Nefrologia xx (2026) 501632

nefrología
Revista de la Sociedad Española de Nefrología
journal homepage: www.revistanefrologia.com

Original article

Day-15 serum rituximab level predicts clinical response in primary membranous nephropathy: A prospective cohort study

Nivel sérico de rituximab en el día 15 como predictor de la respuesta clínica en la nefropatía membranosa primaria: estudio prospectivo de cohortes

Melike Çamoğlu [a,1], Asil Demirezen [b,1,*], Sena Türkmen [c], Özlem Gülbahar [c], Betül Öğüt [d], İpek Işık Gönül [d], Ömer Faruk Akçay [b], Kadriye Altok [b], Yasemin Erten [b], Ülver Derici [b], Galip Güz [b], Özant Helvacı [b]

[a] *Department of Internal Medicine, Faculty of Medicine, Gazi University, Ankara, Turkey*
[b] *Department of Internal Medicine, Division of Nephrology, Faculty of Medicine, Gazi University, Ankara, Turkey*
[c] *Department of Biochemistry, Faculty of Medicine, Gazi University, Ankara, Turkey*
[d] *Department of Pathology, Faculty of Medicine, Gazi University, Ankara, Turkey*

ARTICLE INFO

Keywords:
Membranous nephropathy
Pharmacokinetics
Rituximab
Treatment response

ABSTRACT

Background: Rituximab is first-line therapy for moderate- and high-risk primary membranous nephropathy, yet approximately one-third of patients fail to achieve remission. Accelerated rituximab clearance through urinary loss drives interindividual pharmacokinetic variability; undetectable serum rituximab at month 3 independently predicts treatment failure. We hypothesised that serum rituximab measured on day 15—immediately before the second infusion—predicts composite clinical response at month 6.
Methods: In this single-centre prospective cohort study, 46 adults with moderate- or high-risk pMN initiating rituximab-based therapy (monotherapy, $n = 22$; combination with calcineurin inhibitor and glucocorticoid, $n = 24$) underwent serum rituximab measurement on day 15 using the IDKmonitor ELISA. The primary endpoint was the association between day-15 rituximab level and composite clinical response (complete or partial remission) at month 6.
Results: Composite clinical response at month 6 was achieved in 32 patients (69.6%). Day-15 rituximab was significantly higher in responders than non-responders (113.2 ± 58.0 vs 48.6 ± 55.0 μg/mL; $p = 0.001$). A threshold of 56 μg/mL yielded response rates of 87.1% versus 33.3% (sensitivity 84.4%, specificity 71.4%). Despite significantly lower albumin and higher proteinuria, combination therapy patients achieved day-15 rituximab levels comparable to monotherapy (94.1 ± 54.9 vs 92.9 ± 74.0 μg/mL; $p = 0.95$).
Conclusions: Day-15 serum rituximab predicts month-6 clinical response in pMN, advancing the earliest predictive pharmacokinetic timepoint described in this disease. Combination therapy with a calcineurin inhibitor and low-dose corticosteroid was associated with preserved day-15 rituximab exposure despite more severe baseline disease, suggesting that early proteinuria reduction may help maintain drug bioavailability. If confirmed in multicentre cohorts, day-15 measurement could enable timely treatment individualisation before irreversible underexposure leads to disease progression.

Palabras clave:
Nefropatía membranosa
Farmacocinética
Rituximab
Respuesta al tratamiento

RESUMEN

Antecedentes: El rituximab es un tratamiento de primera línea para la nefropatía membranosa primaria de riesgo moderado y alto; sin embargo, aproximadamente un tercio de los pacientes no logra alcanzar la remisión. La eliminación acelerada de rituximab mediante pérdida urinaria determina la variabilidad farmacocinética interindividual; la ausencia de rituximab sérico detectable en el mes 3 predice de manera independiente el

* Corresponding author.
E-mail address: demirezenasil@gmail.com (A. Demirezen).
[1] Melike Çamoğlu and Asil Demirezen contributed equally to this work.

https://doi.org/10.1016/j.nefro.2026.501632
Received 19 May 2026; Accepted 25 July 2026
Available online xxx
0211-6995/© 2026 Sociedad Española de Nefrología. Published by Elsevier España, S.L.U. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Please cite this article in press as: M. Çamoğlu, A. Demirezen, S. Türkmen, et al., Day-15 serum rituximab level predicts clinical response in primary membranous nephropathy: A prospective cohort study, Nefrologia, https://doi.org/10.1016/j.nefro.2026.501632

fracaso terapéutico. Planteamos la hipótesis de que la concentración sérica de rituximab medida en el día 15—inmediatamente antes de la segunda infusión—predice la respuesta clínica compuesta en el mes 6.
Métodos: En este estudio prospectivo de cohorte realizado en un único centro, 46 adultos con nefropatía membranosa primaria de riesgo moderado o alto que iniciaron un tratamiento basado en rituximab (monoterapia, n = 22; combinación con un inhibidor de la calcineurina y un glucocorticoide, n = 24) fueron sometidos a la medición de rituximab sérico en el día 15 mediante el ensayo ELISA IDKmonitor. El criterio de valoración principal fue la asociación entre la concentración de rituximab en el día 15 y la respuesta clínica compuesta (remisión completa o parcial) en el mes 6.
Resultados: La respuesta clínica compuesta en el mes 6 se alcanzó en 32 pacientes (69,6%). La concentración de rituximab en el día 15 fue significativamente mayor en los pacientes respondedores que en los no respondedores (113,2 ± 58 frente a 48,6 ± 55 μg/mL; p = 0,001). Un valor umbral de 56 μg/mL se asoció con tasas de respuesta del 87,1% frente al 33,3% (sensibilidad del 84,4%, especificidad del 71,4%). A pesar de presentar concentraciones de albúmina significativamente menores y una proteinuria mayor, los pacientes tratados con la terapia combinada alcanzaron concentraciones de rituximab en el día 15 comparables a las observadas con la monoterapia (94,1 ± 54,9 frente a 92,9 ± 74,0 μg/mL; p = 0,95).
Conclusiones: La concentración sérica de rituximab en el día 15 predice la respuesta clínica en el mes 6 en la nefropatía membranosa primaria, adelantando el punto temporal farmacocinético predictivo más precoz descrito en esta enfermedad. La terapia combinada con un inhibidor de la calcineurina y un corticosteroide a dosis bajas se asoció con la preservación de la exposición a rituximab en el día 15, a pesar de una enfermedad basal más grave, lo que sugiere que la reducción temprana de la proteinuria puede contribuir a mantener la biodisponibilidad del fármaco. Si estos hallazgos se confirman en cohortes multicéntricas, la medición en el día 15 podría permitir la individualización oportuna del tratamiento antes de que una infraexposición irreversible conduzca a la progresión de la enfermedad.

