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Prognostic Implications of Serum C3 Levels in Primary Membranous Nephropathy: A Retrospective Cohort Analysis

Implicaciones Pronósticas de los Niveles Séricos de C3 en la Nefropatía Membranosa Primaria: Un Análisis de Cohorte Retrospectivo

Engin Onan[1], Ayşe Serra Artan[2], Ayşegül Oruç[3], Müge Doksan[2], Necmi Eren[4], Serhat Karadağ[5], Gülizar Şahin[6], Kenan Turgutalp[7], Nurhan Seyahi[8], Sim Kutlay[9], Şimal Köksal Cevher[10], Belda Dursun[11], Musa Pınar[12], Mehmet Deniz Ayli[13], Taner Baştürk[14], Garip Şahin Sedat Ustundag[15], Zülfükar Yılmaz[16], Serap Yadigar[17], Özkan Güngör[18], Erhan Tatar[19], Feyza Bora[20], Mürvet Yılmaz[21], Murat Hayri Sipahioğlu[22], Sena Ulu[23], Ramazan Öztürk[24], Meltem Gürsu[25], Bülent Kaya[26], Ömer Faruk Akçay[27], Yelda Deligöz Bildacı[28], Cuma Bülent Gül[29], Hakkı Arıkan[30], Şebnem Karakan[31], Dilek Torun[32], Hacı Hasan Yeter[33], Mansur Kayataş[34], Can Sevinç[35], Yavuz Ayar[36], Kültigin Türkmen[37], Saide Elif Güllülü Boz[38], Sevinç Püren Yücel Karakaya[39], Nazlı Totik Doğan[40], Gulsah Seydaoglu[40], Savaş Öztürk[40]

RESUMEN

Antecedentes: El sistema del complemento desempeña un papel crucial en la patogénesis de la nefropatía membranosa (NM). Los niveles séricos de complemento 3 (C3) pueden ser un marcador pronóstico. Este estudio investiga la importancia pronóstica de los niveles séricos de C3 en pacientes con NM primaria. **Métodos:** Este estudio tiene como objetivo evaluar el valor pronóstico de los niveles séricos de C3 en la NM primaria mediante el análisis de su asociación con las características demográficas, clínicas e histopatológicas, así como con los resultados del tratamiento. Se utilizaron datos del Grupo de Trabajo de Enfermedades Glomerulares de la Sociedad Turca de Nefrología (TSN-GOLD), un registro nacional. **Resultados:** Se incluyó a un total de 1259 pacientes con NM primaria diagnosticada mediante biopsia. Los niveles séricos de C3 fueron bajos en 45 (3,6%) pacientes. Los pacientes con niveles bajos de C3 sérico demostraron creatinina sérica basal más alta (1,3 ± 1,1 mg/dl frente a 0,9 ± 0,8 mg/dl, p = 0,006) y proteinuria (9714 ± 6329 mg/24 h frente a 7052 ± 4463 mg/24 h, p = 0,002), y niveles de albúmina más bajos (2,3 ± 0,8 g/dl frente a 2,7 ± 0,8 g/dl, p = 0,007) en comparación con aquellos con C3 sérico normal. Durante un año de seguimiento, ambos grupos mostraron una disminución significativa de la proteinuria y un aumento de los

Correspondencia:
Engin Onan
ORCID:
0000-0003-1299-229X
onanmd@gmail.com

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1) University, Faculty of Medicine, Adana Dr. Turgut Noyan Training and Research Hospital, Department of Nephrology, Adana, Türkiye.
2) Istanbul University, Faculty of Medicine, Department of Nephrology, İstanbul, Türkiye.
3) Bursa Uludag University, Faculty of Medicine, Department of Nephrology, Bursa, Türkiye.
4) University, Faculty of Medicine, Department of Nephrology, Kocaeli, Türkiye
5) University of Health Sciences, Haseki Tranining and Research Hospital, Department of Nephrology, İstanbul, Türkiye.
6) University of Health Sciences Sultan Abdulhamid Research and Training Hospital, Department of Nephrology, İstanbul, Türkiye.
7) Mersin University, Faculty of Medicine, Department of Nephrology, Mersin, Türkiye.
8) İstanbul Cerrahpasa University, Faculty of Medicine, Department of Nephrology, İstanbul, Türkiye.
9) İstanbul Cerrahpasa University, Faculty of Medicine, Department of Nephrology, İstanbul, Türkiye.

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niveles de albúmina. En medidas repetidas, la creatinina y la proteinuria mostraron una diferencia significativa a lo largo del tiempo entre los grupos de C3; la albúmina no mostró dicha diferencia (p: 0,029, 0,013 y 0,705 respectivamente). Las tasas de remisión no fueron diferentes entre los grupos de C3, 10 casos (62,5%) en el grupo Bajo y 432 casos (76,1%) en el grupo Normal, p: 0,237]. Las tasas de recaída fueron notablemente más altas en pacientes con niveles séricos bajos de C3 en comparación con aquellos con C3 sérico normal (62,5% frente a 38,0%, p = 0,049). El análisis multivariado mostró que la edad (HR: 1,017; IC del 95%: 1,002-1,032; p = 0,025) y los niveles séricos de albúmina (HR: 0,759; IC del 95%: 0,568-1,015; p = 0,063) fueron predictores significativos de remisión, mientras que los niveles séricos de C3 no lo fueron. **Conclusión:** Los niveles séricos bajos de C3 en MN primario se asocian con peores parámetros clínicos e histopatológicos basales y tasas de recaída más altas, pero no son predictivos independientes de remisión. El C3 sérico puede ser un marcador pronóstico útil para el riesgo de recaída.

Palabras Clave: Nefropatía membranosa; Complemento sérico 3; pronóstico; Grupo de Trabajo TSN-GOLD; Turquía.

