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Rituximab Treatment Outcomes in Relapsed Primary Membranous Nephropathy: A Single-center Retrospective Study

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Abstract

Objective: Membranous nephropathy (MN) is a leading cause of nephrotic syndrome in adults, with phospholipase A2 receptor (PLA2R) as its primary target antigen. This study assessed the treatment response to rituximab (RTX) in patients experiencing relapsed primary MN, focusing on its efficacy and impact on disease progression. This study aimed to evaluate treatment response to RTX in patients with relapsed primary MN, focusing on its effectiveness and correlation with anti-PLA2R antibody status.

Methods: Thirty-one patients meeting the inclusion criteria, including biopsy-confirmed MN diagnosis and relapsed disease, with an estimated glomerular filtration rate (eGFR) >30 mL/min/1.73m^2, were enrolled. Treatment response was assessed after six months, and patients were categorized into three groups: complete remission (CR), partial remission (PR), and unresponsive (UR).

Results: CR was observed in 6 patients (14.9%), PR in 13 patients (41.9%), and UR in 12 patients (38.7%). Serum anti-PLA2R antibody was positive in 19 patients (61.2%) pre-RTX, with 16 patients (84.2%) patients experienced seroconversion post-RTX (p=0.003). Significant increases in serum albumin and decreases in proteinuria were observed post-RTX (p<0.001). No significant difference in eGFR was noted (p=0.264).

Conclusion: These findings highlight RTX as a valuable treatment modality for relapsed primary MN, offering potential clinical benefits regardless of anti-PLA2R antibody status.

Keywords: Rituximab, membranous nephropathy, anti-PLA2R antibody, immunologic remission, glomerular filtration rate

INTRODUCTION

Membranous nephropathy (MN) is a prevalent cause of primary nephrotic syndrome in adults and is characterized by non-inflammatory autoimmune mechanisms involving subepithelial immune deposits localized within the glomerular basement membrane and podocyte (1). Primary MN accounts for the majority of cases (75 to 80 percent), driven by circulating autoantibodies against podocyte antigens, whereas secondary MN stems from various underlying conditions (2).

Among primary MN cases, the M-type phospholipase A2 receptor (PLA2R) emerges as a pivotal target antigen, with up to 80% of cases exhibiting anti-PLA2R antibodies, which are correlated with disease activity (3,4). Thrombospondin type-1 domain-containing 7A (THSD7A) is a transmembrane protein expressed on podocytes, and is the target antigen in approximately 3% of the primary MN cases (5). Additionally, several other target antigens such as, NELL1, SEMA3B, EXT1, and EXT2 have been identified, which are often associated with autoimmune disorders (1).

Clinically, MN typically manifests as nephrotic syndrome in 70-80% of patients, often presenting in the 4th to 5th decades of life and more prevalent in males. It is characterized by gradual onset proteinuria with hypertension and microscopic hematuria (6). Although renal biopsy has traditionally confirmed MN diagnosis, recent guidelines advocate for anti-PLA2R antibody testing in the absence of secondary causes (7).

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Initial management involves conservative measures and risk assessment for disease progression, with anti-PLA2R antibody levels as the guide for immunosuppressive therapy, considering the heightened risk of malignancy, especially in anti-PLA2R-negative cases (8). Rituximab (RTX), a monoclonal antibody targeting CD20-positive lymphocytes, has emerged as a promising therapeutic option, particularly in primary MN cases, owing to its B-cell depleting effects (9). Previous studies have demonstrated the efficacy of this regimen in inducing clinical and immunologic remission (IR) in primary MN (10,11).

Understanding the etiology and pathogenesis of MN is crucial for developing effective management strategies. Primary MN predominantly involves autoimmune mechanisms targeting podocyte antigens like PLA2R, whereas secondary MN arises from various underlying conditions. The identification of specific target antigens, particularly PLA2R, has revolutionized diagnostic and therapeutic approaches, emphasizing the importance of anti-PLA2R antibody testing in treatment decision-making. Despite advancements in the understanding of MN pathogenesis, optimal management strategies have remained unclear.

