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Exploration of rituximab treatment strategies for membranous nephropathy adapted to the Chinese healthcare environment

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Abstract

Purpose This study aimed to explore the specific efficacy of rituximab (RTX) in the treatment of membranous nephropathy (MN) and compare and analyze the differences in effectiveness among various treatment regimens, with the objective of identifying the optimal treatment protocol suitable for the medical environment in China.

Patients and methods This retrospective study focused on patients with MN who were treated with RTX and hospitalized at the First Medical Center of PLA General Hospital between January 1, 2019, and December 30, 2022. These patients were followed up for more than one year. We collected clinical data from these patients and categorized them into three groups on the basis of their RTX treatment background: the combined glucocorticoids (GCs) and/or immunosuppressants (IMS) and RTX monotherapy treatment groups, the initial and non-initial treatment groups, and the standard RTX and non-standard RTX treatment groups. The study evaluated the comprehensive outcomes of complete or partial remission during follow-up, as well as relapses after remission. Additionally, Cox regression analysis was conducted to identify risk factors influencing patient remission and relapse.

Results A total of 126 patients were enrolled in this study, with an average age of 49.0 ± 13.4 years. Among them, males accounted for up to 77.8%, with an average BMI of 26.7 ± 4.0. Among these patients, 59.5% (75/126) received RTX combined with GCs and/or IMS. Statistical results revealed that the combined use of GCs and/or the IMS had no significant effect on renal remission ($P = 0.439$), but it accelerated the process of renal remission ($P = 0.010$). A total of 34.9% (42/126) of patients chose RTX as the initial treatment. Compared with the non-initial treatment group, this choice did not significantly differ in terms of efficacy or faster remission speed (all $P > 0.05$). On the other hand, 39.7% (50/126) of patients received the standard RTX treatment regimen. Compared with the non-standard group, the standard RTX treatment group presented a better remission rate ($P < 0.001$) and a faster remission speed ($P = 0.027$). During 13.0 (12.0, 20.0) months of follow-up, the cumulative remission rate reached 73% (92/126), including 47.6% (60/126) of patients with partial remission (PR) and 25.4% (32/126) of patients with complete remission (CR). The cumulative relapse rate was 20.7% (26/126). In addition, 17.5% (22/126) of patients experienced adverse reactions. Multivariate Cox regression analysis revealed that the standard RTX treatment regimen was associated with a better

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remission rate, whereas comorbid diabetes reduced the remission rate. Older age and higher white blood cell counts may lead to a higher relapse rate.

Conclusion This study revealed that RTX treatment has a high remission rate and a low relapse rate in MN patients. The standard RTX treatment regimen can provide better benefits. However, our experience is limited by its retrospective design and relatively small sample size, and further large-scale randomized controlled studies are needed to confirm our preliminary findings.

Keywords Membranous nephropathy, Rituximab, Remission, Relapse

Introduction

Membranous nephropathy has become the primary etiology of nephrotic syndrome (NS) in adults, and its incidence rate is increasing annually in China. According to statistics, in the past 11 years, the proportion of MNs in renal biopsies has increased from 12.2 to 24.9%, with an annual growth rate of 13%, whereas the proportions of other major glomerular diseases have remained relatively stable [1]. The progression of MN is highly uncertain. Although approximately one-third of patients will experience spontaneous partial remission [2], approximately 40% of untreated patients with persistent nephrotic syndrome may progress to end-stage kidney disease (ESKD) [3]. This grim situation highlights the urgency of timely clinical intervention and treatment.

Despite the recommendation of the use of GCs combined with cyclophosphamide as the preferred treatment for MN in the Kidney Disease Improving Global Outcomes (KDIGO) guidelines of 2021 [4], the side effects of this regimen, such as infection and reproductive toxicity [5], cannot be ignored. Calcineurin inhibitors (CNIs), such as cyclosporine A (CsA) and tacrolimus, are also considered effective treatment options. In particular, studies have shown that tacrolimus has a high remission rate for MN patients, with relatively few side effects. However, the high relapse rate after drug withdrawal has become a major drawback [6].

With advancements in medicine, RTX has gradually been introduced into the treatment of MN. RTX is a drug that targets the CD20 molecule on the surface of pre-B cells and mature B lymphocytes, exerting its effect by inducing B-cell apoptosis and inhibiting the formation of autoantibodies (such as anti-phospholipase A2 receptor antibodies, PLA2R) [7]. According to the recommendations of the 2021 KDIGO guidelines [4], RTX has become a first-line treatment for MN, with a remission rate of 60-80% at 12 months [8].

In China, despite the gradual popularization of RTX [9], there are currently multiple diverse treatment approaches in use, given the unique medical environment of the country. This study aimed to compare the effects of these different treatment regimens to identify the best treatment approach. We hope that through this research, we can provide clinicians with more targeted treatment recommendations to optimize the treatment outcome of MN.

Materials and methods

Study design

Patients were diagnosed with MN at the Chinese PLA General Hospital from May 1, 2019, to December 30, 2022. The patient inclusion criteria were as follows: (1) age ≥ 18 years; (2) follow-up time ≥ 1 year after RTX treatment; (3) diagnosed with MN via renal biopsy and treated with RTX; (4) complete medical records; exclusion criteria: (1) diagnosed with secondary MN (including MN caused by hepatitis B, autoimmune diseases, tumors, etc.); (2) suffering from ESRD, estimated glomerular filtration rate (eGFR) < 15 mL/min/1.73 m^2 , undergoing dialysis or other renal replacement therapy; (3) severe complications such as severe infection, tumors, active hepatitis B, HIV, severe liver damage during renal biopsy; and (4) pregnant and lactating women.

Research methods

Data collection

Demographic data, laboratory tests, treatment regimens, comorbidities, renal pathology, follow-up duration, and clinical outcomes were collected from patients who met the inclusion criteria. The date when each patient began treatment with RTX marked the baseline for the study. Following the initial RTX therapy, measurements of 24-hour urine protein (via the pyrogallol red method), serum albumin (via the bromocresol green binding assay), eGFR, PLA2R (via a commercially available ELISA kit), CD20, and B-cell counts (via flow cytometry) were recorded at 3, 6, 9, and 12 months. The eGFR was assessed via the CKD-EPI [10] formula. The patients' prognoses, including partial remission, complete remission, no remission, relapse, and adverse reactions (including lung infections, upper respiratory infections, genitourinary infections, gastrointestinal infections, etc.), were recorded. The follow-up duration was defined as the time interval (in months) from the commencement of RTX treatment to the final visit.