ABSTRACT

Background: The complement system plays a crucial role in the pathogenesis of membranous nephropathy (MN). Serum complement 3 (C3) levels may be a prognostic marker. This study investigates the prognostic significance of serum C3 levels in patients with primary MN. **Methods:** This study aims to evaluate the prognostic value of serum C3 levels in primary MN by analyzing their associations with demographic, clinical, and histopathological features, as well as treatment outcomes. We used data from the Turkish Society of Nephrology Glomerular Diseases (TSN-GOLD) Working Group, a nationwide registry. **Results:** A total of 1,259 biopsy-proven primary MN patients were included. Serum C3 levels were low in 45 (3.6%) patients. Patients with low serum C3 levels demonstrated higher baseline serum creatinine (1.3±1.1mg/dL vs. 0.9±0.8mg/dL, p=0.006) and proteinuria (9714±6329 mg/24h vs. 7052±4463mg/24h, p=0.002), and lower albumin levels (2.3±0.8g/dL vs. 2.7±0.8g/dL, p=0.007) compared to those with normal serum C3. At one-year follow-up, both groups showed significant decreases in proteinuria and increases in albumin levels. In repeated-measures analyses, creatinine and proteinuria showed significant differences over time between the C3 groups; albumin did not (p: 0.029, 0.013, and 0.705, respectively). The remission rates were not different between C3 groups, 10 cases (62.5%) in the Low group, and 432 cases (76.1%) in the Normal group, p: 0.237. Relapse rates were notably higher in patients with low serum C3 levels compared to those with normal serum C3 (62.5% vs. 38.0%, p=0.049). Multivariate analysis showed that age (HR: 1.017, 95% CI: 1.002–1.032, p=0.025) and serum albumin levels (HR: 0.759, 95% CI: 0.568–1.015, p=0.063) were significant predictors of remission, while serum C3 levels were not. **Conclusion:** Low serum C3 levels in primary MN are associated with worse baseline clinical and histopathological parameters and higher relapse rates, but are not independently predictive of remission. Serum C3 may be a useful prognostic marker for relapse risk.

10) Ankara University, Faculty of Medicine, Department of Nephrology, Ankara, Türkiy
11) Pamukkale University, Faculty of Medicine, Department of Nephrology, Denizli, Türkiye.
12) Sakarya University, Faculty of Medicine, Department of Nephrology, Türkiye
13) Etlik City Hospital, Department of Nephrology, Ankara, Türkiye.
14) Health Sciences University, Sıslı Hamidiye Etfal Training and Research Hospital, Department of Nephrology, Istanbul, Türkiye.
15) Eskisehir Osmangazi University, Faculty of Medicine, Department of Nephrology, Eskisehir, Türkiye.
16) Trakya University, Faculty of Medicine, Department of Nephrology, Edirne, Türkiye.
17) Dicle University, Faculty of Medicine, Department of Nephrology, Diyarbakır, Türkiye.
18) Kartal Training and Research Hospital, Department of Nephrology, İstanbul, Türkiye.
19) Maras Sutcu imam University, Faculty of Medicine, Department of Nephrology, Kahramanmaraş, Türkiye.
20) Training and Research Hospital, Department of Nephrology, İzmir, Türkiye.
21) Akdeniz University, Faculty of Medicine, Department of Nephrology, Antalya, Türkiye.

Keywords: Membranous Nephropathy; Serum Complement 3; Prognosis; TSN-GOLD Working Group; Türkiye.

INTRODUCTION

Membranous nephropathy (MN) is a leading cause of idiopathic nephrotic syndrome in adults and is characterized by immune-mediated injury to the glomerular basement membrane [1]. Globally, MN constitutes a substantial proportion of primary glomerulonephritis cases and is associated with significant morbidit [2]. The identification of phospholipase A2 receptor (PLA2R) as a major target antigen has markedly advanced the understanding of MN pathogenesis [3].

Increasing evidence highlights the pivotal role of the complement system in MN. Immune complexes formed by autoantibodies against podocyte antigens activate the complement cascade, leading to glomerular injury by generating inflammatory mediators and forming the membrane attack complex [4]. Glomerular deposition of complement components such as C3 and C4d further supports the involvement of complement activation in disease pathophysiology [5]. Reduced serum C3 levels may reflect systemic complement consumption and have been proposed as a marker of disease activity.

Previous studies have shown that intense glomerular C3 deposition is associated with worse renal outcomes, including higher proteinuria, lower eGFR, and an increased risk of progression to end-stage kidney disease [6,8]. In contrast, the prognostic significance of serum C3 levels in primary MN has been less extensively investigated. Limited data suggest potential associations between serum C3 levels and specific disease phenotypes, but the relationship with clinical outcomes, particularly relapse, remains unclear [3, 4, 7]. In this context, the present study aimed to evaluate the prognostic value of serum C3 levels in primary MN by examining their associations with baseline clinical, laboratory, and histopathological features, as well as treatment outcomes, using data from a large nationwide registry.

METHODS

Study Design and Data Collection

This retrospective study used data from the Turkish Society of Nephrology Glomerular Diseases (TSN-GOLD) Working Group database. This nationwide registry includes demographic, histopathological, clinical, treatment, and outcome data from 56 centers across Türkiye. Patients with primary MN were identified, and available data were analyzed to evaluate the association between baseline serum C3 levels and clinical outcomes.

Patient Selection

Eligible patients were aged ≥18 years, had MN confirmed on kidney biopsy, and a serum C3 measurement obtained at the time of, or within a short interval around, the index biopsy (index date). Follow-up clinical records were required for outcome ascertainment.