Introduction

Primary membranous nephropathy (pMN) is the leading cause of nephrotic syndrome in non-diabetic adults.[1] Rituximab (RTX) is now established as a first-line immunosuppressive therapy for patients at moderate or high risk of progressive disease.[2] Approximately 30–40% of patients, however, fail to achieve remission.[3] Interindividual variability in RTX pharmacokinetics, is a major contributor to treatment failure, driven largely by the degree of proteinuria[4]; hypoalbuminaemia, body weight, and anti-drug antibody formation are additional established covariates.[5] The mean serum half-life of RTX in MN is approximately 11.5 days—roughly half that observed in non-proteinuric autoimmune conditions—and month-3 residual drug levels are dramatically lower than in patients without proteinuria.[6] Urinary loss of intact RTX through the damaged glomerular filtration barrier has been identified as the principal mechanism of this accelerated clearance.[7]

Serum RTX concentration has accordingly been investigated as a predictor of treatment outcome. Teisseyre and colleagues reported that undetectable serum rituximab (< 2 μg/mL) at month 3 independently predicted failure to achieve remission in a 68-patient prospective cohort.[8] However, a three-month delay in identifying treatment failure prolongs exposure to the thromboembolic, infectious, and metabolic complications of persistent nephrotic syndrome, and postpones any treatment modification. The predictive value of serum RTX at earlier timepoints in pMN has not been studied.

In this prospective cohort study, our primary objective was to determine whether serum rituximab measured on day 15, immediately before the second infusion, predicts composite clinical response (complete or partial remission) at month 6 in moderate- and high-risk pMN. Secondary objectives were to assess the association of day-15 levels with response at months 1 and 3, the relationship of day-105 levels and CD19+ B-cell depletion with month-6 response, and to compare rituximab exposure between monotherapy and combination (calcineurin inhibitor plus corticosteroid) regimens.

Materials and methods

Study design and setting

This prospective cohort study was conducted between May 2024 and May 2025 at the Department of Nephrology, Gazi University Faculty of Medicine. The article is reported in concordance with STROBE guidelines.

Participants

Adult patients (aged 18–80 years) diagnosed with pMN and classified as moderate or high risk according to the KDIGO 2021 guideline who were initiated on RTX-based therapy were eligible for inclusion.[2] Risk group definitions are provided in Supplementary Material. Exclusion criteria were rapidly progressive glomerulonephritis, secondary MN, anti-glomerular basement membrane disease or ANCA-associated vasculitis, refusal of RTX therapy, prior steroid and calcineurin inhibitor (CNI) use at the time of RTX initiation, and inability to provide informed consent.

Of 71 patients screened, 25 were excluded at final analysis, yielding a final cohort of 46 patients. Flowchart of patient selection is presented in Fig. 1.

Treatment

Therapeutic regimens were selected in accordance with KDIGO 2021 recommendations, based on clinical status and physician discretion. Twenty-two patients received RTX monotherapy at 1000 mg on days 0 and 15. The remaining 24 were treated with our department's combination regimen. In this regimen, rituximab, the CNI, and the corticosteroid were initiated concurrently; patients already receiving a CNI, with or without a corticosteroid, at the time of rituximab initiation were excluded (Fig. 1) to avoid confounding by prior immunosuppressive exposure.

This regimen adds RTX to the original cyclosporine–corticosteroid protocol of Cattran et al.,[9] incorporating two key modifications. First, the prednisolone schedule was simplified: 15 mg/day for one month, 10 mg/day for one month, 5 mg/day for one month, then stopped. Second, tacrolimus was introduced as an alternative CNI. Cyclosporine was dosed at 3.0–3.5 mg/kg/day and tacrolimus at 0.05 mg/kg/day; trough concentrations (125–225 ng/mL and 5–10 ng/mL, respectively) were used as reference thresholds, with dose adjustments reserved for cases of toxicity or inadequate response. Responders continued the CNI for three months before tapering and discontinuation; non-responders maintained it for up to 12 months.

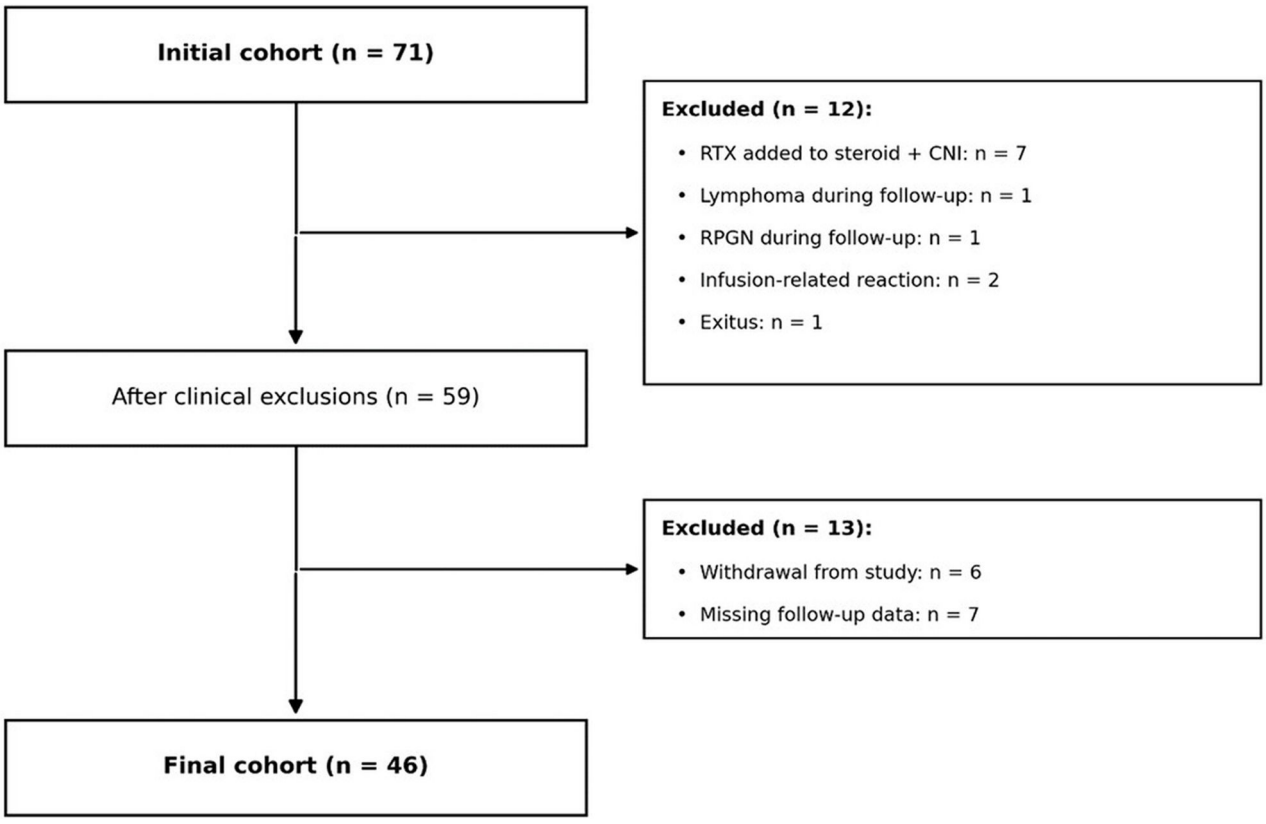


Fig. 1. Flowchart of participant inclusion. CNI: calcineurin inhibitor; RTX: rituximab.