Current therapeutic approaches aim to alleviate symptoms, mitigate disease progression, and minimize treatment-related complications. Immunosuppressive therapy, guided by risk assessment and anti-PLA2R antibody levels, forms a cornerstone in the management of primary MN, highlighting the need for targeted therapies. RTX, with its B-cell depleting effects, presents a promising therapeutic option in primary MN management. Previous studies have demonstrated its efficacy in inducing remission, warranting further exploration of its treatment response, particularly in patients with relapsed disease.

By elucidating the efficacy of RTX in this context, we aim to contribute to the optimization of therapeutic strategies and improve outcomes in patients with primary MN. Therefore, this study was designed to evaluate the treatment response to RTX in patients with relapsed primary MN and assess its efficacy in achieving remission. The hypothesis of this study was that RTX treatment can significantly improve the clinical outcomes of patients with relapsed primary MN.

METHODS

Study Design

This retrospective study was conducted at a single center and was approved by the Erciyes University Clinical Research Ethics Committee (decision number: 2023/715, date: 25.10.2023), which adhered to the principles outlined in the Helsinki Declaration. Written informed consent was obtained from all patients.

The current study aimed to assess treatment response to RTX in patients with relapsed primary MN. Only patients aged 18 years or older were included in the study. The key inclusion criteria comprised a confirmed diagnosis of primary MN via pathological examination and disease relapse following first-line immunosuppressive therapy. Patients with an estimated glomerular filtration rate (eGFR) above 30 mL/min/1.73m^2 were eligible, whereas those with secondary MN, other renal diseases, diabetes mellitus, chronic liver disease, or immunosuppression therapy for another autoimmune condition were excluded.

The RTX treatment protocol consisted of an initial dose of 1 g followed by another 1 g dose after 14 days, in accordance with previous studies (10). Additionally, all patients received renin-angiotensin system (RAS) blockade agents. Treatment response was assessed after six months, and patients were categorized into three groups based on their response: complete remission (CR), partial remission (PR), and unresponsive (UR). CR was defined as a urinary protein-to-creatinine ratio (uPCR) <0.5 mg/mg accompanied by a normal serum albumin concentration, whereas PR was defined as a 50% reduction in proteinuria from the peak value, with uPCR between 0.5 and 3.5 mg/mg and an improvement in serum albumin concentration. UR referred to the failure to achieve either complete or partial response. Disease relapse was characterized by a return of proteinuria to ≥3.5 g/day after achieving CR or PR with immunosuppressive therapy, whereas IR entailed conversion from anti-PLA2R antibody positivity to negativity. Anti-PLA2R antibody detection was performed using enzyme-linked immunosorbent assay (ELISA) method. Antibody levels were measured twice, before RTX treatment and six months after treatment.

Statistical Analysis

To ensure data normality, histograms and q-q plots were examined, and the Shapiro-Wilk's test was applied. Descriptive statistics were employed to summarize numerical variables, which were presented as means and standard deviations or medians and quartiles depending on the data distribution. Categorical variables were summarized using frequencies and percentages. Group differences were assessed using either two-sided independent samples t-tests or Mann-Whitney U test for continuous variables, and Pearson's χ^2 analysis or Fisher's exact test for categorical variables. The Spearman correlation coefficient was used to explore the relationships between numerical variables. The Kruskal-Wallis test was applied to assess differences among the three groups for variables not following

a normal distribution. Statistical analyses were performed using TURCOSA statistical software (Turcosa Analytics Ltd Co, Turkey, www.turcosa.com.tr), with significance set at $p<0.05$.

RESULTS

A total of 31 patients (21 males, 10 females) with a mean age of 49.8±16.5 years were included in the analysis. The median disease duration before RTX therapy was 39 months (range: 21-121). Details regarding the demographic characteristics and laboratory findings of the patients before RTX administration are presented in Table 1.

Prior to RTX, all patients underwent various treatment regimens, with calcineurin inhibitors (CNI) (tacrolimus or cyclosporin A) and glucocorticoid combinations administered to all patients. Additionally, 19 patients (61.2%) received mycophenolate mofetil, while 5 patients (16.1%) received cyclophosphamide plus glucocorticoids. RAS blockade agents were also prescribed to all patients.

Table 1. Demographic features and laboratory results of patients.

