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ORIGINAL RESEARCH

Is Rituximab Suitable for Patients with Idiopathic Membranous Nephropathy Who are Seronegative for Anti-PLA2R Antibody?

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Background: Rituximab (RTX) induces apoptosis in B cells expressing CD20, the efficacy in idiopathic membranous nephropathy (IMN) with seronegative anti-M-type phospholipase A2 receptor (PLA2R) antibody remains unclear.

Methods: This multicenter retrospective study enrolled 111 adult patients with biopsy-proven, medium-to-high-risk IMN, whose serum anti-PLA2R antibodies test results were negative (SAb-). All patients received RTX and non-RTX (tacrolimus or cyclophosphamide) therapy. Subsequently, based on glomerular PLA2R antigen results, they divided into glomerular PLA2R positive (GAg+) and glomerular PLA2R negative (GAg-) groups. Compared the remission rate at 15 months between different treatment groups.

Results: Of the 111 seronegative patients, 36 were assigned to the RTX group and 75 to the non-RTX group. Among the 76 GAg+ patients, 21 were in the RTX group and 45 in the non-RTX group. Kaplan–Meier analysis demonstrated that the complete remission rate was significantly higher in the non-RTX group than in the RTX groups at 15 months, both in total and SAb-/GAg+ cohort ($P = 0.033$, $P = 0.034$, respectively), but there was no statistically significant difference in overall remission (both $P > 0.05$). The non-RTX group exhibited significantly lower proteinuria and higher serum albumin levels than the RTX group at 3, 6, and 9 months after treatment. Of the 19 patients who did not achieve remission by the end of follow-up, seven (36.8%) experienced seroconversion. There were no significant differences in adverse events between two groups.

Conclusion: For seronegative anti-PLA2R antibodies patients, non-RTX immunosuppressive therapy might offer greater benefit for proteinuria remission compared to RTX in short-term, even if their glomeruli are positive for PLA2R antigen.

Keywords: idiopathic membranous nephropathy, anti-PLA2R antibody, proteinuria, remission

Introduction

Since Beck et al identified the M-type phospholipase A2 receptor (PLA2R) as the primary target antigen in idiopathic membranous nephropathy (IMN) in 2009, serum antibodies against the PLA2R have been detected in approximately 70% of patients with IMN.[1] This not only deepens our understanding of the immunopathological mechanisms underlying IMN but also provides a theoretical foundation for therapeutic strategies targeting B cells.

Rituximab (RTX) induces apoptosis in B cells expressing CD20,[2] and its efficacy in treating IMN has been confirmed in the GEMRITUX, MENTOR, STARMEN, and RI-CYCLO studies.[3–6] In the 2021 KDIGO guidelines, RTX is recommended as a first-line immunosuppressive therapy.[7] However, only 60% to 70% of patients achieve sustained clinical remission, and the majority of these included in the studies were positive for serum anti-PLA2R antibodies (SAb+) patients.

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Notably, approximately a proportion of IMN patients exhibit negative anti-PLA2R antibody test results at the serological level, with or without glomerular PLA2R antigen (GAg), yet still present with typical manifestations such as proteinuria and hypoalbuminemia.[8] Previous studies demonstrated that SAb- patients exhibit milder clinical manifestation and less pathological damage compared to SAb+ patients, and achieve a higher rate of clinical remission following treatment with calcineurin inhibitors or cyclophosphamide (CYC).[8,9] A recent study indicated that immunosuppressive therapy may assist in predicting proteinuria remission in SAb-/GAg+ patients.[10] However, data on efficacy of RTX treatment in SAb- patients remain relatively limited.

This study aims to evaluate the clinical efficacy of RTX in patients negative for baseline anti-PLA2R antibodies and to assess PLA2R antigen expression via renal biopsy. A longitudinal design will be employed to systematically analyze the prognostic significance of SAb- outcomes.

Methods

Patients Selection

A total of 111 patients with biopsy-proven, medium-to-high-risk IMN were enrolled from four research centers in Jiangxi Province, China, between July 2022 to April 2024 in this multicenter retrospective study. These patients were anti-PLA2R antibody-negative and all received immunosuppressive therapy. Any data absence or secondary cause of MN (autoimmune diseases, hepatitis B and C, medications, tumors) or patients who were pregnant or lactation or had active infection or life-threatening complications were excluded (Figure 1). The present study was approved by the Ethics Committee of the Second Affiliated Hospital of Nanchang University (IIT-O-2025-196) and conducted according to the ethical principles stated by the Declaration of Helsinki. Informed consent was obtained from all patients.