Variables and measurements

Baseline demographic data, comorbidities, concomitant medications, serum creatinine, estimated glomerular filtration rate (CKD-EPI 2021), serum albumin, lipid profile, proteinuria, and anti-phospholipase A2 receptor (PLA2R) antibody levels were extracted from the electronic health records. Serum RTX concentrations were measured at two timepoints: day 15 (immediately before the second infusion) and month 3 (day 105 from the first infusion). Samples were analysed using the IDKmonitor Rituximab Drug Level ELISA (Immundiagnostik AG, Bensheim, Germany), with a lower detection limit of 5.08 μg/mL and an inter-assay coefficient of variation < 10%.

CD19+ B-cell counts were determined by flow cytometry using CD45-Krome Orange and CD19-ECD antibodies (Beckman Coulter). Complete B-cell depletion was defined as CD19+ cells ≤1% of total lymphocytes.

Anti-PLA2R antibodies were measured using the EUROIMMUN anti-PLA2R ELISA (EUROIMMUN AG, Lübeck, Germany). Results were classified as negative (<14 RU/mL), borderline (14–19 RU/mL), or positive (≥20 RU/mL).

Outcomes

The primary endpoint was the association between day-15 serum rituximab level and composite clinical response at month 6. Clinical response was defined according to KDIGO 2012 criteria as either complete remission (proteinuria < 0.3 g/day on at least two consecutive measurements, with serum albumin normalisation and stable kidney function) or partial remission (proteinuria < 3.5 g/day with at least 50% reduction from baseline, accompanied by improvement in serum albumin and stable kidney function).[10] Complete and partial remission were analysed as a composite endpoint because of the limited sample size.

Secondary endpoints included the association of day-15 RTX levels with clinical response at months 1 and 3, the association of day-105 rituximab levels with month-6 response, the relationship between CD19+ B-cell depletion and month-6 response, and the comparison of RTX levels between the monotherapy and combination therapy groups.

Statistical analysis

Continuous variables were tested for normality using the Shapiro–Wilk test and expressed as mean ± standard deviation or median (interquartile range) as appropriate. Serum RTX concentrations are reported as mean ± SD. Group comparisons were performed using the independent-samples *t*-test or Mann–Whitney *U* test for continuous variables and the Chi-square test or Fisher's exact test for categorical variables.

To determine a clinically informative threshold for day-15 rituximab levels, concentrations were first divided into tertiles. A linear-by-linear association test confirmed a significant trend between increasing tertile category and clinical response rate. Based on this trend, levels were dichotomized at the lower tertile boundary (56 μg/mL). Diagnostic performance of this threshold for predicting composite clinical response was assessed by calculating sensitivity, specificity, positive predictive value, and negative predictive value from the resulting 2 × 2 contingency table. Given the limited event count (14 non-responders), threshold selection was treated as exploratory. Stability of the 56 μg/mL threshold was assessed by bootstrap resampling (2500 resamples; seed 20260707), examining both the distribution of the data-derived optimal cut-point and the diagnostic performance at the fixed threshold. The continuous association between day-15 rituximab and clinical response was quantified by univariable logistic regression, expressed as the odds ratio per 10 μg/mL increment, with discrimination summarised by the

area under the receiver operating characteristic curve and internal optimism estimated by bootstrap. Multivariable modelling was not undertaken given the sample size and event count. As the primary analysis rested on a single dichotomised comparison, no adjustment for multiple comparisons was applied; associations at months 1 and 3 are presented as supportive secondary analyses. All analyses were performed using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). A two-sided p value < 0.05 was considered statistically significant.

Results

Study population

Of 71 patients screened, 25 were excluded (Fig. 1), yielding a final cohort of 46 patients. Baseline age, sex, eGFR, proteinuria, serum albumin, anti-PLA2R positivity, and KDIGO risk classification were comparable between the 25 excluded patients and the final cohort (Supplementary Table 1). The mean age was 57 ± 14 years, 27 (59%) were male, and 30 (65.2%) were classified as high risk. The baseline demographic and clinical characteristics of the entire cohort, stratified by treatment response at month 6, are presented in Table 1. No statistically significant differences were observed between responders and non-responders in age, sex, kidney function, serum albumin, proteinuria, risk classification, or treatment regimen (all $p > 0.05$). Anti-PLA2R antibody positivity was numerically more frequent among non-responders (79% vs 50%), though this difference did not reach statistical significance ($p = 0.07$). Day-15 serum RTX level was the only measured variable that differed significantly between groups (113.2 ± 58.0 vs 48.6 ± 55.0 μg/mL; $p = 0.001$).

Baseline characteristics by risk group

Kidney function was similar between moderate- and high-risk groups; however, serum albumin was significantly lower (3.05 ± 0.57 vs 3.45 ± 0.27 g/dL; $p = 0.003$), LDL cholesterol higher (178 ± 57 vs 151 ± 47 mg/dL; $p = 0.003$), and proteinuria more severe (8.4 ± 5.2 vs 4.5 ± 1.5 g/day; $p = 0.001$) in the high-risk group. The proportion of patients receiving combination therapy was significantly greater in the high-risk group (66.7% vs 25%; $p = 0.007$) (Table 2).

Day-15 serum RTX level and clinical response at month 6

At month 6, 32 patients (69.6%) achieved composite clinical response (16 complete and 16 partial remission) and 14 (30.4%) were non-responders. When day-15 RTX levels were divided into tertiles, a significant linear trend was observed between increasing tertile category and clinical response rate (linear-by-linear association, $p < 0.001$). Based on this trend, levels were dichotomized at the lower tertile boundary (56 μg/mL). Among 31 patients with day-15 RTX > 56 μg/mL, 27 (87.1%) achieved clinical response at month 6, compared with 5 of 15 (33.3%) patients with levels ≤56 μg/mL ($p < 0.001$). Using >56 μg/mL as a predictor of composite response,

Table 1
Baseline clinical and laboratory characteristics of the study cohort, stratified by treatment response at month 6.

	Total ($n = 46$)	Non-response ($n = 14$)	Response ($n = 32$)	p value
Age (years), mean ± SD	57 ± 14	58 ± 16	55 ± 13	0.62
Sex (female/male), n (%)	19/27 (41/59%)	5/9 (36/64%)	14/18 (44/56%)	0.61
Creatinine (mg/dL), mean ± SD	1.01 ± 0.53	1.1 ± 0.56	0.9 ± 0.51	0.11
eGFR (CKD-EPI 2021, mL/min/1.73 m²), mean ± SD	85 ± 32	76 ± 41.2	87 ± 28.6	0.32
Albumin (g/dL), mean ± SD	3.1 ± 0.5	3.1 ± 0.6	3.2 ± 0.5	0.53
Proteinuria (g/day), mean ± SD	7.05 ± 4.68	7.01 ± 4.2	7.06 ± 2.7	0.69
Risk classification, n (%)				0.93
Moderate	16 (34.8%)	5 (35.7%)	11 (34.4%)	
High	30 (65.2%)	9 (64.3%)	21 (65.6%)	
Treatment, n (%)				0.40
RTX monotherapy	22 (47.8%)	8 (57.1%)	14 (43.8%)	
RTX + CNI + steroid	24 (52.2%)	6 (42.9%)	18 (56.2%)	
Anti-PLA2R antibody (absent/present), n (%)	19/27 (41/59%)	3/11 (21/79%)	16/16 (50/50%)	0.07
Day-15 serum RTX level (μg/mL), mean ± SD	93.5 ± 64.3	48.6 ± 55.0	113.2 ± 58.0	**0.001**

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; CNI: calcineurin inhibitor; eGFR: estimated glomerular filtration rate; PLA2R: phospholipase A2 receptor; RTX: rituximab; SD: standard deviation. Bold p values indicate statistical significance ($p < 0.05$). Day-15 serum rituximab was the only variable significantly associated with month-6 clinical response.