Patients were excluded if there was clinical, serologic, or pathologic evidence of secondary MN, including lupus nephritis class V, hepatitis B virus–associated MN, malignancy-associated MN (per age-appropriate screening and directed evaluations), drug-induced MN, or other systemic autoimmune diseases. In cases of diagnostic uncertainty, anti-PLA2R and/or anti-THSD7A serology or tissue staining was used to

22) University of Health Sciences Bakirkoy Dr. Sadi Konuk Training and Research Hospital, Department of Nephrology, İstanbul, Türkiye.
23) Erciyes University, Faculty of Medicine, Department of Nephrology, Kayseri, Türkiye.
24) Bahcesehir University, Faculty of Medicine, Department of Nephrology, İstanbul, Türkiye.
25) Ministry of Health Ankara Training and Research Hospital, Department of Nephrology, Ankara, Türkiye.
26) Bezmialem Vakif University, Faculty of Medicine, Department of Nephrology, İstanbul, Türkiye.
27) Cukurova University, Faculty of Medicine, Department of Nephrology, Adana, Türkiye.
28) Gazi University, Faculty of Medicine, Department of Nephrology, Ankara, Türkiye.
29) Dokuz Eylul University, Faculty of Medicine, Department of Nephrology, İzmir, Türkiye.
30) Bursa Yuksek Ihtisas Training and Research Hospital, Department of Nephrology, Bursa, Türkiye.
31) Marmara University, Faculty of Medicine Department of Nephrology, İstanbul, Türkiye.
32) Ankara City Hospital, Ankara Yıldırım Beyazıt University, Department of Nephrology, Ankara, Türkiye.

support a diagnosis of primary MN. Patients without baseline C3 data or with <6 months of follow-up were also excluded.

Laboratory Measurements and C3 Classification

Serum C3 levels were measured in each center's routine clinical laboratory using standard platforms (e.g., nephelometry or turbidimetry) and reported in the units used locally. To account for inter-laboratory variability, C3 values were classified relative to the lower limit of normal (LLN) defined by each center. Patients with C3 levels below the LLN were categorized as having low C3; those with levels at or above the LLN were categorized as having normal C3. Anti-PLA2R antibody status was recorded in the TSN-GOLD registry as a binary variable (positive/negative). Quantitative titers were unavailable due to the absence of standardized measurement protocols across participating centers.

Variables and Clinical Definitions

Data collected at or near the index date included age, sex, serum creatinine and/or eGFR, serum albumin, proteinuria, comorbidities (hypertension, diabetes), and the use and type of immunosuppression. Proteinuria was analyzed using a single unit within each analysis (either g/24 h or g/g creatinine, depending on data availability), without conversion between units across datasets. Serum creatinine was used as the primary measure of kidney function in longitudinal and multivariate analyses, whereas eGFR was used only for defining stable kidney function within remission criteria.

Definitions followed KDIGO 2021 guidance. Remission and relapse status were assessed at each participating center according to these predefined criteria and recorded by the treating physician; no central adjudication process was used.

-Partial remission: proteinuria between 0.3 and 3.5 g/24 h with at least a 50% reduction from baseline and stable kidney function.

-Complete remission: proteinuria <0.3 g/24 h and stable kidney function, defined as a ≤15% decline in eGFR.

-Any remission: the composite of complete or partial remission, used for primary analyses.

-Relapse: proteinuria>3.5 g/24 h after remission.

Where available, remission and relapse dates were recorded to describe time to remission and time to relapse.

Study Groups

Patients were divided into two groups according to baseline C3 classification:

1) Low C3 group: serum C3 below the center-specific LLN.

2) Normal C3 group: serum C3 at or above the center-specific LLN.

Histopathology

Adequate biopsy material required ≥8 glomeruli on light microscopy. Immunofluorescence for IgG, IgA, IgM, C3, κ, and λ was graded semi-quantitatively (0–3+), considered positive at ≥1+, and evaluated by local pathologists at each center without central review. Interstitial fibrosis/tubular atrophy (IF/TA) and interstitial inflammation were reported as the percentage of cortical area involved and dichotomized as ≤25% versus >25% for uniformity across centers.

33) Baskent University, Faculty of Medicine, Adana Dr. Turgut Noyan Training and Research Hospital, Department of Nephrology, Adana, Türkiye.
34) Hacettepe University, Faculty of Medicine, Department of Nephrology, Ankara, Türkiye.
35) Cumhuriyet University, Faculty of Medicine, Department of Nephrology, Sivas, Türkiye.
36) Ataturk University, Faculty of Medicine, Department of Nephrology, Erzurum, Türkiye.
37) University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Department of Nephrology, Bursa, Türkiye.
38) Necmettin Erbakan University, Meram School of Medicine, Department of Internal Medicine, Division of Nephrology, Konya, Türkiye.
39) Bursa Uludag University, Faculty of Medicine, Department of Nephrology, Türkiye.
40) Cukurova University, Faculty of Medicine, Department of Biostatistics, Türkiye.

Endpoints

-Primary endpoint (remission): achievement of any remission during follow-up, based on treating-physician documentation per center practice. When available, remission dates were used for descriptive analyses only.

-Secondary endpoint (relapse): occurrence of the first relapse among patients who achieved remission, as documented by the treating physician (increase in proteinuria and/or decline in serum albumin consistent with MN activity). When available, relapse dates were used to describe the time from remission to relapse.

Data Analyses

Categorical variables were summarized as counts and percentages, and continuous variables as mean ± standard deviation or median with minimum–maximum values, as appropriate. Between-group comparisons for categorical variables (low vs normal C3) were performed using the chi-square test. The distribution of continuous variables was assessed with the Kolmogorov–Smirnov test. For continuous variables, the Student's t-test was used when normally distributed; otherwise, the Mann–Whitney U test was applied. Changes in serum creatinine, proteinuria, and albumin from baseline to 3, 6, and 12 months were analyzed using repeated-measures methods. Sphericity was assessed with Mauchly's test; when the assumption was violated, the Greenhouse–Geisser correction was applied in repeated-measures ANOVA. Univariate Cox regression analyses were performed to identify predictors of remission among variables showing statistically significant between-group differences in univariate comparisons. Variables with $p<0.05$ in univariate analysis were included in a multivariate Cox regression model. All analyses were conducted using IBM SPSS Statistics, version 26.0 (IBM Corp., Armonk, NY, USA). A two-sided p-value <0.05 was considered statistically significant.