Risk Stratification

Risk stratification was performed according to the 2021 KDIGO guideline: moderate risk was defined as normal estimated glomerular filtration rate (eGFR), 24-hour urinary protein (UP) >3.5 g/d and no decrease >50% after 6 months of conservation therapy with renin-angiotensin-aldosterone system inhibitor and does not meet high-risk criteria. High risk was defined as eGFR <60 mL/min/1.73 m^2 and/or UP >8 g/d for >6 moths, or normal eGFR, UP >3.5 g/d and no decrease >50% after 6 months of conservation therapy with renin-angiotensin-aldosterone system inhibitor and serum albumin <25 g/L.[7]

Intervention

Based on the actual clinical distribution of patients receiving different immunosuppressive therapies during the study period, the patients were divided into RTX and non-RTX (received tacrolimus [TAC] or CYC) group. Patients receiving RTX were administered intravenous infusion at 1 g on days 1 and 15, repeated infusion of 1g at the month 6 if no complete remission (CR) achieved. Non-RTX group received TAC or CYC treatment. Subjects treated with TAC were initiated oral TAC on a dose of 0.05 mg/kg/day in two divided doses. The dose was adjusted according to the target trough blood concentration of 4–8 ng/mL for the first 6 months and tapered gradually until discontinued at the end of 12 months, and they were combined with oral glucocorticoids therapy (20 mg/day). Patients received CYC therapy undergo a cyclical treatment regimen, consisting of three consecutive cycles lasting for 2 months each (for a total of 6 months), where steroids were alternated with CYC every other month (cumulative dosage, 8–10 g).[6]

Data Collection

Baseline patient data were collected, including gender, age, blood pressure, total cholesterol, triglycerides, alanine aminotransferase, aspartate aminotransferase, UP, serum albumin, eGFR, PLA2R titers, and renal pathology data. All basic tests were completed before immunotherapy. eGFR levels were estimated using the Chronic Kidney Disease Epidemiology Collaboration formula.[11] Anti PLA2R-antibody levels were measured using enzyme-linked immunosorbent assay kits (EUROIMMUN, Lübeck, and Germany). A serum anti-PLA2R antibody concentration ≥20 RU/mL was considered positive.