Table 2
Baseline clinical and laboratory characteristics by risk classification.

	Moderate risk ($n = 16$)	High risk ($n = 30$)	p value
Age (years), mean ± SD	56.5 ± 11.6	56.6 ± 15.5	0.993
Sex (female/male)	7/9	12/18	0.806
Kidney biopsy (absent/present)	2/14	11/19	**<0.001**
Anti-PLA2R antibody positivity, n (%)	6 (38%)	21 (70%)	**0.007**
Immunosuppressive therapy, n (%)			**0.007**
RTX monotherapy	12 (75%)	10 (33.3%)	
RTX + CNI + steroid	4 (25%)	20 (66.7%)	
Creatinine (mg/dL), mean ± SD	0.85 ± 0.28	1.09 ± 0.61	0.075
eGFR (CKD-EPI 2021, mL/min/1.73 m²), mean ± SD	92 ± 19	80 ± 36	0.16
Albumin (g/dL), mean ± SD	3.45 ± 0.27	3.05 ± 0.57	**0.003**
LDL (mg/dL), mean ± SD	151 ± 47	178 ± 57	**0.003**
Proteinuria (g/day), mean ± SD	4.5 ± 1.5	8.4 ± 5.2	**0.001**

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; CNI: calcineurin inhibitor; eGFR: estimated glomerular filtration rate; LDL: low-density lipoprotein; PLA2R: phospholipase A2 receptor; RTX: rituximab; SD: standard deviation. Bold p values indicate statistical significance ($p < 0.05$).

Table 3
Association between day-15 serum rituximab level and clinical response at month 6.

Day-15 serum RTX level	Response (n = 32)	Non-response (n = 14)	p value
>56 μg/mL (n = 31)	27 (87.1%)	4 (12.9%)	**<0.001**
≤56 μg/mL (n = 15)	5 (33.3%)	10 (66.7%)	
Sensitivity	**Specificity**	**PPV**	**NPV**
84.4%	71.4%	**87.1%**	66.7%

NPV: negative predictive value; PPV: positive predictive value; RTX: rituximab. The 56 μg/mL threshold was derived from the lower tertile boundary of the day-15 rituximab distribution. Bold p value indicates statistical significance ($p < 0.05$).

sensitivity was 84.4%, specificity 71.4%, positive predictive value 87.1%, and negative predictive value 66.7% (Table 3).

The predictive value of day-15 levels was consistent across earlier timepoints; similar associations were observed at months 1 ($p < 0.001$) and 3 ($p = 0.004$), (Fig. 2, Supplementary Table 2).

Across 2500 bootstrap resamples, the data-derived optimal cut-point clustered around the pre-specified threshold (lower-tertile median 58.4 μg/mL, interquartile range 45.0–64.5; Youden median 52.6 μg/mL), with approximately 70% of resamples falling within 40–70 μg/mL, and three independent derivation rules converged (lower tertile 58.4, Youden 52.6, pre-specified 56 μg/mL). Diagnostic performance at 56 μg/mL was preserved across resamples (sensitivity 84.8% [interquartile range 80.0–88.9], specificity 72.2% [63.6–80.0], positive predictive value 87.5% [83.3–91.4], negative predictive value 66.7% [58.3–75.0]). On the continuous scale, each 10 μg/mL increment in day-15 rituximab was associated with higher odds of month-6 response (odds ratio 1.27, 95% CI 1.07–1.50; $p = 0.005$), with an area under the curve of 0.824 and an optimism-corrected area under the curve of 0.821.

The association between day-15 rituximab category and month-6 response was consistent across clinical strata: response rates for day-15 > 56 versus ≤56 μg/mL were 83% versus 25% in the moderate-risk group and 89% versus 36% in the high-risk group, and 92% versus 22% in the monotherapy group and 83% versus 50% in the combination group (Supplementary Table 3).

Among responders, all 16 complete remissions occurred in patients with day-15 rituximab > 56 μg/mL (16 complete, 11 partial), whereas all 5 responders with day-15 ≤56 μg/mL achieved partial remission only. The composite endpoint was pre-specified for the primary analysis given the event count, with this distribution provided as an exploratory observation.

Day-105 serum RTX level

At day 105, RTX was undetectable (0 μg/mL) in 19 patients (41.3%) and detectable in 27 (58.7%). Among patients with undetectable day-105 levels, 8 (42.1%) achieved clinical response at month 6, compared with 24 of 27 (88.9%) with detectable levels ($p = 0.001$) (Supplementary Table 4).

CD19+ B-cell depletion

CD19+ B-cell depletion was achieved in 44 of 46 patients (95.6%) at day 15 and in all patients by day 105. All 32 responders demonstrated CD19+ B-cell depletion at day 15, whereas depletion was absent in 2 of 14 non-responders (14.3%). This difference did not reach statistical significance ($p = 0.088$) (Supplementary Table 5).