RESULTS

A total of 1595 patients were analyzed. Patients with missing or unrecorded C3 levels (n:152), secondary MN patients (n:168), and patients younger than 18 years of age (n:16) were excluded from the analysis.

A total of 1259 primary MN patients were included in the study. 45 (3.6%) patients had low baseline serum C3 levels, and 1214 (96.4%) had normal baseline serum C3 levels. (**Figure 1**)

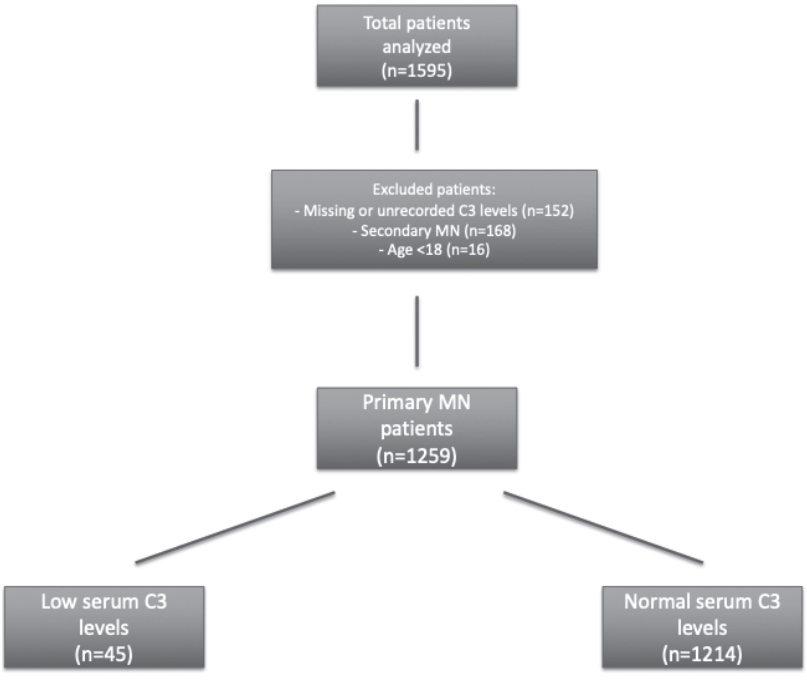


Figure 1: Flowchart of Patient Selection and Classification Based on Baseline Serum C3 Levels

Baseline Data of the Patients

The median age of patients in the low C3 group was 50 years (range: 19-76), compared to 55 years (range: 4-99) in the normal C3 group (p=0.121). The proportion of males was comparable between groups (53.3% vs. 59.0%, p=0.450). There were no significant differences in symptom duration, prior hypertension, or diabetes rates between the study groups (Table 1). Patients in the low C3 group had significantly higher baseline BUN levels (21.0 mg/dL vs. 14.0 mg/dL, p<0.001) and lower hemoglobin (11.2 g/dL vs. 13.2 g/dL, p<0.001) and hematocrit levels (34.0% vs. 39.0%, p<0.001). Pyuria and hematuria were more common in the low C3 group (27.3% vs. 12.7%, p=0.010; 50.0% vs. 34.4%, p=0.034, respectively). There was no significant difference in glucose, uric acid, total protein, or antiphospholipase A2 receptor antibody positivity between the groups. (**Table 1**)

Table 1: Baseline Demographic, Clinical and Laboratory Characteristics of the Study Groups

Variables	Low C3 Group (n=45)	Normal C3 Group (n=1214)	p
Age (year)	47(26-57)	48(37-57)	0.121
Gender (Male)	24(53.3)	716(59.0)	0.450
Symptom duration(year)	39.0(1.0-73.0)	46.0(0.0-86.0)	0.060
Hypertension before kidney disease	12(27.3)	417(34.6)	0.313
Diabetes Mellitus before kidney disease	3(6.7)	141(11.8)	0.295
Smoking	7(30.4)	181(23.6)	0.660
Systolic Blood Pressure (mmHg)	125(120-140)	130(120-140)	0.475
Diastolic Blood Pressure (mmHg)	80(70-80)	80(70-90)	0.228
Laboratory characteristics			
Glucose (mg/dL)	94(85-111)	92(85-103)	0.222
Blood urea nitrogen (mg/dL)	21(14-33)	14(11-20)	<0.001
Creatinine (mg/dL)	0.9(0.5-1.2)	0.8(0.5-1.2)	<0.001
Uric acid (mg/dL)	5.6(5-7)	5.8(5-7)	0.633
Hemoglobin (g/dL)	11.2(10-14)	13.2(12-15)	<0.001
Total protein (g/dL)	5.0(4-6)	5.2(5-6)	0.154
Albumin (mg/dL)	2.4(2-3)	2.6(2-3)	0.007
Pyuria-leukocyte count (>5)	12(27.3)	149(12.7)	0.010
Hematuria- erythrocyte count (>5)	22(50.0)	405(34.4)	0.034
Proteinuria (g/g)	8.6(5.3-10.4)	6.0(3.6-9.1)	0.002
Anti-PLA2R (+)	5/8 (62.5)	204/335(60.9)	0.927
Outcomes			
Immunosuppressive treatment	21 (72.4)	638(72.6)	0.984
Remission	10(62.5)	432(76.1)	0.237
Relapse	10(62.5)	150(38.0)	0.049
Time from treatment to remission (month)	6(3-11)	6(3-8)	0.513
Duration of stay in first remission (month)	17(12-36)	13(6-36)	0.792
Total follow-up time since biopsy (month)	31(26-36)	24(10-66)	0.744

Data were expressed as median (**IQR**) or n (%), Abbreviations: **Anti-PLA2R**: phospholipase A2 receptor antibody.