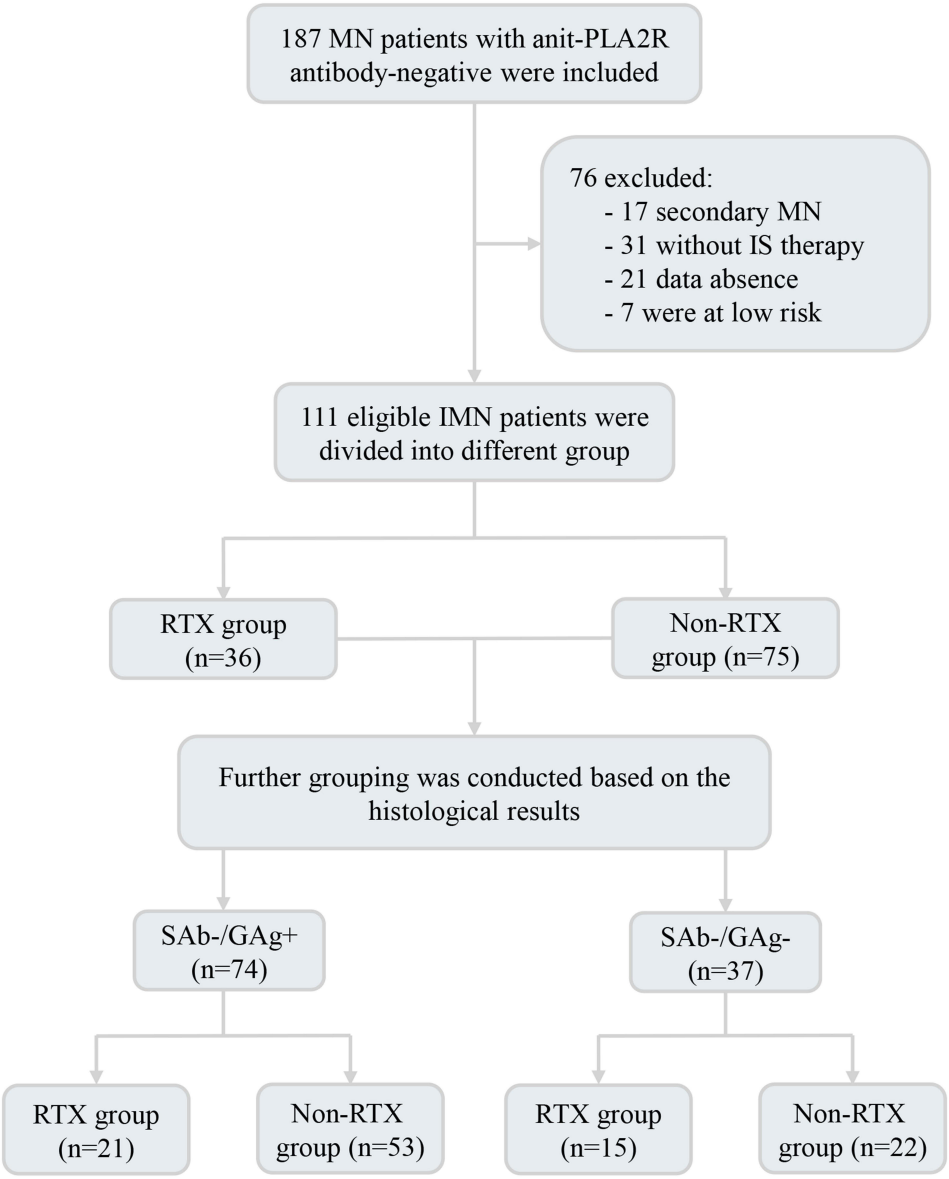

Figure 1 Flow diagram.

Outcomes and Follow-Up

The primary outcome including CR and overall remission (OR). CR was defined as UP ≤ 0.3 g/24 h with stable renal function (eGFR declined less than 20% from baseline), and OR was CR plus partial remission, with partial remission was defined as a proteinuria level <3.5 g/d and a 50% or greater reduction from baseline. Relapse is UP >3.5 g/d after achieving complete or partial remission. Secondary outcome measures included changes in UP, eGFR, albumin levels, and adverse events. Follow-up will be conducted on all patients at 3, 6, 9, 12 and 15 months after treatment to collect relevant clinical data.

Statistical Analysis

Statistical analyses were performed using R (version 4.4.3) and GraphPad (version 9.0). Continuous variables with skewed distribution were expressed as median (interquartile range IQR) and compared with Mann–Whitney *U*-test; variables following a normal distribution were presented as mean ± standard deviation (SD) and analyzed using *t*-test.

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Categorical variables were expressed as frequency (percentages) and analyzed using the chi-square test or Fisher exact test. Kaplan–Meier survival analysis method and the Log rank test. Multivariable Cox regression models were constructed to estimate adjusted hazard ratios (HR) for 15-month CR. Three models were utilized: model 1 was adjusted for age, sex and blood pressure; model 2 included model 1 plus UP, eGFR and serum albumin; and model 3 included model 2 along with the risk level and pathological stage. Additionally, Subgroup analyses were conducted based on baseline characteristics using a stratified Cox proportional hazards model, with results presented in a forest plot. Interactions between subgroups were assessed using the likelihood ratio test. Significance was defined as $P < 0.05$.

Results

Baselines Clinical Characteristics

One hundred and eleven IMN with anti-PLA2R antibodies-negative patients were retrospectively collected, 36 (32.43%) receiving RTX and 75 (67.57%) non-RTX (66 treated with TAC and 9 treated with CYC). Baseline clinical characteristics were comparable between two groups (Table 1). The median (IQR) UP in the RTX group was 5.77 (3.88, 7.54) and 5.26 (4.04, 7.12) in non-RTX group. The median (IQR) PLA2R titer was 8.57 (1.10, 13.57) and 5.66 (1.25, 12.62) in RTX and non-RTX groups, respectively.