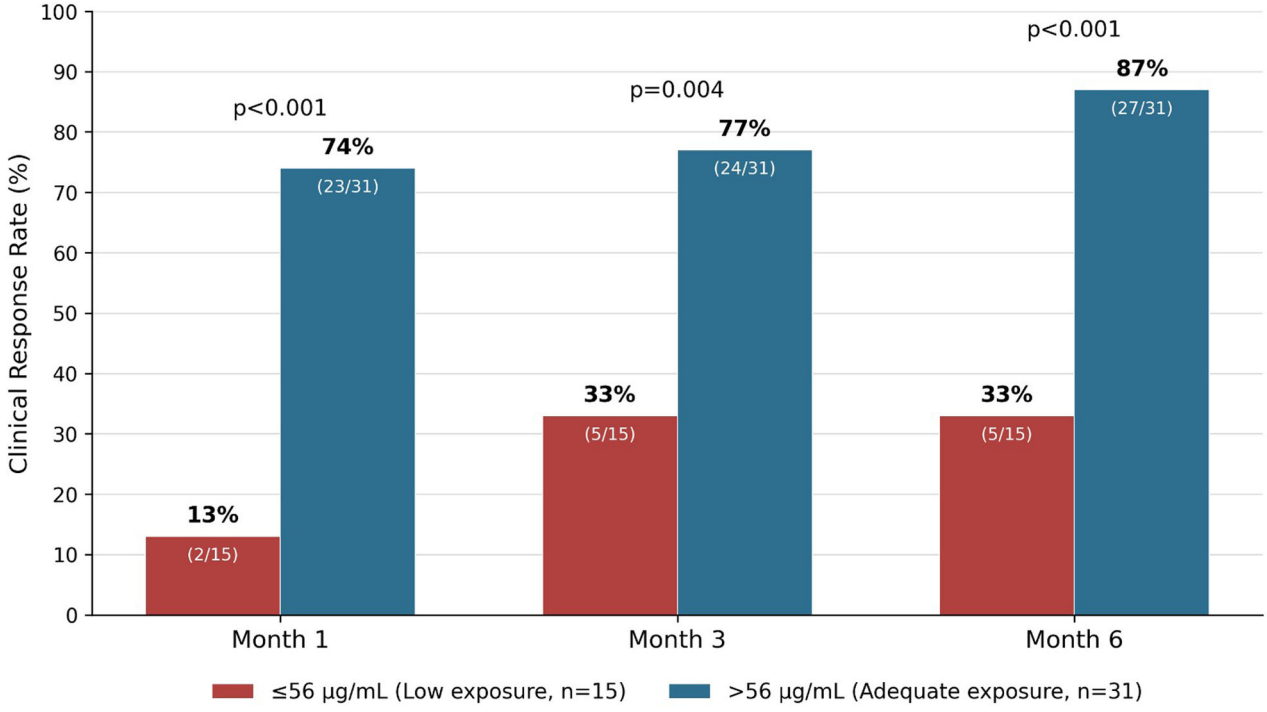


Fig. 2. Clinical response rates at 1/3/6 months by day 15 rituximab category.

Table 4
Comparison of clinical and laboratory characteristics across treatment groups.

	RTX monotherapy (n = 22)	RTX + CNI + steroid (n = 24)	p value
Age (years), mean ± SD	53.8 ± 16	59.1 ± 12	0.21
Sex (female/male)	10/12	9/15	0.58
Creatinine (mg/dL), mean ± SD	1.06 ± 0.66	0.96 ± 0.38	0.55
eGFR (CKD-EPI 2021, mL/min/1.73 m²), mean ± SD	87 ± 36.5	82 ± 28.2	0.56
Albumin (g/dL), mean ± SD	3.2 ± 0.5	2.8 ± 0.4	**0.046**
Proteinuria (g/day), mean ± SD	5.96 ± 3.23	8.05 ± 5.59	**0.039**
Risk classification, n (%)			**0.007**
Moderate	12 (55%)	4 (17%)	
High	10 (45%)	20 (83%)	
PLA2R antibody (absent/present), n (%)	11/11 (50/50%)	8/16 (33/67%)	0.25
Day-15 RTX level (μg/mL), mean ± SD	92.89 ± 74.03	94.10 ± 54.89	0.95
Day-15 RTX category, n (%)			0.25
Low (≤56 μg/mL)	9 (41%)	6 (25%)	
Moderate–High (>56 μg/mL)	13 (59%)	18 (75%)	
Day-105 RTX level (μg/mL), mean ± SD	6.55 ± 6.25	7.79 ± 12.89	0.68
Day-105 RTX category, n (%)			0.24
Undetectable (0 μg/mL)	7 (32%)	12 (50%)	
Detectable (>0 μg/mL)	15 (68%)	12 (50%)	
Treatment response, n (%)			0.40
Non-response	8 (36%)	6 (25%)	
Response	14 (64%)	18 (75%)	

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration; CNI: calcineurin inhibitor; eGFR: estimated glomerular filtration rate; PLA2R: phospholipase A2 receptor; RTX: rituximab; SD: standard deviation. Bold p values indicate statistical significance ($p < 0.05$).

Serum RTX levels by treatment group

Twenty-two patients received RTX monotherapy and 24 received RTX combined with a CNI and glucocorticoid. Patients in the combination group had significantly lower albumin (2.8 ± 0.4 vs 3.2 ± 0.5 g/dL; $p = 0.046$), higher proteinuria (8.05 ± 5.59 vs 5.96 ± 3.23 g/day; $p = 0.039$), and a greater proportion of high-risk classification (83% vs 45%; $p = 0.007$). Despite this more severe disease profile, day-15 serum RTX levels were comparable between groups (94.10 ± 54.89 vs 92.89 ± 74.03 μg/mL; $p = 0.95$). Day-105 levels were also similar (7.79 ± 12.89 vs 6.55 ± 6.25 μg/mL; $p = 0.68$) (Table 4).

No deaths occurred in the final cohort during the 6-month follow-up. In the monotherapy group ($n = 22$), infections were recorded in 3 patients and hospitalisation in 2, with no new-onset diabetes; in the combination group ($n = 24$), infections occurred in 4 patients (5 episodes), hospitalisation in 1, and new-onset diabetes in 3.

Discussion

We hypothesised that serum rituximab measured on day 15, immediately before the second infusion, predicts composite clinical response at month 6 in moderate- and high-risk pMN—a hypothesis this study supports. Building on the observation that undetectable month-3 serum RTX independently predicts treatment failure, we advance this monitoring window by approximately 10 weeks—to our knowledge the earliest timepoint at which serum RTX has been shown to carry prognostic value in pMN.[8]

Bootstrap resampling supported the 56 μg/mL operating threshold: independent derivation rules converged on 52–58 μg/mL, and diagnostic performance at 56 μg/mL was maintained across resamples, while the continuous exposure–response relationship was strong and internally validated (optimism-corrected area under the curve 0.82). Defining the precise decision limit within the 50–60 μg/mL range, and confirming its transferability across assay platforms, is the task of larger externally validated cohorts using standardised assays; our data indicate that a clinically actionable pharmacokinetic signal is measurable as early as day 15.

The biological plausibility of day 15 as a predictive timepoint is supported by the pharmacokinetic profile of RTX in pMN. Fogueri et al. reported a mean serum half-life of approximately 11.5 days—roughly half that observed in non-proteinuric conditions.[6] Larrosa-García and colleagues confirmed a similar half-life and identified proteinuria > 2.4 g/day as a threshold for significantly accelerated clearance,[11] while Boyer-Suavet and colleagues provided direct clinical evidence that month-3 residual RTX levels in pMN are approximately sixfold lower than in non-proteinuric patients receiving an identical regimen.[12] Day 15 therefore corresponds to approximately one elimination half-life after the first infusion, making it a physiologically rational timepoint at which underexposure first becomes detectable.

The mechanism driving this underexposure was rigorously characterised by Allinovi and colleagues in a prospective Florentine cohort, who demonstrated that urinary loss of intact RTX through non-selective proteinuria directly reduces serum peak levels and predicts treatment failure—a commendable methodological contribution that nonetheless requires laboratory expertise not readily available outside specialist centres.[7] Broader implementation of RTX monitoring faces additional practical barriers: LISA-TRACKER, employed by both Teisseyre[8] and Destere,[13] requires dedicated automated instrumentation and carries higher per-test costs.[14] Inter-assay variability compounds this—Truffot and colleagues showed that the Promonitor kit underestimates RTX concentrations by a mean of 69% relative to LC-MS/MS, whereas LISA-TRACKER shows only 4% bias.[15] The IDKmonitor kit employed in the present study has not been evaluated in such head-to-head analyses, and our 56 μg/mL threshold may not be directly transferable across platforms. Nevertheless, measuring serum RTX at day 15 with a widely available ELISA on a conventional microplate reader represents a pragmatic alternative that could extend pharmacokinetic monitoring beyond the handful of centres currently equipped for it.