Histopathological Characteristics

The histopathological characteristics of the study groups are detailed in **Supplementary Table 1**. A comparison between the low-C3 and normal-C3 groups revealed several significant differences. Both groups had a notably lower total number of glomeruli in the low C3 and normal C3 groups [medians: 11 (IQR 9–17) and 16 (IQR 10–24), respectively; p=0.021]. Similarly, the global sclerotic count was reduced

in both groups; the low C3 group and the normal C3 group [medians: 0 (IQR 0–2) and 1 (IQR 0–2), respectively, p=0.019].

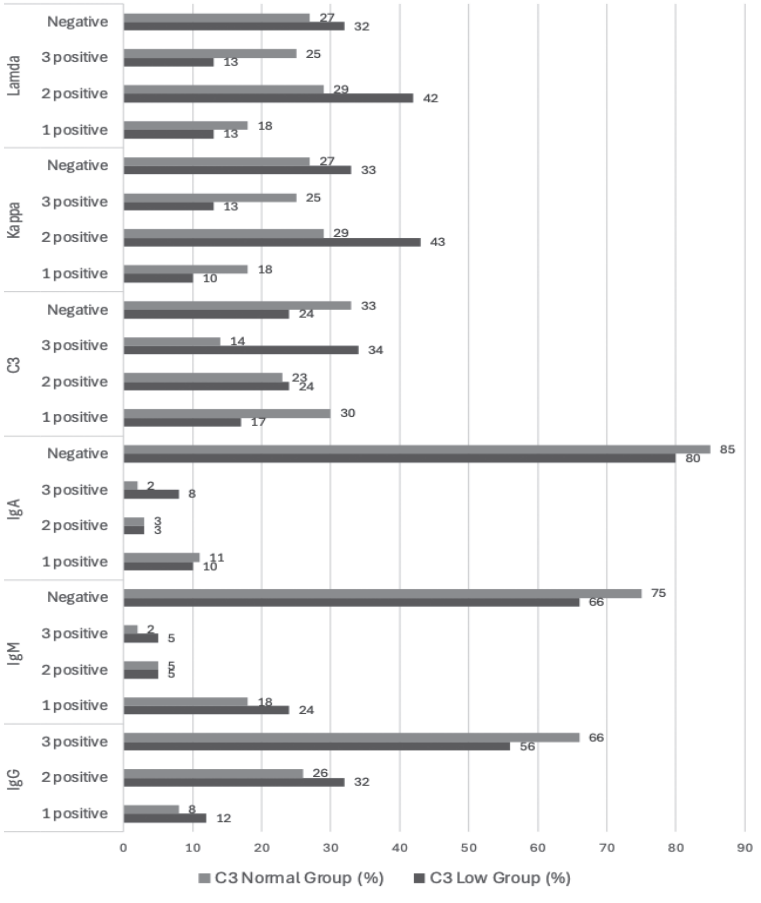
Interstitial inflammation was significantly more common in the low C3 group, occurring in 75.6% of cases. In the normal C3 group, it was presented in 57.2% of cases (p=0.022). However, no statistically significant differences were observed for segmental sclerotic glomeruli, interstitial fibrosis, vascular changes, or tubular atrophy between the groups.

Comparison of immunofluorescence findings between the C3 low and C3 normal groups revealed significant differences in C3 deposition (C3 low: 34% +3 positivity; C3 normal: 14%, p = 0.004) (**Supplementary Figure 1**). IgA deposition showed a near-significant trend (C3 low: 8% +3 positivity; C3 normal: 2%, p = 0.063). Other parameters, including IgG, IgM, kappa, and lambda chains, did not show significant differences.

Supplementary Table 1: Histopathological Characteristics of the Study Groups

Variables	Low C3 Group (n=45)	Normal C3 Group (n=1214)	p
Total number of glomeruli	11(9-17)	16(10-24)	0.021
Global sclerotic count	0(0-2)	1(0-2)	0.019
Number of segmental sclerotic glomeruli	0(0-0)	0(0-0)	0.240
Interstitial inflammation	34(75.6)	677(57.2)	0.022
Endocapillary proliferation	6(17.1)	65(6.4)	0.013
Interstitial fibrosis	18(40.9)	556(46.8)	0.538
Vascular changes	19(42.2)	444(37.6)	0.640
Tubular atrophy	25(56.0)	558(46.0)	0.205

Data were expressed as median (IQR) or n(%)



Supplementary Figure 1: Comparison of Immunofluorescence Parameters Between C3 Low and Normal Groups in Primary Membranous Nephropathy

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Treatment data were extracted from the TSN-GOLD registry, which records immunosuppressive and supportive therapies at the time of biopsy and follow-up. Treatment protocols were not pre-specified and varied across centers. Among patients with available data (n=1086), 72% received immunosuppressive therapy and 28% were managed conservatively. Most centers applied conventional regimens such as corticosteroids combined with calcineurin inhibitors, the Ponticelli protocol (cyclophosphamide plus corticosteroids), or rituximab-based therapy. A minority received mycophenolate mofetil, azathioprine, or other agents. Median treatment duration was 12 months (IQR 6–24). These findings are summarized in **Supplementary Table 2.**

Supplementary Table 2: Changes in Serum Creatinine, Proteinuria, and Albumin Levels Over Time Based on Serum C3 Levels