Subsequently, patients were categorized into SAb-/GAg+ (n=74) and SAb-/GAg- (n=37) groups based on the presence of PLA2R antigen in the glomeruli. Among the 74 SAb-/GAg+ patients, based on their treatment regimen, 21 patients received RTX (RTX group), with baseline median age, 24 h UP, and PLA2R titer of 54 years, 5.78 g/d, 8.58 RU/mL, respectively. The remaining 53 patients received non-RTX treatment, including TAC and CYC, and their corresponding baseline median age, 24 h UP, and PLA2R titer of 53 years, 5.85 g/d, 5.32 RU/mL, respectively. Table 2 summarizes the baseline information of the cohort. In SAb-/GAg- subgroup, no patients were reported to have glomerular thrombospondin type-1 domain-containing 7A positivity.

Table 1 Baseline Characteristic Between RTX and Non-RTX Groups Under Anti-PLA2R Antibody-Negative IMN Patients

Characteristic	All (N=111)	RTX Group (N=36)	Non-RTX Group (N=75)	*P*
Male, n (%)	68 (61.3)	21 (58.3)	47 (62.7)	0.818
Age, years	53.00 [47.00, 61.00]	55.50 [48.00, 63.25]	52.00 [46.50, 58.50]	0.197
Risk, n (%)				0.948
Moderate	76 (68.47)	24 (66.67)	52 (69.33)	
High	35 (31.53)	12 (33.33)	23 (30.67)	
Systolic blood pressure, mmHg	125.02 (10.42)	126.31 (11.61)	124.40 (9.82)	0.369
Diastolic blood pressure, mmHg	73.27 (8.81)	74.11 (9.86)	72.87 (8.30)	0.489
Total cholesterol, mmol/L	7.47 [5.97, 8.88]	7.80 [5.78, 8.70]	7.35 [6.28, 9.14]	0.303
Triglyceride, mmol/L	2.74 [1.91, 3.54]	2.70 [1.92, 3.17]	2.78 [1.91, 3.78]	0.708
Alanine aminotransferase, U/L	18.60 [14.70, 26.00]	19.76 [15.40, 26.01]	18.36 [13.93, 25.90]	0.359
Aspartate aminotransferase, U/L	22.95 [14.91, 30.76]	24.02 [18.12, 30.34]	22.56 [14.73, 31.12]	0.277
Albumin, g/L	30.40 [23.40, 33.46]	29.65 [23.33, 32.52]	31.14 [23.50, 33.46]	0.410
Proteinuria, g/d	5.32 [4.01, 7.24]	5.77 [3.88, 7.54]	5.26 [4.04, 7.12]	0.525
eGFR, mL/min/1.73 m^2	96.07 (17.30)	98.79 (13.88)	94.77 (18.67)	0.253
PLA2R titer, RU/mL	5.75 [1.23, 13.20]	8.57 [1.10, 13.57]	5.66 [1.25, 12.62]	0.821
Glomerular PLA2R positive, n (%)	74 (66.67)	21 (58.33)	53 (70.67)	0.282
Pathological stage, n (%)				0.435
Stage I	3 (2.70)	2 (5.56)	1 (1.33)	
Stage II	74 (66.67)	23 (63.89)	51 (68.0)	
Stage III	34 (30.63)	11 (30.56)	23 (30.67)	

Abbreviations: eGFR, estimated glomerular filtration rate; PLA2R, M-type phospholipase A2 receptor; IMN, idiopathic membranous nephropathy; RTX, rituximab.

Table 2 The Impact of RTX on Clinical Baseline and Outcomes in the SAb-/GAg+ Subgroup