Despite more severe disease at baseline—lower albumin, higher proteinuria, and a greater proportion of high-risk classification—patients receiving combination therapy achieved day-15 and day-105 rituximab concentrations comparable to those on monotherapy. This was unexpected, as hypoalbuminaemia and heavy proteinuria are established drivers of accelerated RTX clearance.[6,16] A pharmacoki-

netically plausible explanation is that CNI-mediated podocyte stabilisation[17] may attenuate urinary rituximab loss and thereby help preserve systemic exposure, although causal inference is not possible in this observational dataset. This would be consistent with the striking remission rates reported by Waldman and colleagues with concurrent RTX and cyclosporine—92% CR + PR at 9 months[18]—and with subsequent data showing that CNI addition to RTX in refractory disease improves outcomes.[19,20] To our knowledge, no prior study has directly compared serum RTX levels between monotherapy and combination therapy in pMN; this finding is hypothesis-generating and requires confirmation in a prospective, adequately powered cohort.

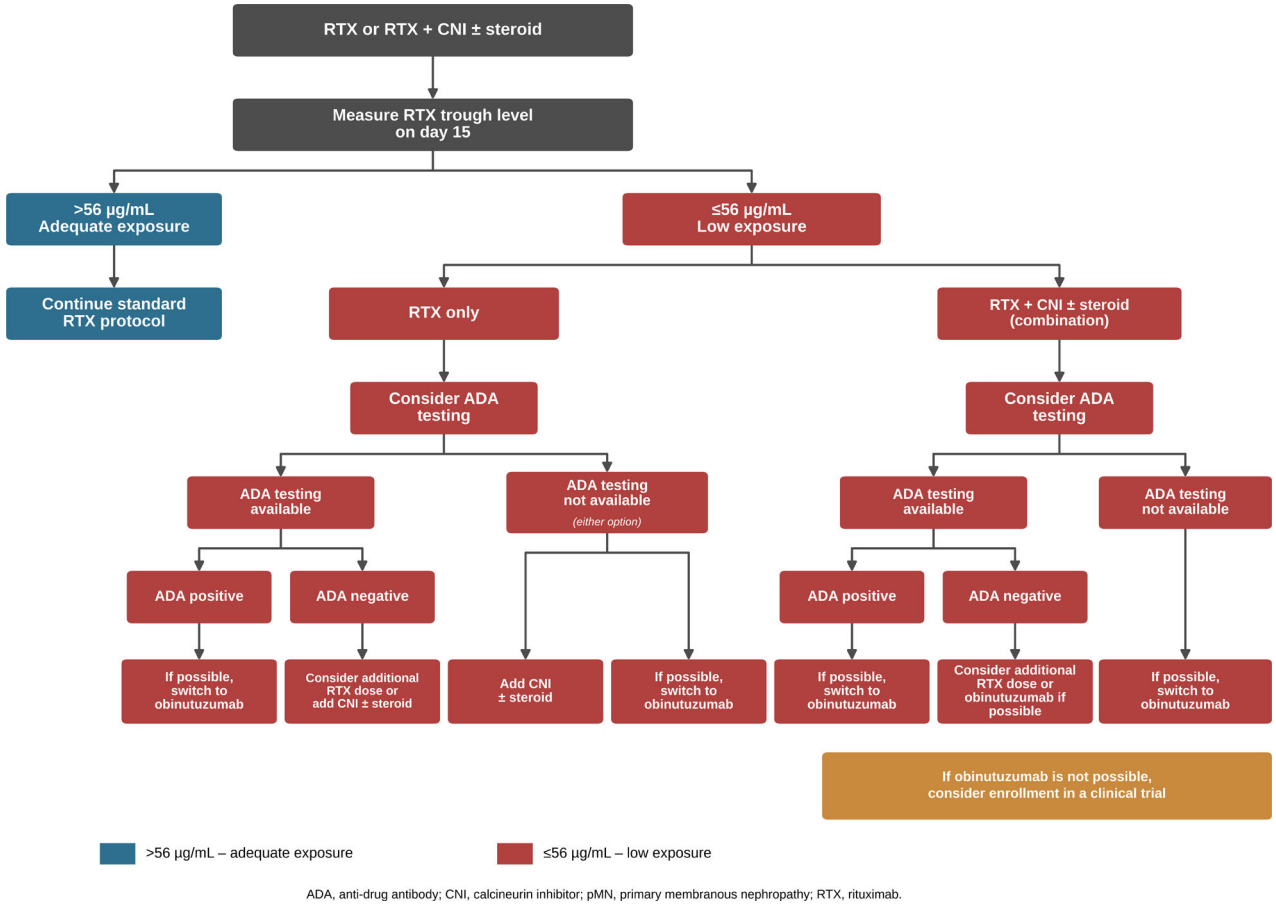
It should be noted that proteinuria is not the sole determinant of rituximab pharmacokinetics. In addition to several measurable clinical factors, urinary rituximab loss has been documented even in patients with selective proteinuria, and FcRn polymorphisms represent a further source of inter-individual variability.[7,8,12] Hartinger and colleagues, through population pharmacokinetic modelling, identified body weight, serum albumin, disease type, treatment duration, and anti-drug antibody formation as independent covariates of rituximab exposure, and argued that dosing strategies should differ across glomerular diseases including FSGS, SLE, and MN.[5] In the present cohort, the major measurable clinical determinants of rituximab clearance were largely comparable between responders and non-responders, yet day-15 serum RTX concentrations differed substantially—further supporting the value of direct pharmacokinetic measurement over any single clinical surrogate.

Established prediction models in pMN rely on parameters assessed at month 3 or later. Beck et al. showed that anti-PLA2R antibody kinetics precede clinical remission by months.[21] In a prospective cohort of 132 patients, Ruggenenti and colleagues showed that a 50% fall in anti-PLA2R antibody levels preceded an equivalent reduction in proteinuria by approximately 10 months.[22] A secondary analysis of the MENTOR trial reported a C-statistic of 0.93 when combining 3-month anti-PLA2R and albumin changes.[23] Day-15 RTX measurement may offer a prognostic signal at a timepoint when none of these parameters have yet had time to evolve—though validation in larger cohorts is needed before firm conclusions can be drawn.

Our day-105 data are consistent with previously published month-3 findings. Detectable RTX at day 105 was associated with significantly higher response rates compared with undetectable levels (Supplementary Table 4), consistent with earlier month-3 immunomonitoring findings.[8] Day-15 measurement, however, provides a prognostic signal at a timepoint when therapeutic intervention remains most feasible.