Variables	Low C3 Group (n=45)	Normal C3 Group (n=1214)	p*	pǂ
	Creatinine (mg/dl)			
Baseline	1.3±1.1	0.9±0.8	0.006	p=0.072 (time) p=0.029 (time×group)
3rd month	0.9±0.3	0.9±0.5	0.131	
6th month	0.9±0.4	0.9±0.5	0.328	
12th month	0.9±0.3	1.0±0.5	0.567	
	Proteinuria (mg/day)			
Baseline	9714.1±6329	7052.5±4463.6	0.002	
3rd month	5610.4±5363.1	4431.5±3702.4	0.131	
6th month	3456.1±2940.6	3190.4±3195.9	0.786	
12th month	1951.7±2065.1	2265.3±3243.0	0.584	
	Serum albumin (g/dl)			
Baseline	2.3±0.8	2.7±0.8	0.007	
3rd month	2.8±0.7	3.1±0.7	0.040	
6th month	3.1±0.8	3.5±0.7	0.039	
12th month	3.3±0.9	3.8±0.7	0.002	

p*: Comparison between Low C3 and Normal C3 groups at each time point.
pǂ: Interaction effect over time using repeated measures ANOVA.
Data presented as mean±standard deviation

Comparison of immunofluorescence findings between the C3 low and C3 normal groups revealed significant differences in C3 deposition (C3 low: 34% +3 positivity; C3 normal: 14%, p = 0.004. While IgA showed a near-significant trend (C3 low: 8% +3 positivity; C3 normal: 2%, p = 0.063), no significant differences were observed in other parameters, including IgG (C3 low: 56% +3 positivity; C3 normal: 66%, p = 0.459), IgM (C3 low: 5%; C3 normal: 5%, p = 0.313), kappa (C3 low: 13%; C3 normal: 25%, p = 0.177), and lambda (C3 low: 13%; C3 normal: 25%, p = 0.225). **Figure 2**

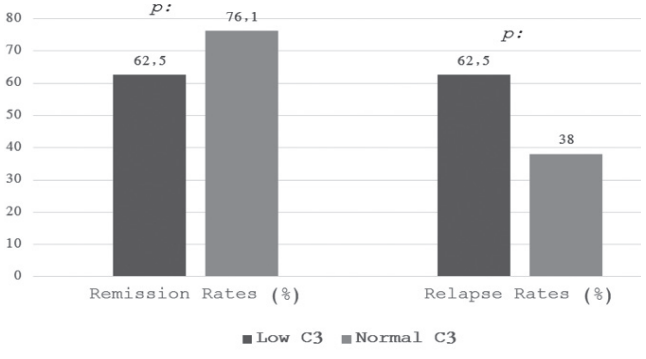

Figure 2: Comparison of Remission and Relapse Rates Between Low and Normal Serum C3 Levels in Primary Membranous Nephropathy

Outcome Data

Remission rates were slightly lower in the low C3 group (62.5% vs. 76.1%, p=0.237), while relapse rates were significantly higher compared to the normal C3 group (62.5% vs. 38.0%, p=0.049) (**Figure 1**). Patients with low serum C3 levels had a shorter remission duration, with a median of 17 months compared to 24 months in the normal C3 group, though this difference was not statistically significant (p=0.792) (**Table 1**).

Patients who achieved remission were compared with those who did not, based on demographic, laboratory, and histopathological variables (**Table 2**). There were no significant differences in age (median 44 years vs. 49 years, p=0.097) or gender distribution (37.5% female in the remission group vs. 29.1% in the no-remission group, p=0.450). Similarly, low C3 levels were rare and comparable between the groups (2.5% in remission vs. 3.5% in no-remission, p=0.501). Patients with remission had significantly better baseline kidney function, as indicated by lower creatinine levels (median 0.8 mg/dL vs. 1.0 mg/dL, p=0.003), higher serum albumin levels (median 2.5 g/dL vs. 2.3 g/dL, p=0.007), and higher hemoglobin levels (median 13.4 g/dL vs. 13.0 g/dL, p<0.001). Although proteinuria was slightly higher in the remission group (median 7.2 g/g vs. 6.9 g/g, p=0.002), this difference was statistically significant. Histopathological features revealed that interstitial fibrosis (42.2% vs. 54.6%, p=0.01) and interstitial inflammation (53.5% vs. 70.8%, p<0.001) were significantly lower in patients with remission, indicating less severe tissue damage. Tubular atrophy was similar between the groups (45.4% in remission vs. 49.6% in no-remission, p=0.376).

Changes in creatinine, proteinuria, and serum albumin values over time were presented in Supplementary **Table 2** and **Supplementary Figure 1.** Baseline creatinine levels were higher in the low serum C3 group (1.3±1.1 mg/dL) compared to the normal serum C3 group (0.9±0.8 mg/dL, p=0.006). No significant differences were observed between the groups at subsequent time points (3rd, 6th, and 12th months). However, a significant interaction between time and group was noted (p=0.029), indicating differing trends over time.

At baseline, proteinuria was significantly higher in the low serum C3 group (9714.1±6329 mg/day) compared to the normal serum C3 group (7052.5±4463.6 mg/day, p=0.002). This difference decreased over time, with no significant differences at 3rd, 6th, and 12th months. Significant effects of time (p<0.001) and the time×group interaction (p=0.013) indicate improvements in both groups, with varying rates of change.

Baseline serum albumin levels were lower in the low serum C3 group (2.3±0.8 g/dL) than in the normal serum C3 group (2.7±0.8 g/dL, p=0.007). Serum albumin levels increased significantly over time in both groups, with persistent differences at 3rd (p=0.040), 6th (p=0.039), and 12th months (p=0.002). Time had a significant overall effect (p<0.001), but there was no significant interaction between time and group (p=0.705).

Treatment protocols were not pre-specified and varied across centers. Among patients with available data (n=1086), 72% received immunosuppressive therapy and 28% were managed conservatively. Most centers applied conventional regimens such as corticosteroids combined with calcineurin inhibitors, the Ponticelli protocol (cyclophosphamide plus corticosteroids), or rituximab-based therapy. A minority received mycophenolate mofetil, azathioprine, or other agents. Median treatment duration was 12 months (IQR 6–24). These findings are summarized in **Supplementary Table 3.**

Table 3 presents the predictors of remission in both univariate and multivariate Cox models of patients who used immunosuppressive treatment. The significant predictors in the univariate analysis include age (HR: 1.014, 95% CI: 1.001-1.028, p=0.035) and serum albumin (HR: 0.728, 95% CI: 0.562-0.943, p=0.016). In the multivariate analysis, age (HR: 1.017, 95% CI: 1.002-1.032, p=0.025) remains a significant predictor, while serum albumin shows a trend towards significance (HR: 0.759, 95% CI: 0.568-1.015, p=0.063). Other variables, including gender, C3 level, hemoglobin, creatinine, proteinuria, interstitial fibrosis,

tubular atrophy, and interstitial inflammation, did not show significant associations with remission in either univariate or multivariate analyses.