Rituximab treatment regimen

On the basis of the therapeutic background of RTX, the following treatment regimens are categorized:

Combined with GCs and/or IMS: In addition to RTX, patients were treated with GCs and/or IMS, which included mainly GCs, tacrolimus, and CsA in this study. If such a combination approach is not adopted, it is referred to as an RTX monotherapy regimen.

Initial RTX: This regimen specifically applies to patients who have not previously received any GCs and/or IMS therapy, with RTX serving as the first-choice treatment drug upon the diagnosis of MN. If this condition is not met, it is classified as a non-initial RTX treatment regimen.

Standard RTX [9]: RTX was administered weekly at a dose of 375 mg/m^2 for a total of 4 courses or 1 g of RTX every 2 weeks for a total of 2 courses. If neither of these standards is met, it is considered a non-standard RTX treatment regimen.

Related definitions

The definition of nephrotic syndrome is characterized by urine protein excretion ≥ 3.5 g/24 h and serum albumin ≤ 30 g/L. PR was defined as urine protein excretion between 0.3 and 3.5 g/24 h, with a reduction of ≥ 50% in 24-hour proteinuria compared with baseline, accompanied by stable renal function. CR was defined as urine protein excretion < 0.3 g/24 h, and the level of serum ALB was > 35 g/L. Non-remission (NR) was defined as a serum ALB concentration < 30 g/L, with a < 50% reduction in 24-hour urine protein excretion compared with baseline, and deterioration of renal function. Remission is the combined outcome of complete or partial remission during follow-up. Relapse is defined as the recurrence of proteinuria consistent with nephrotic syndrome (three consecutive measurements of urine protein > 3.5 g/24 h) after achieving complete or partial remission.

Refractory nephrotic syndrome [11] refers to nephrotic syndrome that is unresponsive to standard corticosteroid therapy, is corticosteroid dependent, is corticosteroid resistant, or experiences multiple relapses in a short period after remission. For example, two or more relapses within six months, or three or more relapses within one year.

Prognostic indicators

Primary outcome: The combined result of complete or partial remission during the entire follow-up period.

Secondary outcomes: Relapse during the follow-up period and complete or partial remission at the 12-month mark.

Statistical analysis

The normality of the data was tested via the Kolmogorov–Smirnov test. Continuous variables with a normal or symmetrical distribution are expressed as the mean ± standard deviation, whereas those with a non-normal distribution are expressed as the median (interquartile range), i.e., [M (O1, Q3)]. Categorical data are expressed as n (%). Student's t test was used to compare normally distributed continuous variables between groups, the Mann–Whitney U test was used to compare non-normally distributed continuous variables between groups, and Pearson's $\chi 2$ test was used to compare ratios or composition ratios.

To delve deeper into and compare the disparities among different treatment groups, taking into account potential confounding factors, we employed linear regression and Cox regression analyses.

In the initial screening phase for risk factors, we utilized univariate Cox regression analysis. Subsequently, indicators with P values less than 0.1 were included in the multivariate Cox regression model to assess the combined effects of various risk factors on patient prognosis more accurately.

SPSS software (v22.0; IBM Corp., Armonk, NY, USA) was used for statistical analysis. All tests were two-sided, and P values < 0.05 were considered statistically significant.

Results

Demographic characteristics, comorbidities, treatment and adverse reactions

This study screened patients with MN who were hospitalized and received RTX treatment at the First Medical Center of the PLA General Hospital from January 1, 2019, to December 30, 2022. Patients with a follow-up period of no less than 12 months were selected. After 7 patients with incomplete data and 3 patients with comorbid tumors were excluded, a total of 126 eligible patients with MN were ultimately included in the study. The follow-up time for these patients was 13.0 (12.0, 20.0) months, and the remission rate reached 73% ($n = 126$). The details of patient enrollment are shown in Table 1.

The average age of the study population was 49.0 ± 13.4 years, with male patients accounting for 77.8% of the sample. The mean BMI was 26.7 ± 4.0. Among these patients, 80 (63.5%) had comorbid hypertension, 39 (31%) had diabetes, 7 (5.6%) had coronary heart disease, and 52 (41.3%) were diagnosed with refractory nephrotic syndrome.

Fifty-two (34.9%) patients chose RTX as their initial treatment during the course of the disease, and 75 (59.5%) RTX-treated patients were also treated with GCs and/or IMS. Among them, 19 (15.1%) patients were given tacrolimus, 50 (39.7%) patients were given GCs, and 11

Table 1 Comparison of baseline parameters, comorbidities, treatments, adverse reactions, clinical characteristics at admission, and differences between patients who achieved remission and those who did not, in the final study population