Parameters and Outcomes	SAb-/GAg+ (N=74)		*P*
	RTX Group (N=21)	Non-RTX Group (N=53)	
Male (%)	11 (52.4)	34 (64.2)	0.502
Age, years	54.00 [50.00, 62.00]	53.00 [44.00, 58.00]	0.134
Risk, n (%)			0.877
Moderate	13 (61.90)	30 (56.60)	
High	8 (38.10)	23 (43.40)	
Systolic blood pressure, mmHg	124.29 (10.93)	125.74 (9.09)	0.561
Diastolic blood pressure, mmHg	72.00 [66.00, 78.00]	72.00 [67.00, 81.00]	0.610
Total cholesterol, mmol/L	8.38 [6.00, 8.80]	7.47 [6.48, 9.14]	0.962
Triglyceride, mmol/L	2.84 (1.15)	2.95 (1.08)	0.720
Alanine aminotransferase, U/L	20.64 (5.80)	19.66 (7.09)	0.575
Aspartate aminotransferase, U/L	24.48 [18.08, 30.29]	19.90 [14.14, 29.49]	0.172
Albumin, g/L	29.45 [23.30, 31.49]	29.60 [23.20, 32.90]	0.876
Proteinuria, g/d	5.78 [4.55, 8.22]	5.85 [4.67, 7.84]	0.640
eGFR, mL/min/1.73 m^2	97.52 (14.45)	95.14 (19.81)	0.618
PLA2R titer, RU/mL	8.58 [0.72, 13.66]	5.32 [1.00, 12.86]	0.824
Pathological stage (%)			0.885
Stage II	14 (66.67)	38 (71.70)	
Stage III	7 (33.33)	15 (28.30)	
Outcomes			
CR, n (%)	2 (9.52)	19 (35.85)	0.024
OR, n (%)	12 (57.14)	43 (81.13)	0.033
Relapse, n (%)	2 (9.52)	3 (5.66)	0.618

Abbreviations: CR, complete remission; eGFR, estimated glomerular filtration rate; OR, overall remission; PLA2R, M-type phospholipase A2 receptor; RTX, rituximab; SAb-/GAg+, serum anti-PLA2R antibody negative and glomerular PLA2R antigen positive.

Remission Rates

In the overall population, the CR rate at 15 months was 27.8% in the RTX group and 49.3% in the non-RTX group (odds ratio 1.91, 95% CI 1.02–3.57, P = 0.031), and the OR was 75.0% vs. 86.7% (odds ratio 1.61, 95% CI 0.91–2.86, P = 0.127). The Kaplan–Meier survival curve results demonstrated that the incidence of CR was significantly higher in the non-RTX group than in the RTX groups at 15 months (P = 0.033, Figure 2A), but there was no statistically significant difference in OR (P = 0.063, Figure 2B). After full variable adjustment (model 3), multivariate Cox regression analysis revealed that patients in the RTX group exhibited a 73% reduction in the probability of achieving CR compared with the non-RTX group (HR: 0.27, 95% confidence interval [CI] 0.12–0.61, P = 0.001, Table 3).

In the SAb-/GAg+ cohort, a significantly higher rate of CR and OR was observed in the non-RTX group at the month 15 (35.85% vs. 9.52%, P = 0.024; 81.13% vs. 57.14%, P = 0.033, respectively, Table 2). Kaplan–Meier analysis further indicated a statistically significant difference in CR between the two group (P = 0.034, Figure 2C), while no significant difference was observed in OR (P = 0.079, Figure 2D). Multivariate Cox regression analysis still suggests that receiving non-RTX therapy correlates with achieving CR at 15 months (HR: 0.05, 95% CI 0.01–0.34, P = 0.002, Table 3). At the follow-up visit of 15 months, 2 of 21 RTX patients (9.52%) had a relapse, whereas 3 of 53 non-RTX patients (5.66%) had a relapse (odds ratio 0.688, 95% CI: 0.22–2.15; P = 0.618).