CD19+ B-cell depletion was achieved in 95.6% of patients at day 15 but did not predict clinical response (Supplementary Table 5), consistent with the GEMRITUX trial,[24] a retrospective cohort evaluating B-cell depletion thresholds in MN,[25] and a prospective study of post-RTX CD19 kinetics demonstrating that depletion was near-universal within weeks regardless of subsequent clinical outcome.[26] Peripheral CD19 depletion reflects circulating B-cell elimination but does not capture tissue-resident B cells, long-lived plasma cells, or other effector mechanisms relevant to pMN pathogenesis.[27] KDIGO 2021 does not endorse CD19 as a primary monitoring tool, reserving it as one factor in the evaluation of treatment failure.[2]

We propose a hypothetical, stepwise algorithm for the management of day-15 underexposure, summarised in Fig. 3 and outlined below. A day-15 RTX level below the exploratory threshold identifies



ADA, anti-drug antibody; CNI, calcineurin inhibitor; pMN, primary membranous nephropathy; RTX, rituximab.

Fig. 3. Proposed stepwise treatment algorithm for moderate- or high-risk primary membranous nephropathy based on day-15 rituximab levels. ADA: anti-drug antibody; CNI: calcineurin inhibitor; pMN: primary membranous nephropathy; RTX: rituximab.

insufficient drug exposure at a timepoint when therapeutic modification remains feasible. Additional RTX dosing may suffice in patients whose low levels reflect urinary loss—an approach supported by Allinovi and colleagues, in whom re-treatment of underexposed non-responders yielded clinical response in five of eight patients.[7] CNI addition could simultaneously reduce proteinuria and preserve drug exposure, as our combination group data suggest and as the high remission rates with concurrent RTX-cyclosporine reported by Waldman and colleagues support.[18] For patients in whom anti-drug immunity rather than urinary loss underlies treatment failure, agent substitution is preferable to dose escalation. Obinutuzumab has demonstrated markedly higher response rates than rituximab in refractory pMN,[28–30] and its humanised structure may additionally overcome pre-existing anti-RTX immunity, which accumulates progressively with repeated courses.[29,31–33] Obinutuzumab was not evaluated in the present study; the cited data support this management rationale rather than representing outcomes of our cohort. Because anti-drug antibodies were not measured, this algorithm cannot yet distinguish urinary loss from immunogenicity as the mechanism underlying day-15 underexposure. Accordingly, it cannot be used to infer the cause of underexposure in individual non-responders. Clinical implementation would require external validation in independent cohorts, together with cross-platform assay comparison. At present, the data support risk stratification only and do not justify protocolised dose adjustments.

This study has several limitations. No formal a priori sample-size or power calculation was performed; this is an exploratory single-centre cohort of 46 patients with 14 non-responders, which constrains multivariable modelling and external validation of the 56 μg/mL threshold, derived from tertile distribution and regarded as exploratory, and residual confounding cannot be excluded. RTX was measured at only two timepoints (days 15 and 105), leaving intermediate kinetics undefined; tacrolimus and cyclosporine were not analysed separately, and the IDKmonitor ELISA has not been evaluated against LISA-TRACKER or LC-MS/MS, so the threshold may not transfer directly across platforms. Anti-drug antibodies were not measured, although 31 of 46 patients (67%) were rituximab-naïve, in whom pre-existing anti-rituximab antibodies would not be expected. Nevertheless, de novo anti-drug antibodies may have developed during follow-up, but this was not characterised in the present cohort. Complete and partial remission were analysed as a composite endpoint owing to limited event count, and follow-up was limited to 6 months, leaving the predictive value of day-15 levels for longer-term outcomes unknown.

Conclusions

In conclusion, day-15 serum RTX concentration predicts clinical response at month 6 in moderate- and high-risk pMN, offering the earliest pharmacokinetic signal yet described in this disease. The 56 μg/mL threshold identified in this study should be considered an exploratory operating value for hypothesis generation and internal risk stratification. It should not be interpreted as a decision threshold ready for clinical application. If confirmed in multicentre cohorts, this measurement could enable a precision medicine approach to pMN, individualising treatment intensity from the outset rather than correcting course only after failure.

Ethical disclosures

The study was approved by the Ankara Etlik City Hospital Ethics Committee (Decision No: AEŞH-EK1-2024-0050, May 5, 2024) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used Claude (Anthropic) to assist with language editing and figure preparation. The authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

Funding

This work was supported by the Gazi University Scientific Research Projects Coordination Unit (BAP) [project code TTU-2025-9663].

Conflicts of interest

Authors declare no conflicts of interest.

Data availability statement

Data are available upon reasonable request from the corresponding author.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.nefro.2026.501632.

References

1. Couser WG. Primary membranous nephropathy. Clin J Am Soc Nephrol. 2017;12:983–97, http://doi.org/10.2215/CJN.11761116
2. Kidney Disease: Improving Global Outcomes Glomerular Diseases Work Group. KDIGO 2021 clinical practice guideline for the management of glomerular diseases. Kidney Int. 2021;100:S1–276, http://doi.org/10.1016/j.kint.2021.05.021
3. Nayak S, Chauhan P, Jha V, Ramachandran R, Bose B. Outcomes of primary membranous nephropathy refractory to immunosuppressants. Clin Kidney J. 2024;17sfae113, http://doi.org/10.1093/ckj/sfae113
4. Teisseyre M, Cremoni M, Boyer-Suavet S, Ruetsch C, Graca D, Esnault VLM, et al. Advances in the management of primary membranous nephropathy and rituximab-refractory membranous nephropathy. Front Immunol. 2022;13859419, http://doi.org/10.3389/fimmu.2022.859419
5. Hartinger JM, Sima M, Hruskova Z, Pilkova A, Kratky V, Rysava R, et al. A novel dosing approach for rituximab in glomerular diseases based on a population pharmacokinetic analysis. Biomed Pharmacother. 2024;175116655, http://doi.org/10.1016/j.biopha.2024.116655
6. Fogueri U, Cheungapasitporn W, Bourne D, Fervenza FC, Joy MS. Rituximab exhibits altered pharmacokinetics in patients with membranous nephropathy. Ann Pharmacother. 2019;53:357–63, http://doi.org/10.1177/1060028018803587
7. Allinovi M, Accinno M, Finocchi C, Mazzierli T, Caroti L, Lazzarini D, et al. Urinary rituximab loss and rate of treatment failure in membranous nephropathy. Nephrol Dial Transplant. 2025;41:92–101, http://doi.org/10.1093/ndt/gfaf127
8. Teisseyre M, Cremoni M, Boyer-Suavet S, Crepin T, Benzaken S, Zorzi K, et al. Rituximab immunomonitoring predicts remission in membranous nephropathy. Front Immunol. 2021;12:738788, http://doi.org/10.3389/fimmu.2021.738788
9. Cattran DC, Appel GB, Hebert LA, Hunsicker LG, Pohl MA, Hoy WE, et al. Cyclosporine in patients with steroid-resistant membranous nephropathy: a randomized trial. Kidney Int. 2001;59:1484–90, http://doi.org/10.1046/j.1523-1755.2001.0590041484.x
10. Kidney Disease: Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group. KDIGO clinical practice guideline for glomerulonephritis. Kidney Int Suppl. 2012;2:139–274, http://doi.org/10.1038/kisup.2012.9
11. Larrosa-Garcia M, Agraz Pamplona I, Sanz Martinez MT, Bury Macias RP, Martinez Gallo M, Colobran R, et al. Pharmacokinetic characterization of rituximab in patients with glomerular diseases. Ther Drug Monit. 2025;47:e142–9, http://doi.org/10.1097/FTD.0000000000001353
12. Boyer-Suavet S, Andreani M, Cremoni M, Brglez V, Benzaken S, Bernard G, et al. Rituximab bioavailability in primary membranous nephropathy. Nephrol Dial Transplant. 2019;34:1423–5, http://doi.org/10.1093/ndt/gfz041
13. Destere A, Teisseyre M, Merino D, Cremoni M, Gerard AO, Crepin T, et al. Optimization of rituximab therapy in adult patients with PLA2R1-associated membranous nephropathy with artificial intelligence. Kidney Int Rep. 2024;9:134–44, http://doi.org/10.1016/j.ekir.2023.10.023