Project	Remission(*N* = 92)	Non-remission (*N* = 34)	Total (*N* = 126)	*P*
Age (years)	48.5 ± 14.7	50.1 ± 11.0	49.0 ± 13.4	0.571
Sex (male)	71(77.2%)	27(79.4%)	98(77.8%)	0.789
BMI	26.6 ± 4.1	26.9 ± 3.9	26.7 ± 4.0	0.744
Laboratory examination				
HB (g/L)	123.3 ± 20.3	122.5 ± 22.4	123.1 ± 20.8	0.863
WBC (10^9/L)	6.8(5.6, 8.6)	6.8(5.2, 10.4)	6.8(5.6, 8.8)	0.822
PLT (10^9/L)	234.0(190.0, 283.0)	238.5(203.3, 302.8)	238.0(191.0, 285.0)	0.562
CRP (mg/dL)	0.1(0.09, 0.1)	0.1(0.09, 0.1)	0.1(0.09, 0.1)	0.540
IL-6 (pg/ml)	2.1(2.0, 4.5)	2.0(2.0, 3.8)	2.1(2.0, 3.9)	0.990
TP(g/L)	46.4 ± 7.5	43.6 ± 8.4	45.7 ± 7.8	0.083
ALB (g/L)	25.1 ± 6.1	23.6 ± 6.2	24.7 ± 6.1	0.238
CH (mmol/L)	5.6(4.5, 6.9)	6.1(5.6, 7.7)	5.8(4.8, 6.9)	0.027
TC (mmol/L)	2.0(1.4, 3.0)	2.9(2.6, 3.3)	2.3(1.5, 3.2)	0.001
GLU (mmol/L)	4.9(4.3, 5.5)	5.0(4.3, 6.2)	4.9(4.4, 5.6)	0.524
UN (mmol/L)	6.0(4.9, 8.0)	7.1(5.8, 8.9)	6.2(5.0, 8.2)	0.056
SCR (umol/L)	86.8(72.7, 112.6)	99.4(82.8, 116.0)	90.3(77.8, 113.4)	0.071
eGFR ml/min/1.73m^2	83.1 ± 27.8	73.7 ± 24.5	80.5 ± 27.2	0.087
UTP (g/24 h)	5.8(4.5, 8.0)	6.7(5.3, 8.6)	6.0(4.7, 8.4)	0.090
PLA2R (RU/ml)	46.0(5.1, 93.3)	72.5(53.9, 89.3)	51.3(9.6, 113.3)	0.029
B cell count	256.4(138.3, 414.5)	314.0(172.3, 376.0)	265.7(145.0, 412.0)	0.823
CD20	0.12 ± 0.05	0.11 ± 0.05	0.12 ± 0.05	0.819
T cell count	1610.0(1265.5, 2089.0)	1642.0(1013.5, 2479.0)	1630.0(1221.0, 2130.0)	0.741
Comorbidities				
Hypertension (n, %)	57(62.0%)	23(67.6%)	80(63.5%)	0.556
Diabetes (n, %)	23(25.0%)	16(47.1%)	39(31.0%)	0.017
Coronary heart disease (n, %)	6(6.5%)	1(2.9%)	7(5.6%)	0.673
RNS (n, %)	30(32.6%)	22(64.7%)	55(41.3%)	0.001
Renal pathology				
Pathological stage (n, %)				0.289
I	56(50.0%)	16(47.1%)	42(49.2%)	
II	42(45.7%)	14(41.2%)	56(44.4%)	
III	4(4.3%)	3(8.8%)	7(5.6%)	
IV	0(0.0%)	1(2.9%)	1(0.8%)	
Renal arteriosclerosis (n, %)	29(31.9%)	9(26.5%)	38(30.4%)	0.559
Renal tubulointerstitial injury (n, %)	6(6.6%)	1(2.9%)	7(5.6%)	0.429
Glomerulosclerosis %	4.6(0.0, 11.5)	6.0(0.0, 10.0)	5.0(0.0, 10.0)	0.941
Balloon adhesion %	2.5 ± 6.8	4.5 ± 9.4	2.9 ± 7.4	0.404
Mesangial appreciation (n, %)	19(20.7%)	7(20.6%)	26(20.6%)	0.994
Treatment				
Initial RTX (n, %)	37(40.2%)	7(20.6%)	42(34.9%)	0.040
Combined with GCs and/or IMS (n, %)	55(59.8%)	20(58.8%)	75(59.5%)	0.992
With GCs	37(40.2%)	13(38.2%)	50(39.7%)	
With tacrolimus	14(15.2%)	5(14.7%)	19(15.1%)	
With CsA	8(8.7%)	3(8.8%)	11(8.8%)	
Standard RTX (n, %)	39(42.4%)	11(32.4%)	50(39.7%)	0.307
Time to use (months)	12.0(4.3, 36.0)	15.5(7.8, 61.3)	13.0(6.0, 37.3)	0.111
RTX dose				
Standard dose (g)	2.0(2.0, 2.0)	2.0(2.0, 2.0)	2.0(2.0, 2.0)	1.000
Non-standard dose (g)	1.1(1.0, 1.1)	1.1(1.0, 1.1)	1.1(1.0, 1.1)	0.873
First year dose (g)	2.0(1.1, 2.2)	1.6(1.1, 2.0)	2.0(1.1, 2.1)	0.231
Total dose (g)	2.0(1.1, 2.8)	1.6(1.1, 2.0)	2.0(1.1, 2.6)	0.153
ARB or ACEI(n, %)	78(84.4%)	26(76.5%)	104(82.5%)	0.275

Table 1 (continued)

Project	Remission(*N* = 92)	Non-remission (*N* = 34)	Total (*N* = 126)	*P*
Adverse reactions (n, %)				0.028
Pulmonary infection	7(7.6%)	6(17.6%)	13(10.3%)	
Upper respiratory infection	2(2.2%)	3(8.8%)	5(4.0%)	
Gastrointestinal infection	0(0.0%)	2(5.9%)	2(1.6%)	
Urinary and reproductive tract infection	1(1.1%)	0(0.0%)	1(0.8%)	
Others	1(1.1%)	0(0.0%)	1(0.8%)	
Follow up time (months)	14.0(12.0, 22.5)	12.0(12.0, 15.5)	13.0(12.0, 20.0)	0.011

Abbreviations: BMI, body mass index; HB, hemoglobin; WBC, white blood cell; PLT, platelets; CRP, C-reactive protein; IL-6,interleukin-6; TP, total protein; ALB, albumin; CH, cholesterol; TC, triglyceride; GLU, glucose; UN, urea nitrogen; SCR, serum creatinine; eGFR, estimated glomerular filtration rate; UTP, urinary protein; PLA2R, Phospholipase A2 Receptor; RNS, refractory nephrotic syndrome; RTX, rituximab; GCs, Glucocorticoids; IMS, immunosuppressants; CsA, Cyclosporine; ARB, Angiotensin II Receptor Blocker; ACEI, angiotensin-Converting Enzyme Inhibitors