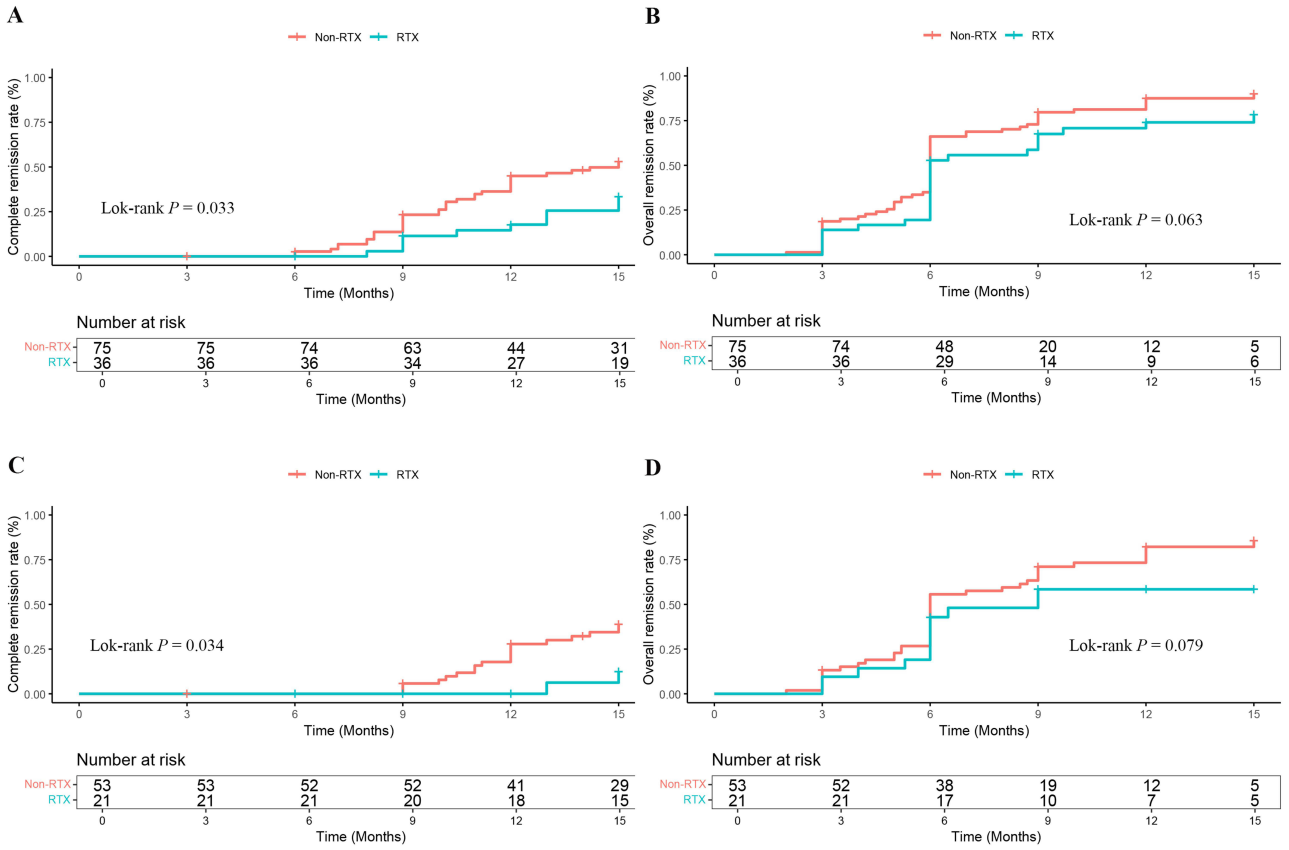

Figure 2 Kaplan-Meier analysis in anti-PLA2R antibody-negative idiopathic membranous nephropathy patients between RTX and non-RTX group. (**A**) complete remission and (**B**) overall remission in total population. (**C**) complete remission and (**D**) overall remission in SAb-/GAg+ cohort. RTX: rituximab.

Secondary Outcomes

In the overall cohort, the mean 24 h UP in the Non-RTX group decreased from 5.67 g/d to 0.85 g/d at 15 months, compared with a decrease from baseline level of 6.03 g/d to 1.50 g/d in the RTX group. At the 3, 6, and 9-month follow-up, UP levels in the non-RTX group remained consistently lower than those in the RTX group, and serum albumin levels were higher (Supplementary Figure 1A). There was no significant difference in eGFR between the two groups (Supplementary Figure 1B).

Table 3 Multivariable Cox Proportional Hazards Model Comparing the Time to Complete Remission Between Treatment Regimens in Different Group

	Model 1		Model 2		Model 3	
	HR 95% CI	***P***	**HR 95% CI**	***P***	**HR 95% CI**	***P***
Total population						
Non-RTX	Ref.	0.044	Ref.	0.015	Ref.	0.001
RTX	0.48 (0.24–0.98)		0.40 (0.19–0.84)		0.27 (0.12–0.61)	
SAb-/GAg+ cohort						
Non-RTX	Ref.	0.040	Ref.	0.004	Ref.	0.002
RTX	0.20 (0.05–0.93)		0.08 (0.02–0.45)		0.05 (0.01–0.34)	

Notes: Model 1: adjusted for age, sex, SBP, and DBP; Model 2: adjusted for model 1 plus UP, eGFR, and serum albumin; Model 3: adjusted for model 2 plus risk and pathological stage.
Abbreviations: RTX, rituximab; SAb-/GAg+, serum anti-PLA2R antibody negative and glomerular PLA2R antigen positive.