Parameters	Results
Gender	
Male Female	21 (67.7%) 10 (32.3%)
Age (years)	49.8±16.5
BMI (kg/m^2)	26.82±4.36
Disease duration (months)	39 (21-121)
eGFR (mL/min/1.73m^2)	73 (50-112)
uPCR (mg/mg)	5.4 (2.9-7.8)
Albumin (g/dL)	2.8±0.3
Total protein (g/dL)	5.7±0.9
BUN (mg/dL)	20.6±6.6
Creatinine (mg/dL)	1.0 (0.8-1.3)
Sodium (mEq/L)	139.8±3.1
Potassium (mEq/L)	4.3±0.6
Calcium (mg/dL)	8.8 ±0.7
Phosphorus (mg/dL)	4.6±1.0
Uric acid (mg/dL)	6.3±1.7
Glucose (mg/dL)	97 (90-115)
LDL-Cholesterol (mg/dL)	143.8 (97-169)
Leukocytes (cell/µL)	9.380±3.068
Hemoglobin (g/dL)	13.51±1.52
Platelet (10^3/µL)	279.0±57.5

Values are expressed as mean ± standard deviation, median (1st-3rd quartiles)
BUN: Blood urea nitrogen, eGFR: Estimated glomerular filtration rate, uPCR: Urinary protein to creatinine ratio, BMI: Body mass index, LDL: Low density lipoprotein

Following RTX treatment, the treatment response was as follows: CR in 6 patients (14.9%), PR in 13 patients (41.9%), and unresponsiveness in 12 patients (38.7%). Before RTX administration, serum anti-PLA2R antibody was positive in 19 patients (61.2%) and negative in 12 patients (38.8%). Post-RTX, 16 patients (84.2%) exhibited IR. Furthermore, among patients with IR, 14 (87.5%) achieved either CR or PR. A statistically significant correlation was observed between the antibody response of the patients and the treatment response ($p=0.003$). However, no significant correlation was found between initial proteinuria (or uPCR) and treatment response ($p=0.145$), nor between initial eGFR and treatment response ($p=0.179$), as illustrated in Figure 1. Moreover, age, body mass index, disease duration, serum albumin, electrolyte, and hemogram parameters were not correlated with treatment response. Similarly, no correlation was found between these variables and IR.

Sustained anti-PLA2R antibody positivity post-RTX was observed in only 3 patients (15.8%), all of whom exhibited UR. Conversely, among patients with negative anti-PLA2R antibodies at baseline, CR was observed in 2 patients (16.6%) and PR in 3 patients (25%). The distribution of IR according to treatment response groups is depicted in Figure 2.

Comparisons between the initial and sixth-month results revealed a statistically significant increase in serum albumin levels ($p<0.001$), as shown in Figure 3. Additionally, there was a significant decrease in uPCR values ($p<0.001$). However, no significant difference in eGFR values was observed ($p=0.264$), as summarized in Table 2.

DISCUSSION

In this study, we present the outcomes of RTX in patients with relapsed primary MN based on a single-center experience.

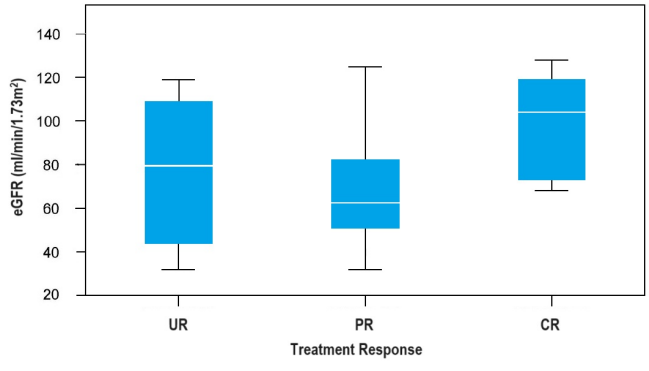

Figure 1. Initial eGFR of the patient's according to treatment response
eGFR: Estimated glomerular filtration rate, UR: Unresponsive, PR: Partial remission, CR: Complete remission

The data presented encompass the sixth month post-RTX treatment. Particularly noteworthy is the high treatment success rate observed in patients with anti-PLA2R antibody positivity. In patients with anti-PLA2R antibody positivity, complete or partial remission was achieved in 73.6% of patients receiving RTX. This outcome is not surprising for a disease with antibody-associated pathogenesis because RTX is known for its potent B cell depletion.