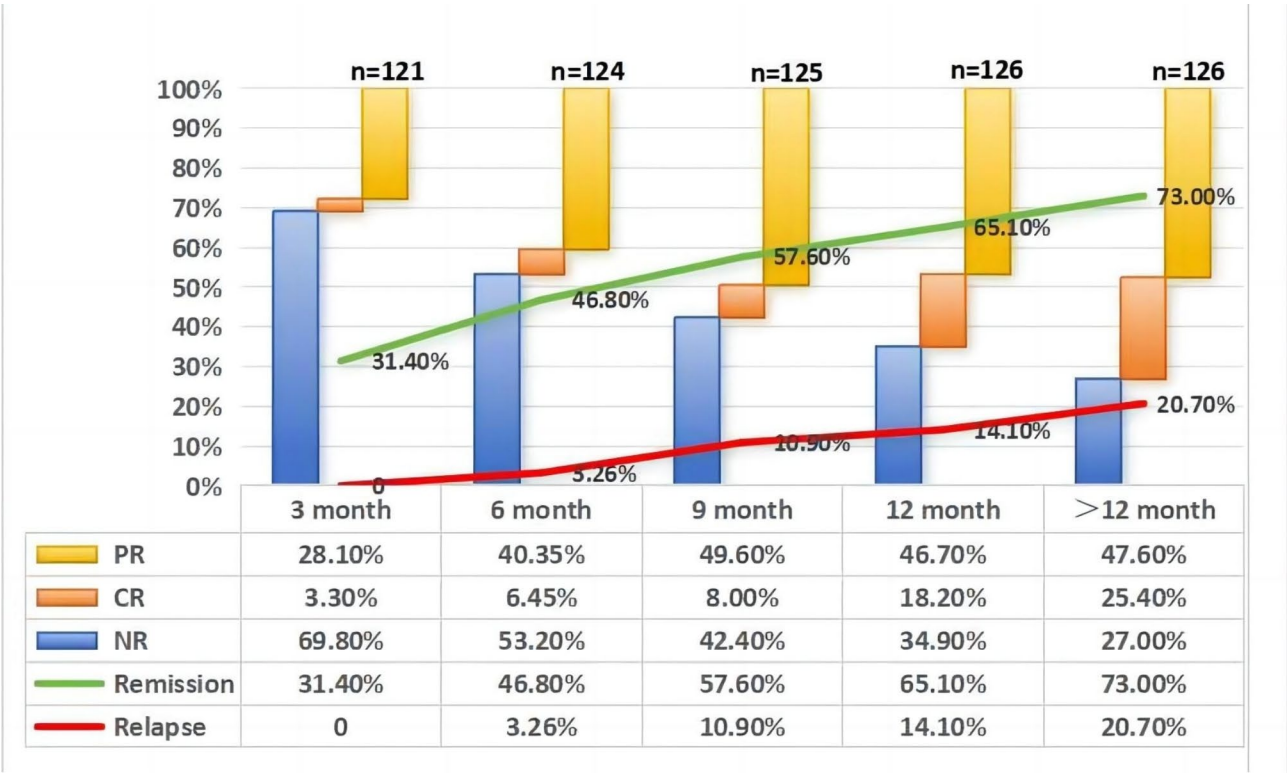

Fig. 1 Patient remission and relapse at different follow-up points
Abbreviations: PR, partial remission; CR, complete remission; NR, non-remission

(8.8%) patients were given CsA. Additionally, 50 (39.7%) patients were treated with the standard RTX regimen. The time from the diagnosis of kidney disease to the initiation of RTX treatment was 13.0 (6.0–37.3) months. The standard RTX dose is 2.0 (2.0, 2.0) g, whereas the non-standard RTX treatment dose is 1.1 (1.0, 1.1) g, with the non-standard group primarily receiving low-dose treatment.

Twenty-two (17.5%) patients experienced adverse reactions during the follow-up period. The main adverse reaction was pulmonary infection in 13 patients (10.3%), followed by upper respiratory infection in 5 patients (4.0%), gastrointestinal infection in 2 patients (1.6%), urinary and reproductive tract infection in 1 patient (0.8%), and other reactions in 1 patient (0.8%).

The main clinical characteristics of the remaining laboratory tests, renal pathology, and other baselines are shown in Table 1.

Patient remission and relapse at different follow-up points

Figure 1 shows that during the 13.0 (12.0, 20.0) months of follow-up in this study, the cumulative remission rate was 73%, and the cumulative relapse rate was 20.7%. Detailed information about patient remission and relapse at different follow-up time points is also presented.

Third Month Follow-Up: PR was observed in 34 patients (28.1%, $n = 121$). CR was achieved in 4 patients

(3.3%, $n=121$). Sixth Month Follow-Up: The number of patients with a PR increased to 50 (40.3%, $n=124$). CR was achieved in 8 patients (6.45%, $n=124$). Relapse was observed in 3 patients (3.2%, $n=92$). Ninth Month Follow-Up: PR continued to increase in 62 patients (49.6%, $n=125$). CR increased in 10 patients (8.00%, $n=125$). The number of patients who experienced relapse increased to 10 (10.9%, $n=92$). Twelfth Month Follow-Up: Fifty-nine patients (46.7%, $n=126$) achieved a PR. Twenty-three patients (18.2%, $n=126$) achieved CR. The cumulative remission rate was 65.1%. The number of patients who experienced relapse increased to 12 (13, $n=92$). Follow-Up Period Over 12 Months: The number of patients with a PR further increased to 60 (47.6; $n=126$). CR was achieved in 32 patients (25.4%, $n=126$). The number of patients who experienced relapse also increased to 19 (20.7, $n=92$).

The impact of different treatment regimens on patient remission and remission speed

Among the different treatment regimens, there was no significant difference in remission rates between the combined GCs and/or IMS therapy group and the RTX monotherapy group ($P=0.439$). However, the median remission time in the combined therapy group was 3 (3, 9) months, which was faster than that in the RTX monotherapy group, with a median remission time of 6 (3, 12) months ($P=0.010$). Figure 2 shows the differences in remission and relapse rates between the different treatment groups, whereas Fig. 3 shows the differences in