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Table 4 Summary of Adverse Events in Different Treatments Groups

Adverse Events	RTX Group (N=36)	Non-RTX Group (N=75)	*P*
Infections	3 (8.3%)	6 (8.0%)	> 0.999
Infusion-related reactions	1 (2.8%)	0 (0)	0.324
Hyperglycemia	0 (0)	3 (4.0%)	0.550
Gastrointestinal reactions	2 (5.6%)	5 (6.7%)	> 0.999
Hepatotoxicity	2 (5.6%)	3 (4.0%)	0.659

Abbreviation: RTX, rituximab.

Similarly, in the SAb-/GAg+ cohort, the UP, serum albumin and eGFR also exhibited analogous patterns of change (Supplementary Figure 1C-D). Notably, we re-assessed the anti-PLA2R antibody titer of 19 patients who had not achieved remission at the end of follow-up. Seven patients (36.84%) underwent seroconversion, including four in the RTX and three in the non-RTX group. One of them is a relapse case. Their mean anti-PLA2R antibody titers from 5.07 UR/mL at baseline to 46.65 UR/mL, and all were GAg+.

Subgroup Analysis for 15-Month CR and Adverse Events

In subgroup analyses of gender, age, blood pressure, risk level, pathological staging, proteinuria, serum albumin, and eGFR, the proportion effect of non-RTX on achieving CR at 15 months was consistent across all subgroups in both total cohort and SAb-/GAg+ cohort (all interaction $P \geq 0.05$, Supplementary Figure 2).

Among all adverse events reported in both groups, infections were the most common occurrence, with three cases in RTX group and six cases in non-RTX group (8.3% vs. 8.0%, $P > 0.999$). In RTX group, infusion-related reactions events were more common, whereas hyperglycemic events occurred more frequently in the non-RTX group. Additionally, gastrointestinal reactions and hepatic impairment were reported, with no statistically significant differences between the two groups. No serious adverse events were reported in either group (Table 4).

Sensitivity Analysis

In a sensitivity analysis excluding the CYC subgroup (n = 9), the comparison between the RTX group and the TAC-only group yielded consistent results for the primary outcome (CR: 27.8% vs. 56.1%, odds ratio 2.22, 95% CI 1.20–4.11, $P = 0.006$; OR: 75.0% vs. 90.9%, odds ratio 1.93, 95% CI 1.15–3.25, $P = 0.030$).

Discussion

This multicenter, retrospective study represents the first evaluation of the differences between RTX and non-RTX treatments in the IMN patients with negative for serum anti-PLA2R antibodies. Our findings suggest that during the 15-month follow-up period, patients in the non-RTX group demonstrated a higher probability of achieving CR and a greater reduction in proteinuria compared with the RTX group.

2021 KDIGO guidelines state that immunosuppressive therapy should be considered for patients at risk of progressive renal injury,[7] therefore, this study enrolled medium-to-high-risk SAb- patients. As indicated by the research of Wu et al, for patients who are negative for serum anti-PLA2R antibodies yet present with severe clinical symptoms, receiving immunosuppressive therapy may be associated with a higher likelihood of reduced urinary protein excretion.[10] RTX is a chimeric monoclonal IgG1 targeting CD20, a specific marker of B lymphocytes.[12] It acts on B cells through mechanisms including apoptosis, antibody-dependent cellular cytotoxicity, and complement-dependent cytotoxicity. Simultaneously, RTX has been found to directly target podocytes, maintain the expression of podocyte functional proteins such as nephrin and podocin within the glomeruli, and stabilize the cytoskeleton, thereby exerting an anti-proteinuric effects independent of B lymphocytes.[13] Patients with SAb- may also have other antigens, such as thrombospondin type-1 domain-containing 7A, serine protease high temperature requirement protein A1 or unknown antigen,[14] the specificity of RTX for these antigens-induced B-cell depletion remains unclear. Moreover, both the

excretion of RTX in urine and the generation of anti-RTX antibody may result in serum concentration of RTX that below the levels required for therapeutic efficacy.[2] These factors may partially explain why RTX proves effective in SAb- patients, yet the rate of CR does not exceed that of the non-RTX group.