Our results emphasize the significance of anti-PLA2R therapy in disease prognosis and follow-up. No PR or CR proteinuria responses were observed in any patient in whom IR could not be achieved. Additionally, there was no proteinuria response (PR or CR) in only 2 patients (12.5%) in whom IR was attained. The kidney disease improving global outcomes (KDIGO) glomerular disease guidelines recommend anti-PLA2R antibody levels measured by ELISA and are valuable for informing initial treatment decisions when used in combination with clinical and laboratory parameters. Serum anti-PLA2R antibody level above 50 RU/mL is considered a high-risk predictor. Furthermore, high anti-PLA2R antibody levels and epitope spreading were associated with low RTX response in clinical studies (12).

We can approach MN treatment under the following two main headings; conservative approach and immunosuppression. KDIGO's recent glomerular disease guidelines recommend that in the treatment of MN, disease progression risk first be considered and then decide on an immunosuppression protocol. Treatment options include CNI, cytotoxic agents (such as cyclophosphamide), mycophenolate mofetil, RTX, and glucocorticoids.

RTX has taken its place in today's glomerulonephritis treatment guidelines (7). However, the optimal dosing regimen for RTX remains uncertain. The two most frequently preferred options are as follows. RTX 1 g was initially administered, followed 14 days later by another 1 g dose. An alternative regimen is to administer RTX 375 mg/m^2 weekly for 4 weeks. Furthermore, some experts offer B-cell monitoring for effective RTX dosage decisions (13). At our center, we administer RTX 1 g using a two-dose treatment protocol.

In the MENTOR study published in 2019, which examined 130 patients with nephrotic proteinuria, the RTX and CNI treatment arms were compared. At 12 months, 39 of 65 patients (60%) in the RTX group and 34 of 65 (52%) in the cyclosporine group had complete or partial remission. As a result, RTX was non-inferior to CNI in attaining complete or PR of proteinuria at 12 months (10). In another study recently conducted in Turkey in which two different dose groups were compared among 36 MN participants, a similarity was found between the two doses in terms of remission response (14). When compared with the literature data, the response rates to RTX treatment is similar.

In this investigation, we focused on RTX treatment outcomes in patients who received the first series of non-RTX immunosuppression therapy and subsequently developed disease relapse. We defined disease relapse as a return of proteinuria to ≥3.5 g/day after achievement of CR or PR with immunosuppressive therapy (7). In a study evaluating the prognosis of MN disease: the rate of remaining in remission was

Table 2. Alterations of laboratory parameters after RTX administration

Parameters	Initial	Sixth-month	Pearson χ^2
Serum albumin (g/dL)	3.3 (2.7-3.9)	3.9 (3.4-4.4)	p<0.001
uPCR (mg/mg)	5.4 (2.9-7.8)	1.7 (0.9-5.0)	p<0.001
eGFR (mL/min/1.73m^2)	73 (50-112)	75 (41-113)	p=0.264

RTX: Rituximab, uPCR: Urinary protein to creatinine ratio, eGFR: Estimated glomerular filtration rate

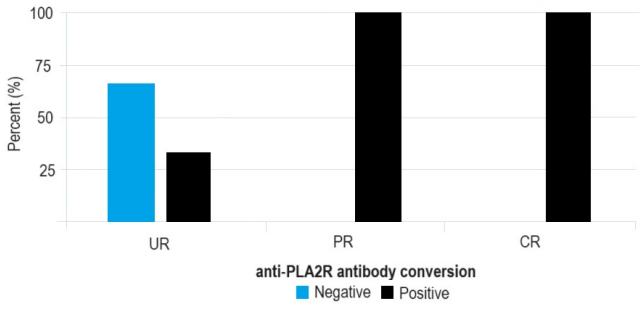

Figure 2. Anti-PLA2R antibody seroconversion according to the treatment response groups
UR: Unresponsive, PR: Partial remission, CR: Complete remission, PLA2R: Phospholipase A2 receptor