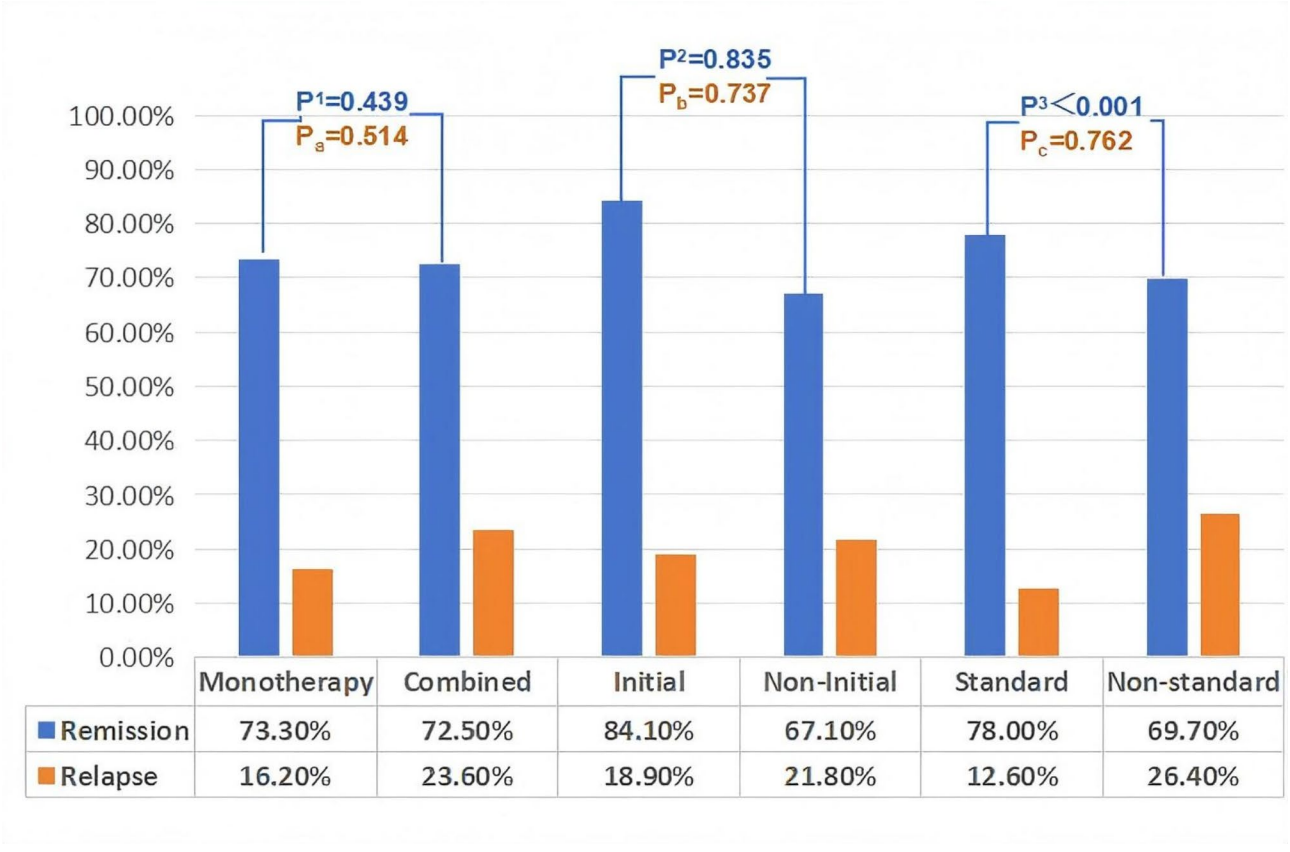

Fig. 2 The impact of different treatment regimens on patient remission and relapse

Abbreviations: Blue P represents the remission difference between different treatment groups

P [1]: After correcting for hypertension, the differences between patient groups treated with or without a combination of GCs and/or IMS were compared; P [2]: After correcting for hemoglobin, CD20, UN, and PLA2R; time to use; glomerular arteriosclerosis; standard treatment; and RNS; the differences between the initial treatment and non-initial treatment patient groups were compared; P [3]: After correcting for RNS; time to use; and initial treatment. compared the differences between the standard treatment and non-standard treatment patient groups

Yellow P represents the difference in relapse between the different treatment groups

P_a: After hypertension was corrected, the differences between patient groups treated with or without a combination of GCs and/or the IMS were compared. P_b: After the hemoglobin, UN, PLA2R, time to use, standard treatment, and RNS were corrected, the differences between the initial treatment and non-initia treatment patient groups were compared. Pc: After UN, RNS, the time to use, and initial treatment were corrected. compared the differences between the standard treatment and non-standard treatment patient groups

GCs, glucocorticoid; IMS, immunosuppressant; UN, urea nitrogen; PLA2R, phospholipase A2 receptor; RNS, refractory nephrotic syndrome