Table 2: Comparative Data of Remission in the Patients Used Immunosuppressive Treatment

	Remission present	No remission	p
Age	44 (37-57)	49 (39-62)	0.097
Gender (female)	166(37.5)	41(29.1)	0.450
Serum C3 level (low)	11(2.5)	5(3.5)	0.501
Creatinine (mg/dl)	0.8(0.5-1.1)	0.8(0.5-1.1)	0.003
Serum albumin (g/dL)	2.5(2-3)	2.3(2-3)	0.007
Proteinuria (g/g)	7.2(5-10)	6.9(5-10)	0.002
Hemoglobin (gr/dl)	13.4(12-15)	13(12-15)	<0.001
Interstitial fibrosis	187(42.2)	77(54.6)	0.01
Tubular atrophy	201(45.4)	70(49.6)	0.376
Interstitial inflammation	234(53.5)	97(70.8)	<0.001

Data were expressed as median (IQR) or n (%)

Supplementary Table 3: Distribution of Immunosuppressive Treatment Regimens Among Patients with Available Therapy Data in the TSN-GOLD Primary MN Registry

Treatment Category	n (%) or Median (IQR)
Patients with treatment data available	1,086 (68.1%)
Only conservative (non-immunosuppressive) management	304 (19.1%)
Immunosuppressive therapy	782 (49.0%) of total / 72.0% of treated
Corticosteroid + Calcineurin inhibitor (CsA/Tacrolimus)	41%
Ponticelli-type regimen (Cyclophosphamide + Steroid)	27%
Rituximab-based therapy	18%
Mycophenolate mofetil (MMF)-based therapy	8%
Other regimens (Azathioprine, Chlorambucil, etc.)	6%
Median duration of immunosuppressive therapy (months)	12 (6–24)

Table 3: Predictors of Remission in Univariate and Multivariate Cox models

Variable	Univariate Analyses				Multivariate analyses			
	HR	95.0% CI for HR		Sig.	HR	95.0% CI for HR		Sig.
		Lower	Upper			Lower	Upper	
Age	1.014	1.001	1.028	0.035	1.017	1.002	1.032	0.025
Gender male/female	0.800	0.551	1.160	0.238	0.663	0.408	1.077	0.097
Serum C3 level (normal/low)	1.158	0.161	8.354	0.884	1.868	0.239	14.570	0.551
Hemoglobin (gr/dl)	0.937	0.849	1.034	0.196	0.981	0.862	1.116	0.771
Creatinine (mg/dl)	0.925	0.666	1.285	0.643	0.899	0.619	1.306	0.576
Serum albumin (g/dl)	0.728	0.562	0.943	0.016	0.759	0.568	1.015	0.063
Proteinuria(g/g)	1.017	0.981	1.055	0.364	1.003	0.961	1.047	0.893
Interstitial fibrosis	1.064	0.737	1.536	0.741	1.212	0.689	2.134	0.504
Tubular atrophy	1.049	0.724	1.520	0.802	0.812	0.466	1.416	0.463
Interstitial inflammation	1.151	0.798	1.662	0.452	1.020	0.664	1.565	0.929

Supplementary Table 1: Histopathological characteristics of the study groups

Variables	Low C3 Group (n=45)	Normal C3 Group (n=1214)	p
Total number of glomeruli	11(9-17)	16(10-24)	0.021
Global sclerotic count	0(0-2)	1(0-2)	0.019
Number of segmental sclerotic glomeruli	0(0-0)	0(0-0)	0.240
Interstitial inflammation	34(75.6)	677(57.2)	0.022
Endocapillary proliferation	6(17.1)	65(6.4)	0.013
Interstitial fibrosis	18(40.9)	556(46.8)	0.538
Vascular changes	19(42.2)	444(37.6)	0.640
Tubular atrophy	25(56.0)	558(46.0)	0.205

Data were expressed as median (IQR) or n (%)

DISCUSSION

This study evaluated the role of serum C3 in the characteristics of primary MN and in predicting disease activity. Our findings showed that patients with lower baseline serum C3 levels had higher baseline serum creatinine and proteinuria, and lower baseline serum albumin levels, all of which are associated with more severe renal disease. Despite these differences, both groups (low and normal serum C3) showed improvements in proteinuria and albumin levels during follow-up. However, patients with lower serum C3 levels had a significantly higher recurrence rate. These results suggest that although lower serum C3 levels are associated with more severe disease, they may also serve as a useful prognostic marker for the risk of recurrence in primary MN, consistent with and expanding on existing literature pointing to the critical role of the complement system in MN pathophysiology [4,5].

Nevertheless, the consistency of this finding with the observed worse baseline clinical and histopathological features in the low C3 group supports the biological plausibility of an association between complement activation and relapse risk. The observed association between lower C3 levels and heightened disease severity, as indicated by higher creatinine and proteinuria, and lower albumin levels, resonates with recent studies highlighting the clinical significance of the complement system in MN [5]. The correlation between low serum C3 levels and adverse clinical outcomes underscores the role of complement activation in the disease course, suggesting that serum C3 may be a potential biomarker of disease progression. There is literature supporting the correlation between low serum C3 levels and adverse clinical outcomes in primary membranous nephropathy (PMN) [8]. This association underscores the role of complement activation in disease progression and suggests that serum C3 may be a potential biomarker for monitoring PMN [9,10].

