2
gingival hyperplasia: 1			1

Discussion

In the present study, we found that the combination of CNIs and RTX can effectively relieve IMN in the first 3 months, which was comparable to that of CNIs combined with GC, and the effectiveness of both treatments was superior to that of RTX alone at the end of 3 months. The remission rate among the three groups were similar at the end of 12 months. However, the incidence of adverse events in CNIs+RTX group was lower than the CNIs+GC group. We also found a gradul increase plasma albumin level and a decrease PLA2R antibody, 24h-urinary protein levels after the treatment in the 3 groups during the follow-up time. In addition, more stable renal function in both CNIs+ RTX and RTX groups compared with CNIs+GC group was observed and no fatal adverse events occured in the 12-month monitoring period.

Treatment options for idiopathic membranous nephropathy are constantly being studied and optimized. The management of PMN has been recommended by the 2021 KDIGO guidelines [8] to include the use of RTX, which has been proven effective and safe for clinical use. Positive effectiveness of rituximab on IMN remission occurred about 6 months later [11]. Waldman M et al. [11]reported 13 high-risk patients had six months of combination induction therapy consisting of RTX and cyclosporine in a single arm study, 92% of patients experienced composite remission in nine months, and 54% attained complete remission in twelve months. In our present study, patients in the

CNIs+RTX group had an obvious effect of composite remission at the end of 3 months, which was comparable to that of CNIs+GC gruop and was ealier than that of RTX group. In terms of complete response rate, the three groups were similar from 3 to 12 months. Our findings demonstrated that the combined remission and the complete remission rates of RTX+CNIs group at 12 months were 88.24% and 52.94% respectively, which parallelled with the previous study [11].

Recent data suggest that the combination of CNIs+RTX is more effective compared to the combination of GC and CNIs [6]. Considering the delay in treatment response and the potential risks associated with persistent nephrotic syndrome, coupled with the excellent safety record of RTX, it can be argued that therapy should be initiated promptly upon diagnosis of idiopathic membranous nephropathy [28]. Short-term induction of CNIs can reduce the probability of renal impairment.The benefits of this treatment approach include a limited duration of CNIs usage, reduced risk of complications from the NS due to faster remission onset, and avoiding the short-and long-term adverse effects that are frequently linked to the use of drugs for nephrotic syndrome [12].

Anti-PLA2R antibody titers rise during the course of clinical activity and fall before clinical remission [13]. As a result, anti-PLA2R antibodies serve as a key indicators for monitoring the efficacy of membranous nephropathy   [13].In the present study, although the anti-PLA2R antibody level in CNIs+GC group was much higher than RTX and CNIs+GC groups at the baseline, the difference among 3 groups dispeared after treatment at the end of 3 months. At 12 months, all patients with positive anti-PLA2R antibody in the CNIs+GC group achieved antibody negativity. This result is closely associated with a higher response rate of complete remissions, and these data also suggested that the significant effectiveness of CNIs combined with RTX in eliminating anti-PLA2R antibodies in high risk IMN patientts.

Of the 58 patients in this study, 7 patients did not respond to treatment at 12 months of treatment, including 1 patient with diabetes, negative anti-PLA2R antibody, serum creatinine increased at 12 months. 4 patients without remission were positive for PLA2R antibody at baseline, but all of the 4 patients achieved antibody negativity at 12 months, suggesting that these patients may gain remission in the future. 2 patients were still had positive antibody at 12 months. In the present study, we also found that patients with lower initial levels of the antibody were more likely to achieve remission. The correlation between the level of disease remission, relapse, and drug resistance was significant [14].Ruggenenti et al. [15] reported that the degree of PLA2R-Ab and the activity of IMN illness are correlated. Moreover, when the antibody levels increase again, it strongly indicates a recurrence of the disease. In the present study, one patient relapsed during the 12-month treatment with a re-elevation of antibodies, which confirmed the relationship between serum PLA2R-Ab levels and disease activity and proteinuria.

In this study, we also found that albumin had been significantly increased at 3 months of treatment, while the significant reduction of urinary protein occurred at 6 months in the CNIs+RTX group. The significant changes may be explained by several causes. First, the level of PLA2R antibody in RTX+CNIs group was the highest among three groups,furthmore, there were 11 patients with the PLA2R antibody more than 150RU/ml, which may prolong the remission time. Remuzzi et al. [15]found in RTX treatment with nephrotic syndrome that patients with high antibody titers had lower possibility of achieving clinical remission than patients with low antibody levels. Second, the restoration of the glomerular filtration barrier's structure and operation takes time due to extensive immunologic damage. Third,Anti-PLA2R antibody levels become negative earlier than achieving clinical remission, and the immunological response to drugs occurs prior to the clinical response [16–18].

Adverse events were prevalent in patients using immunosuppressive agent or biological agents. In the present study, the incidence of adverse events was 31.01% in all patients, and most of which occurred in the CNIs+GC group. None of these adverse events were malignant or fatal. The CNIs+RTX group had the lowest incidence of side effects among the three groups. Fervenza FC et al. reported that the incidence of infusion-related reactions ranges from 50% to 80% [19]. However, it has been observed that the incidence tends to decrease to 0-17% in subsequent infusions [20–22], which was consistent with our results. In this study, infusion groups were well tolerated, the

combined incidence of infusion-related events in the RTX group and the RTX+CNIs group was 8.62%. Acute kidney injury was a common complication during the treatment period with CNIs compared with RTX [18].In the present study, the CNIs+GC group had higher serum creatinine and serum urea nitrogen levels than the other two groups during the 12-month observational phase, which further confirmed that CNIs caused kidney damage, and the change in the hemodynamics of the kidneys and the imbalance of endogenous diastolic-systolic factors caused by CNIs [18] may be contribute to this result [13]. However, the renal function in CNIs+RTX group was stable, the reason may be explained as follow. First, CNIs was tapering after 6 months, which shortens the time for patients to be exposed to drug toxicity and weaken the potential risks associated with CNIs. Second, RTX treatment made renal function persistent stable on the basis of maintaining remission.

In conclusion, the effective approach for IMN using CNIs combined with RTX could achieve better clinical results and less complications. Patients using CNIs+RTX could gain more rapid onset of remissions, compared with RTX alone, the treatment regimen of CNIs+RTX effectively shortened the induction period by 3 months and reduced the risk of patients with complications such as infection and thromboembolism caused by long-term exposure to nephrotic syndrome. In addition, this kind of therapy has a favorable safety profile,no patients had malignant or fatal adverse events during the 12-months period. So CNIs combined with RTX could be recommended as a superior therapeutic method for IMN treatment and the therapeutic method has a good application prospect.

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Informed Consent: Written and verbal consents were obtained from all individual participants included in the study.

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