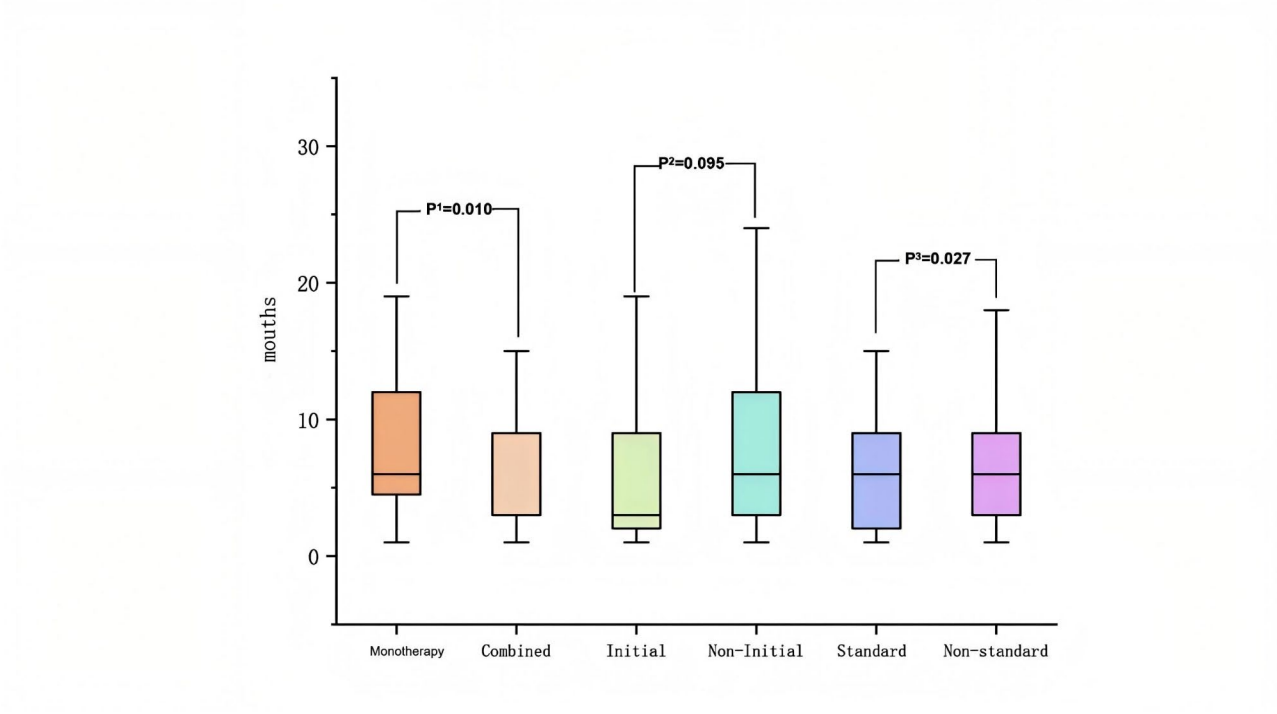

Fig. 3 The impact of different treatment regimens on patient remission speed
Abbreviations: P [1]: After hypertension was corrected, the differences between patient groups treated with or without a combination of GCs and/or the IMS were compared
P [2]: After the hemoglobin, CD20, UN, and PLA2R levels, time to use, initial dose, degree of glomerular arteriosclerosis, standard treatment, and RNS were corrected, the differences between the initial treatment and non-initial treatment patient groups were compared
P [3]: After correcting for RNS, the time to use is the initial treatment. compared the differences between the standard treatment and non-standard treatment patient groups
GCs, glucocorticoid; IMS, immunosuppressant; UN, urea nitrogen; PLA2R, phospholipase A2 receptor; RNS, refractory nephrotic syndrome

the speed of remission between the various treatment groups.

The remission rate in the initial RTX treatment group was 84.1%, which was higher than the 67.1% reported in the non-initiall treatment group. However, after adjusting for confounding factors, there was no significant difference between the two groups ($P = 0.835$). The median remission time was 3 (2, 9) months, and there was no difference in remission speed compared with non-initial treatment patients after adjusting for confounding factors ($P = 0.095$).

The remission rate in the standard treatment group was 78%, which was higher than the 69.7% reported in the non-standard treatment group. After adjusting for confounding factors, there was a significant difference in remission rates between the two groups ($P < 0.001$). This result indicates that the standard treatment group had a significant advantage in terms of the treatment effect. In terms of remission time, there was also a significant difference between the standard treatment group and the non-standard treatment group ($P = 0.027$).

After adjusting for confounding factors, there were no significant differences in relapse rates across all treatment regimens (all $P > 0.05$).

Differences in baseline parameters, comorbidities, treatment, adverse reactions, and clinical characteristics at admission between remission patients and non-remission patients

Compared with the non-remission group, our study revealed that the remission group had significantly lower cholesterol ($P = 0.027$), triglyceride ($P = 0.001$), and PLA2R values ($P = 0.029$). Additionally, the proportions of diabetic patients ($P = 0.017$) and patients with refractory nephrotic syndrome ($P = 0.001$) were significantly lower in the remission group.

Further analysis revealed that more patients in the remission group chose to use RTX as their initial treatment ($P = 0.040$). Moreover, during the longer follow-up period ($P = 0.011$), the remission group experienced significantly fewer adverse reactions ($P = 0.028$).

Apart from the aforementioned significant differences, no other parameters were notably different between the

Table 2 Cox regression analysis of risk factors affecting remission of patients

Univariate Cox regression analysis					Multivariate Cox regression analysis			
Influencing factors	**HR**	**95% CI**		***P*-value**	**HR**	**95% CI**		***P*-value**
		Lower Limit	**Upper Limit**			**Lower Limit**	**Upper Limit**	
BMI	0.938	0.891	0.988	0.016				
TP (g/L)	1.025	0.996	1.054	0.091				
UTP (g/24 h)	0.927	0.851	1.009	0.081				
Diabetes (n, %)	0.603	0.374	0.972	0.038	0.585	0.362	0.947	0.029
Standard RTX (n, %)	2.208	1.406	3.468	<0.001	2.262	1.434	3.568	<0.001
Combined With tacrolimus	1.903	1.057	3.427	0.032				
First year dose (g)	1.193	0.982	1.450	0.076				

Abbreviations: BMI, body mass index; TP, total protein; UTP, urinary protein; RTX, rituximab

Table 3 Cox regression analysis of risk factors affecting relapse of patients

Univariate Cox regression analysis					Multivariate Cox regression analysis			
Influencing factors	**HR**	**95% CI**		***P*-value**	**HR**	**95% CI**		***P*-value**
		Lower Limit	**Upper Limit**			**Lower Limit**	**Upper Limit**	
Age (years)	1.061	1.019	1.105	0.004	1.082	1.034	1.132	<0.001
WBC (10^9/L)	1.184	1.016	1.370	0.030	1.285	1.104	1.496	0.001
UN (mmol/L)	1.134	1.003	1.284	0.045				
Hypertension (n, %)	2.406	0.795	7.281	0.120				
Laboratory indicators when remission								
ALB (g/L)	0.965	0.885	1.052	0.417				
UTP (g/24 h)	0.953	0.594	1.531	0.844				
eGFR ml/min/1.73m^2	0.994	0.977	1.011	0.480				
PLA2R (RU/ml)	0.995	0.913	1.085	0.917				
B cell count	0.979	0.942	1.018	0.293				

Abbreviations: WBC, white blood cell; UN, urea nitrogen; ALB, albumin; UTP, urinary protein; PLA2R, Phospholipase A2 Receptor

two groups. For detailed information, please refer to Table 1.

Cox regression analysis of risk factors affecting patient remission

Univariate Cox regression analysis revealed that a lower BMI, the absence of diabetes, the adoption of standard RTX, the combined use of tacrolimus improved the remission rate. The detailed data are presented in Table 2.