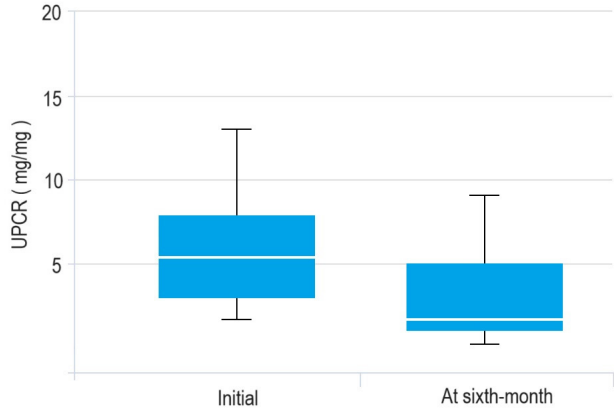

Figure 3. Changing in proteinuria level after rituximab administration
uPCR: Urinary protein to creatinine ratio

determined to be 67%, the rate of proteinuric relapse was 20%, and the rate of relapse accompanied by loss of kidney function was determined to be 13% (15).

The initial anti-PLA2R antibody seronegativity rate in our patient group was found to be 38.8%. This outcome is not far from the literature. The serum anti-PLA2R antibody positivity rate is reported in approximately 80% of primary MN patients (3). Antibodies developed against different antigens other than anti-PLA2R antibodies have been held responsible for the pathogenesis of the disease. The RTX treatment response rate was determined as 41.6% in patients with negative anti-PLA2R antibodies. When considered based on the mechanism of action of RTX, this rate can be thought high. However, there are a few suppositions that can be explanatory. First, there is the possibility of other responsible antibody positivity, such as anti-THSD7A antibodies, in these patients (16). Unfortunately, other antibodies responsible for pathogenesis are not commercially used in our country and are not assessed. Another possibility is that the antibody levels may have decreased to undetectable levels in the blood as a result of previous immunosuppression treatments, but the disease could be activated. The last option is that laboratory kits may not detect positivity.

Another important clinical consequence of immunosuppression is side effects. There were no RTX-related major side effects in these patients during the follow-up period. No major side effects, such as drug infusion-related anaphylaxis, life-threatening infections, or hepatitis B virus reactivation, were observed. Additionally, no life-threatening nephrotic syndrome complications were observed during the follow-up period of the patients, such as pulmonary embolism.

Study Limitations

The following are some limitations of our study: the treatment responses of the patients could be evaluated using histopathological data. The relationship between kidney biopsy findings and treatment response could be analyzed. However, this was a retrospective study. The kidney biopsy dates of some patients were too old, so we considered that these biopsies could not accurately reflect the actual nephron injury. However, Mirioğlu et al. (14) did not determine the relationship between RTX response and histological injury markers, such as sclerotic glomeruli, interstitial fibrosis, and tubular atrophy.

Furthermore, the follow-up period could be extended for long-term outcomes. In an RTX response analysis performed with 18 MN patients, 11.9 g/day baseline proteinuria decreased to 4.2 g/day and 2.0 g/day at 12 and 24 months, respectively (17).

CONCLUSION

Our data showed that RTX is an effective treatment agent for relapsed primary MN, and it can also be effective in patients with negative anti-PLA2R antibody. Due to this effect, it has already taken its place in first-line treatment in the current treatment guidelines. Optimal RTX dosing and treatment tips will be revealed with increasing clinical experience. We hope that the results we present will contribute to the global experience. Further randomized, controlled trials are needed to confirm these findings.

Ethics

Ethics Committee Approval: This retrospective study was conducted at a single center and was approved by the Erciyes University Clinical Research Ethics Committee (decision number: 2023/715, date: 25.10.2023), which adhered to the principles outlined in the Helsinki Declaration.

Informed Consent: Written informed consent was obtained from all patients.

Authorship Contributions

Surgical and Medical Practices: T.Y., İ.K., B.T., Concept: C.U., İ.K., M.H.S., B.T., Design: C.U., İ.K., Data Collection or Processing: T.Y., H.Ç., Analysis or Interpretation: C.U., H.Ç., M.H.S., B.T., Literature Search: T.Y., İ.K., Writing: C.U., H.Ç., M.H.S.