14. Freeman K, Connock M, Auguste P, Taylor-Phillips S, Mistry H, Shyangdan D, et al. Clinical effectiveness and cost-effectiveness of use of therapeutic monitoring of tumour necrosis factor alpha (tnf-alpha) inhibitors [lisa-tracker(r) enzyme-linked immunosorbent assay (ELISA) kits, tnf-alpha-blocker elisa kits and promonitor(r) ELISA kits] versus standard care in patients with Crohn's disease: systematic reviews and economic modelling. Health Technol Assess. 2016;20:1–288, http://doi.org/10.3310/hta20830
15. Truffot A, Jourdil JF, Seitz-Polski B, Malvezzi P, Brglez V, Stanke-Labesque F, et al. Simultaneous quantification of rituximab and eculizumab in human plasma by liquid chromatography–tandem mass spectrometry and comparison with rituximab elisa kits. Clin Biochem. 2021;87:60–6, http://doi.org/10.1016/j.clinbiochem.2020.10.007
16. Buse M, Dounousi E, Kramann R, Floege J, Stamellou E. Newer B-cell and plasma-cell targeted treatments for rituximab-resistant patients with membranous nephropathy. Clin Kidney J. 2025;18sfaf088, http://doi.org/10.1093/ckj/sfaf088
17. Faul C, Donnelly M, Merscher-Gomez S, Chang YH, Franz S, Delfgaauw J, et al. The actin cytoskeleton of kidney podocytes is a direct target of the antiproteinuric effect of cyclosporine a. Nat Med. 2008;14:931–8, http://doi.org/10.1038/nm.1821
18. Waldman M, Beck LH Jr, Braun M, Wilkins K, Balow JE, Austin HA 3rd. Membranous nephropathy: pilot study of a novel regimen combining cyclosporine and rituximab. Kidney Int Rep. 2016;1:73–84, http://doi.org/10.1016/j.ekir.2016.06.005
19. Chen X, Jiao S, Li S, Li J, Li P, Song F, et al. Combination of rituximab and low-dose tacrolimus in the treatment of refractory membranous nephropathy: a retrospective cohort study. Balkan Med J. 2023;40:287–93, http://doi.org/10.4274/balkanmedj.galenos.2023.2022-9-7
20. Zhu F, Chu X, Guo Y, Li Y, Cao C, Wu J, et al. Combination of ultra-low dose rituximab and low dose tacrolimus versus tacrolimus alone in the treatment of non-responsive idiopathic membranous nephropathy: a Chinese retrospective cohort study. Am J Transl Res. 2021;13:7622–31. PMID: 34465978.
21. Beck LH Jr, Fervenza FC, Beck DM, Bonegio RG, Malik FA, Erickson SB, et al. Rituximab-induced depletion of anti-PLA2R autoantibodies predicts response in membranous nephropathy. J Am Soc Nephrol. 2011;22:1543–50, http://doi.org/10.1681/ASN.2010111125
22. Ruggenenti P, Debiec H, Ruggiero B, Chianca A, Pelle T, Gaspari F, et al. Anti-phospholipase A2 receptor antibody titer predicts post-rituximab outcome of membranous nephropathy. J Am Soc Nephrol. 2015;26:2545–58, http://doi.org/10.1681/ASN.2014070640
23. Barbour SJ, Fervenza FC, Induruwage D, Brenchley PE, Rovin B, Hladunewich MA, et al. Anti-PLA2R antibody levels and clinical risk factors for treatment nonresponse in membranous nephropathy. Clin J Am Soc Nephrol. 2023;18:1283–93, http://doi.org/10.2215/CJN.0000000000000237
24. 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, http://doi.org/10.1681/ASN.2016040449
25. Gaggar P, Madipally R, Raju SB. Rituximab, use and B cell depletion in patients with membranous nephropathy- a retrospective, observational study. Indian J Nephrol. 2023;33:356–61, http://doi.org/10.4103/ijn.ijn_62_22
26. Sevinc M, Shukkur M, Hamilton P, Thet M, Bate S, Ragy O, et al. Kinetics of CD19 (+) B-cell depletion post-rituximab in membranous nephropathy. Clin Kidney J. 2025;18:sfaf153, http://doi.org/10.1093/ckj/sfaf153
27. Tomas NM. Therapeutic targets in membranous nephropathy: plasma cells and complement. Clin Kidney J. 2024;17sfae243, http://doi.org/10.1093/ckj/sfae243
28. Xu M, Wang Y, Wu M, Chen R, Zhao W, Li M, et al. Obinutuzumab versus rituximab for the treatment of refractory primary membranous nephropathy. Nephrol Dial Transplant. 2025;40:978–86, http://doi.org/10.1093/ndt/gfae267
29. Díaz-García JD, Nanwani AC, Villalobos Navarro A, Robiou Vivero EJA, Guerrero Hinzpeter E, Beck LH Jr, et al. Anti-CD20 monoclonal antibodies in membranous nephropathy. Nefrología. 2026, http://doi.org/10.1016/j.nefro.2026.501553
30. de la Prada Alvarez FJ, Cintra Cabrera M, Almenara Tejederas M, Burgos Martin J, Alonso Garcia F, Salgueira Lazo M. Obinutuzumab in the treatment of PLA2R-positive membranous glomerulonephritis resistant to treatment. Nefrologia (Engl Ed). 2024;44:306–7, http://doi.org/10.1016/j.nefroe.2024.03.004
31. Boyer-Suavet S, Andreani M, Lateb M, Savenkoff B, Brglez V, Benzaken S, et al. Neutralizing anti-rituximab antibodies and relapse in membranous nephropathy treated with rituximab. Front Immunol. 2019;10:3069, http://doi.org/10.3389/fimmu.2019.03069
32. Allinovi M, Teisseyre M, Accinno M, Finocchi C, Esnault VLM, Cremoni M, et al. Anti-rituximab antibodies occurrence and clinical outcomes in patients with primary membranous nephropathy. Kidney Int Rep. 2025;10:2621–9, http://doi.org/10.1016/j.ekir.2025.04.059
33. Wu D, Xu Z, Xu R, Liu W, Su Z, Li K, et al. Association between anti-rituximab antibodies and treatment efficacy of rituximab in membranous nephropathy. Nephrol Dial Transplant. 2026, http://doi.org/10.1093/ndt/gfag054