Indicators with $P<0.05$ (BMI, diabetes, standard treatment, combined use of tacrolimus) were included in the multivariate Cox regression analysis. The results revealed that the standard RTX ($HR=2.262$, 95% CI [1.434 ~ 3.568], $P<0.001$) improved the remission rate, whereas comorbidity with diabetes ($HR=0.585$, 95% CI [0.362 ~ 0.947], $P=0.029$) reduced the remission rate.

Cox regression analysis of risk factors affecting patient relapse

Univariate Cox regression analysis revealed that advanced age, higher white blood cell count, and urea can increase the relapse rate of patients. See Table 3 for details.

Indicators with $P<0.05$ (age, white blood cell count, urea) were included in the multivariate Cox regression analysis. Advanced age ($HR=1.082$, 95% CI [1.034 ~ 1.132], $P<0.001$) and higher white blood cell count ($HR=1.285$, 95% CI [1.104 ~ 1.496], $P=0.001$) can lead to a higher relapse rate.

Discussion

In recent years, RTX has gradually attracted attention in the treatment of MN. Its therapeutic mechanism may involve reducing B-cell levels, thereby reducing the production of circulating antibodies. Through this approach, RTX can prevent the deposition of immune complexes under the glomerulus, thereby reducing damage to the glomerular filtration barrier and effectively alleviating MN [12]. This study conducted a retrospective analysis, and the results revealed that at the 12th month after medication, the cumulative remission rate of patients reached 65.1%. This result is similar to the 62% reported in the RI CYCLO study [13] and the 60% reported in the MENTOR study [14] but slightly lower than the 80.2% reported in the Zhang S et al. [15] study. During the average follow-up period of 13.0 (12.0–20.0) months, 73% of patients achieved clinical remission. Although this remission rate is slightly lower than the 82% reported in the 24-month follow-up of the RI CYCLO study [13], it is significantly higher than the 64.9% reported in the

17-month follow-up of the GEMRITUX study [16]. These findings provide strong evidence to support the efficacy and safety of RTX in the treatment of MN.

In China, despite the gradual popularization of RTX, achieving patient cooperation remains a major challenge. This is primarily because patients often need to travel long distances to seek treatment at large tertiary hospitals—as exemplified by the patients in this study, who hailed from 11 different provinces and cities. Additionally, the currently recommended RTX treatment regimen [9] (standard treatment) demands extended hospitalization and incurs substantial costs, making patient compliance a considerable hurdle. Taking these factors into account, Chinese doctors frequently need to explore treatment options that are more suitable for local patients. To better meet the actual needs of local patients, these adjustments may involve reducing the RTX dosage, combining RTX with GCs and/or IMS, or adjusting the dosing interval and extending the dosing time. Therefore, in this study, 59.5% of patients received RTX in combination with GCs and/or IMS. In contrast, only 39.7% of patients strictly followed standard RTX treatment. On the other hand, owing to economic considerations, patients tend to choose RTX only after GCs, and IMS treatments have proven ineffective. This explains why only 34.9% of patients in this study selected RTX as their initial treatment. Moreover, the high proportion of refractory nephrotic syndrome patients in the present study (41.3%) indirectly reflects this phenomenon.

RTX is effective in the treatment of MN, but the remission speed is often slow. A study [17] revealed that during RTX treatment, when CD20-positive B cells were depleted, antibody titers did not decrease. These findings suggest that non-CD20-positive B cells may be involved in the production of pathogenic antibodies, which could lead to RTX treatment results falling below expectations. Adding IMS to RTX may achieve better treatment outcomes, accelerate disease remission, and delay the progression of kidney disease. Ma Q et al. [18] compared the efficacy of RTX combined with GCs with that of RTX alone. The study revealed that during the 12-month follow-up period, the combined remission rates in the RTX/GC group and RTX group were 74.3% and 67.7%, respectively. The median remission time in the RTX/GC group was shorter than that in the RTX group. Kaplan-Meier survival analysis revealed that the cumulative CR rate and cumulative combined remission rate were better in the RTX/GC group than in the RTX group ($P = 0.043$, $P = 0.040$). Chen X et al. [19] reported that the total effective rate of RTX + tacrolimus in the treatment of refractory IMN was 87.14%, with a median time to complete remission of 9 (6.0, 12.0) months. The total effective rate of RTX alone was 65.87%, with a median time to complete remission of 10.5 (6.0, 12.0) months. The RTX + tacrolimus group showed better efficacy without a significant increase in adverse reactions. In this study, although there was no significant difference in remission rates between the combined with GCs and/or IMS therapy group and the RTX monotherapy group (72.5% and 73.3%, respectively), it is worth noting that the remission rate in the combination therapy group was significantly faster than that in the RTX monotherapy group ($P = 0.010$).

Chen P et al. [20] reported that the total remission rates in the initial group and the refractory/relapse group were 84.21% and 82.76%, respectively. There was no statistically significant difference in total remission rates between the two groups ($P > 0.05$), which is consistent with the findings of this study. Additionally, this study revealed that in terms of the speed of response, the initial treatment group did not demonstrate a significant advantage over the non-initial treatment group ($P = 0.095$). This result may indicate that RTX as an initial treatment does not possess special advantages. Therefore, in clinical practice, choosing RTX as an initial treatment may require comprehensive consideration on the basis of the specific situation of the patient and the experience of the doctor.

There is still some controversy regarding the optimal dose of RTX for the treatment of MN, as RTX dosages vary widely across different studies. The mainstream consensus is that full-dose therapy may contribute to a better treatment response [21]. Currently, there is a lack of randomized controlled studies comparing low-dose and full-dose RTX infusions both domestically and internationally, which warrants further investigation [22]. This study revealed that patients receiving the standard RTX dose had a significantly greater remission rate than did those receiving the non-standard dose ($P < 0.001$), and the remission speed of the standard dose group was also faster ($P = 0.027$). This result is consistent with previous research [21], indicating that full-dose RTX treatment may be more beneficial in improving patients' remission rates and accelerating remission. Although Chinese doctors have explored treatment options that are more suitable for local patients, such as the use of low-dose RTX in combination with GCs and/or IMS, the results of this study indicate that the standard RTX treatment regimen currently remains the best choice.