Conflict of Interest: No conflicts of interest were declared by the authors.

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REFERENCES

1. Özer H, Baloğlu I, Fervenza FC, Türkmen K. Membranous Nephropathy: Current Understanding in The Light of New Advances. Turk J Nephrol. 2023;32:103-11.
2. Comparison of idiopathic and systemic lupus erythematosus-associated membranous glomerulonephropathy in children. The Southwest Pediatric Nephrology Study Group. Am J Kidney Dis. 1986;7:115-24.
3. Beck LH Jr, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009;361:11-21.
4. Hofstra JM, Beck LH Jr, Beck DM, Wetzels JF, Salant DJ. Anti-phospholipase A2 receptor antibodies correlate with clinical status in idiopathic membranous nephropathy. Clin J Am Soc Nephrol. 2011;6:1286-91.
5. Tomas NM, Beck LH Jr, Meyer-Schwesinger C, Seitz-Polski B, Ma H, Zahner G, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. N Engl J Med. 2014;371:2277-87.
6. Sherman RA, Dodelson R, Gary NE, Eisinger RP. Membranous nephropathy. J Med Soc N J. 1980;77:649-52.

7. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerular Diseases Work Group. KDIGO 2021 Clinical Practice Guideline for the Management of Glomerular Diseases. Kidney Int. 2021;100:1-276.

8. Timmermans SA, Ayalon R, van Paassen P, Beck LH Jr, van Rie H, Wirtz JJ, et al. Anti-phospholipase A2 receptor antibodies and malignancy in membranous nephropathy. Am J Kidney Dis. 2013;62:1223-5.

9. M MacIsaac J, Siddiqui R, Jamula E, Li N, Baker S, Webert KE, et al. Systematic review of rituximab for autoimmune diseases: a potential alternative to intravenous immune globulin. Transfusion. 2018;58:2729-35.

10. Fervenza FC, Appel GB, Barbour SJ, Rovin BH, Lafayette RA, Aslam N, et al. Rituximab or Cyclosporine in the Treatment of Membranous Nephropathy. N Engl J Med. 2019;381:36-46.

11. Dahan K, Debiec H, Plaisier E, Cachanado M, Rousseau A, Wakselman L, et al. Rituximab for severe membranous nephropathy: a 6-month trial with extended follow-up. J Am Soc Nephrol. 2017;28:348-58.

12. Seitz-Polski B, Debiec H, Rousseau A, Dahan K, Zaghrini C, Payré C, et al. Phospholipase A2 Receptor 1 Epitope Spreading at Baseline Predicts Reduced Likelihood of Remission of Membranous Nephropathy. J Am Soc Nephrol. 2018;29:401-8.

13. Cravedi P, Ruggenenti P, Sghirlanzoni MC, Remuzzi G. Titrating rituximab to circulating B cells to optimize lymphocytolytic therapy in idiopathic membranous nephropathy. Clin J Am Soc Nephrol. 2007;2:932-7.

14. Mirioğlu Ş, Akyıldız A, Uçar AR, Uludağ Ö, Özlük Y, Dirim AB, et al. Low Versus Standard Dose of Rituximab in Adult Patients with Relapsed or Refractory Primary Membranous Nephropathy: Does It Make Any Difference? Turkish J Nephrol. 2023;32:209-15.

15. Laluck BJ Jr, Cattran DC. Prognosis after a complete remission in adult patients with idiopathic membranous nephropathy. Am J Kidney Dis. 1999;33:1026-32.

16. Zaghrini C, Seitz-Polski B, Justino J, Dolla G, Payré C, Jourde-Chiche N, et al. Novel ELISA for thrombospondin type 1 domain-containing 7A autoantibodies in membranous nephropathy. Kidney Int. 2019;95:666-79.

17. Fervenza FC, Abraham RS, Erickson SB, Irazabal MV, Eirin A, Specks U, et al. Rituximab therapy in idiopathic membranous nephropathy: a 2-year study. Clin J Am Soc Nephrol. 2010;5:2188-98.