Interestingly, among our cohort of 111 SAb- individuals, 74 (66.67%) were found to be positive for glomerular PLA2R antigen, consistent with current literature.[8,14,15] Van de Logt et al reported a case of SAb-/GAg+ patient in whom serum anti-PLA2R antibody became detectable following recurrence.[16] This suggests that "seroconversion" may occur in patient initially lacking anti-PLA2R antibody. Subsequently, Ramachandran et al also reported that four out of six SAb-/GAg+ patient developed positive anti-PLA2R antibody during follow-up.[17] These findings support the hypothesis that antibodies become detectable only after the renal buffering capacity is exceeded.[16] In our cohort, 36.8% of patients who did not achieve remission were found to be anti-PLA2R antibody positive, one of whom was a relapsed patient. As not all patients underwent repeat anti-PLA2R testing during follow-up, it is difficult to ascertain precisely when seroconversion occurred. Moreover, it is impossible to rule out the possibility of false negative results for baseline anti-PLA2R antibody titer, as study have demonstrated that time-resolved fluoroimmunoassay enhance the sensitivity and specificity than ELISA for detecting these antibody.[18] Therefore, repeated testing of serum anti-PLA2R antibody levels in SAb-/GAg+ patients is particularly important.

The results of our study indicate that the CR rate in non-RTX group was significantly superior to that in RTX group. Although the difference in OR between the two groups did not reach statistical significance ($P = 0.063$), non-RTX group also demonstrated a trend towards superiority. Moreover, within SAb-/GAg+ cohort, the remission rate in the non-RTX group was significantly higher than that in the RTX group. In our non-RTX group, the majority of patients were received TAC treatment, it exhibits greater potency in inhibiting antigen-driven T-cell activation, cytokine production, and lymphocyte proliferation in vitro.[19] Meanwhile, TAC can directly act on the podocytes to inhibit Ang II–induced damage on podocyte structures, and regulates cytoskeletal proteins.[20] Its effect in reducing proteinuria may be dose-dependent.[21] TAC may also augment the efficacy of glucocorticoids by enhancing glucocorticoid receptor affinity.[22] CYC can effectively alleviate proteinuria by inhibiting the proliferation of B and T cells.[23] In RI-CYCLO Randomized Trial, 34% of patients were SAb-, and RTX showed no significant advantage over CYC.[6] It is important to note that previous studies have demonstrated the superiority of RTX in terms of long-term efficacy, whereas TAC has been reported to have a higher recurrence rate following treatment discontinuation.[3–5] Given that our follow-up period was only 15 months, we are unable to draw conclusions regarding differences in long-term efficacy between the two groups. Consistent with published studies, adverse events between two groups were similar.[6,23,24]

This study has several limitations. First, as a non-randomized retrospective study, it is subject to inherent selection bias and unmeasured confounding factors. Second, serum anti-PLA2R antibody levels were not comprehensively monitored during follow-up. Repeated antibody testing allows for the timely identification of patients who have seroconverted, providing valuable information regarding treatment efficacy and disease activity while avoiding the potential risks associated with repeated kidney biopsies. Due to the lack of longitudinal anti-PLA2R antibody data, our study was unable to more accurately assess the immune response and its correlation with clinical outcomes. Third, the non-RTX control group was heterogeneous, although the results of the sensitivity analysis were similar, the heterogeneity within the control treatment regimen should still be carefully considered. Fourth, the small sample size may have limited the statistical power to detect differences in secondary outcomes. Finally, the relatively short follow-up period may not have been sufficient to fully capture the delayed therapeutic effects of RTX or to assess the durability of long-term remission and the recurrence rate. Given these limitations, the observed results may represent only preliminary explorations findings and is intended solely for patients with moderate-to-high-risk IMN in that region. Future prospective studies with larger sample sizes, longer follow-up periods, standardized treatment, and systematic monitoring of anti-PLA2R antibody are required to validate these findings.

In summary, for patients with negative serum anti-PLA2R antibodies, non-RTX immunosuppressive therapy may be associated with higher complete remission rates and greater reduction in proteinuria compared with RTX during the 15-month follow-up period. Repeated anti-PLA2R antibody testing may prove crucial for patients initially testing negative.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article or supplementary material.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Second Affiliated Hospital of Nanchang University and included in the study and obtained from all individual participants (IIT-O-2025-196).

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Disclosure

The authors declare that they have no competing interests. Yang Yang and Xiaojie Xie share first authorship.

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