Through multivariate Cox regression analysis, this study also identified several risk factors that affect patient remission and relapse. Among them, the adoption of the standard RTX treatment regimen was significantly associated with a higher remission rate, which once again confirms the effectiveness of the standard treatment regimen. However, the presence of comorbid diabetes was found to reduce the remission rate of patients, which deserves further exploration [23]. It has

been documented in the literature that MN accounts for up to 50% of non-diabetic nephropathy confirmed by pathology in diabetic patients. During the treatment of MN, the use of GCs or calcineurin inhibitors may exacerbate glucose metabolism disorders in diabetic patients, which may have further negative impacts on the kidneys. Therefore, MN patients with diabetes often face a worse renal prognosis. Qian et al. [24] found that the remission rate of MN patients with diabetes was lower than that of patients without diabetes, although this difference was not statistically significant (33.3% vs. 45.5%, $P=0.53$). Similarly, Xie H et al. [25] reported that baseline diabetes is associated with an increased risk of failure to achieve remission in patients receiving IMS or GCs, but it is not directly related to the relapse of MN or a decline in renal function. In addition, when exploring the role of the lectin complement pathway and diabetes in the pathogenesis of MN, Zdravkova et al. [26] In the context of chronic inflammation, increased activation of the lectin pathway in diabetic patients may trigger a "switch" from diabetic nephropathy to MN. These findings provide a new perspective on the relationship between diabetes and MN. In summary, there is a clear association between diabetes and failure of proteinuria remission, and this association appears to be reliable and strong. However, the underlying mechanism of this association is currently not fully understood and therefore deserves further investigation.

The relapse rate in this study was 20.7%, which was slightly lower than the 29.8% relapse rate reported by Ruggenenti et al. [27] in their study, but their follow-up period was 37.7 (24.8–49.6) months. In this study, advanced age and higher WBC counts were identified as risk factors for relapse. Compared with younger patients, elderly patients have a lower eGFR, a greater incidence of hypertension, a greater incidence of glomerular sclerosis, more severe renal tubular atrophy and interstitial fibrosis, and a poorer response to GCs and IMS therapy [12]. Previous studies have shown that male sex and advanced age (>50 years) are associated with poor prognosis in MN patients [9]. Notably, this study is the first to identify elevated WBC counts as a risk factor for MN relapse. This discovery provides a new perspective for monitoring and preventing the relapse of MN, but more research is needed to further validate the specific role and guiding significance of WBC counts in predicting the relapse of MN.

In addition, 17.5% of patients in this study experienced adverse reactions, a proportion similar to that reported in previous studies [15]. Notably, the incidence of adverse reactions was lower in the remission group than in the non-remission group. Among all adverse reactions, the main serious event was pulmonary infection, whereas other adverse reactions were relatively minor. We conducted detailed data verification for these serious adverse events and confirmed that none of them resulted in malignant or fatal consequences. This outcome further demonstrates the safety of RTX in the treatment of MN.

The present study is retrospective in nature and inherently relies on the completeness and accuracy of existing data records. This approach may introduce biases related to data capture and documentation practices, which can vary over time and across different clinical settings. Additionally, our study is based on a hospital-based sample, which may not be representative of the broader population. The relatively small sample size of 126 patients could also limit the generalizability of our findings to other populations or settings. Selection bias may have occurred because of the non-random selection of patients from a single center, potentially skewing the results and affecting the external validity of our conclusions. In addition, the follow-up time in this study was not long enough, which may affect the distinction of factors such as relapse in different treatment regimens. Finally, although the study attempted to control potential confounding factors through statistical analysis, there may still be unconsidered variables or unknown influencing factors, which may have had some impact on the results. We acknowledge these limitations and suggest that future studies employ a prospective design with larger, diverse samples to better understand the treatment effects across different patient populations.

Conclusion

This study revealed that whether patients had previously used GCs and the IMS had no significant effect on the efficacy of RTX in the treatment of MN. Although the combined use of GCs and/or IMS can accelerate the speed of remission in patients, it does not significantly improve the long-term prognosis of the disease. The results showed that the standard RTX treatment regimen remains the most effective in treating MN and is closely associated with a higher remission rate. On the basis of these findings, we recommend the standardization of RTX treatment protocols in China to ensure consistency in treatment approaches and to optimize patient outcomes. Additionally, we emphasize the need to address the economic barriers that limit patient access to RTX treatment. This includes exploring strategies to reduce the cost of therapy, such as incorporating the drug into medical insurance coverage and centralized procurement to lower drug prices and implementing policies that can improve patient affordability and accessibility.

Abbreviations

RTX	Rituximab
MN	Membranous nephropathy
GCs	Glucocorticoids
IMS	Immunosuppressants
PR	Partial remission
CR	Complete remission

NR	Non-remission
NS	Nephrotic syndrome
ESKD	End-stage kidney disease
KDIGO	Kidney Disease Improving Global Outcomes
BMI	Body mass index
HB	Hemoglobin
WBC	White blood cell
PLT	Platelets
CRP	C-reactive protein
IL-6	Interleukin-6
TP	Total protein
ALB	Albumin
CH	Cholesterol
TC	Triglyceride
GLU	Glucose
UN	Urea nitrogen
SCR	Serum creatinine
eGFR	Estimated glomerular filtration rate
UTP	Urinary protein
PLA2R	Phospholipase A2 Receptor
RNS	Refractory nephrotic syndrome
CsA	Cyclosporine
ARB	Angiotensin II receptor blocker
ACEI	Angiotensin-converting enzyme inhibitor

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Author contributions
Jie Wu and Xueying Cao designated the research direction, while Xiaolong Wang was responsible for collecting cases, data acquisition, and manuscript writing. Jian Yang polished the manuscript, and Shuang Liang together with Hong Wang conducted data analysis and statistics.All authors agreed on the journal to which the article has been submitted; and agreed to be accountable for all aspects of the work.

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Data availability
The original dataset is stored on the server of the First Medical Center of the Chinese PLA General Hospital and can be obtained by contacting the corresponding author, Xueying Cao, whose email address: 18911622536@163.com.

Declarations

Ethics approval and informed consent
This study was reviewed by the Medical Ethics Committee of the General Hospital of the People's Liberation Army of China ((Approval No: S2024-319-01). The requirement for written informed consent was waived by this institution because of the retrospective nature of the study. The decision letter of the Ethics Committee covered patient data confidentiality and compliance with the Declaration of Helsinki.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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