Our findings support the concept that complement activation plays a relevant role in disease activity in primary membranous nephropathy. In this context, emerging complement-targeted therapies warrant further investigation. However, no conclusions regarding therapeutic efficacy can be drawn due to the observational nature of the present study. Future prospective and interventional studies are needed to clarify whether modulation of the complement pathway may have a role in the management of selected patients with MN.

The findings of the present study support the concept that complement activation is involved in disease activity and relapse risk in primary membranous nephropathy. From a clinical perspective, serum C3 may serve as a marker for risk stratification and closer monitoring. However, given the observational design of this study, as we said, no conclusions regarding therapeutic efficacy can be drawn. Further prospective and interventional studies are required to determine whether modulation of the complement pathway may have a role in the management of selected patients with MN. In this context, emerging complement-targeted therapies, such as C3 or factor B inhibition, which have been explored in other complement-

mediated kidney diseases, including lupus nephritis, warrant further investigation in appropriately designed clinical trials [4,11].

Comparing our results with global data reveals both consistencies and unique patterns in the Turkish cohort. The frequency of low serum C3 levels and their association with poor outcomes align with international studies. However, the specific demographic and clinical characteristics of our cohort highlight the need for region-specific data to fully understand the disease. Such comparative analyses can inform both local and global treatment strategies, fostering a more personalized approach to MN management.

It is noteworthy that in our study, the prevalence of endocapillary proliferation and interstitial inflammation increased in patients with low serum C3 levels. This finding suggests that these patients have a more active or severe disease process. Endocapillary proliferation, often indicative of an active inflammatory response in the glomeruli, may indicate that immune-mediated damage is more pronounced in patients with lower serum C3 levels [2]. Similarly, the higher interstitial inflammation observed is consistent with a more aggressive disease phenotype and potentially contributes to worse renal outcomes. The relationship between low serum C3 levels and these histopathological features underscores the role of the complement system in MN pathogenesis and strengthens the hypothesis that complement activation significantly contributes to disease activity and progression [12]. Interestingly, the low C3 group showed fewer globally sclerosed glomeruli, a finding that may reflect sampling variability inherent to multicenter registry biopsies rather than true disease attenuation. One possible explanation is that patients with lower serum C3 levels may have undergone kidney biopsy earlier in the disease course, when active inflammatory lesions predominate and chronic scarring is less established. This interpretation should be considered hypothesis-generating and requires confirmation in prospective studies with standardized biopsy timing.

Although IgG deposition is central to MN pathogenesis, no significant difference in IgG staining intensity was observed between the low and normal serum C3 groups. This finding suggests that the extent of IgG deposition may not solely explain differences in complement activation and clinical outcomes.

The presence of C3 staining in the tissue, especially the statistical difference observed in 3+ staining between groups, emphasizes the local activation of the complement system in the kidney [13]. This finding is consistent with the idea that local complement activation, resulting in C3 deposition in the glomeruli, plays an important role in the pathophysiology of MN. Differential staining between serum C3 groups (low and normal) may reflect the systemic and local dynamics of complement activation and its relationship to disease severity. Although our study did not directly investigate the relationship between low hemoglobin, high fibrinogen staining, and low serum C3 levels, we hypothesize that the activation of the inflammatory cascade may contribute to these findings. Further studies are needed to explore the role of fibrinogen and complement activation in the pathogenesis and prognosis of primary MN.

The study has several limitations that should be noted. The retrospective design and the use of data from multicenter studies introduce variability in laboratory measurements and in data recording. The small size of the low-C3 group limits statistical power to detect differences in certain outcomes, particularly in multivariate analyses. This increases the risk of type II error, and therefore, the absence of an independent association between serum C3 levels and remission should be interpreted with caution. Importantly, this finding does not exclude a potential biological or clinical effect of low serum C3, but may reflect insufficient power to detect such an effect after adjustment for multiple covariates.

Although remission and relapse were defined according to KDIGO 2021 criteria, outcome ascertainment relied on treating physicians' documentation at individual centers, without central adjudication. This may have introduced inter-observer variability. In addition, exact remission and relapse dates were not available for all patients, and analyses involving both should therefore be interpreted cautiously.

Another significant limitation is the lack of long-term renal outcome data, such as progression to end-stage kidney disease or doubling of serum

creatinine levels, which are critical for understanding the full impact of serum C3 levels on kidney prognosis. Besides, this study is limited by the lack of standardized anti-PLA_2R titer data, as the registry recorded only qualitative results across centers. Hence, we could not assess correlations between C3 and anti-PLA_2R titers. Nevertheless, similar positivity rates between groups suggest that complement activation, reflected by low serum C3, is not solely explained by PLA2R antibody status.

Treatment heterogeneity represents another limitation of this registry-based analysis. Protocols were not standardized across the centers. The multivariate analyses were limited by treatment heterogeneity and the absence of standardized immunosuppressive protocols across centers, which precluded robust adjustment for treatment type and increased the risk of instability in the models. Therefore, large prospective studies with standardized treatment protocols will be needed in the future to confirm the present findings and further investigate the dynamic changes in serum C3 throughout the disease course.

In summary, low serum C3 levels are associated with worse baseline clinical and histopathological features and higher relapse rates in primary MN. Serum C3 levels are not independently predictive of remission, but their measurement provides significant insight into disease activity and relapse risk. These findings point to the potential benefit of incorporating serum C3 testing into clinical practice to enhance risk stratification and personalize treatment in MN.

Authors' contributions

Concept and Design of the Study: Engin Onan, Savas Ozturk, Ayse Serra Artan, Aysegul Oruc, Muge Doksan, Kenan Turgutalp, Nurhan Seyahi, Sim Kutlay, Serap Yadigar, Simal Koksal Cevher, Belda Dursun, Musa Pinar, Mehmet Deniz Ayli, Taner Basturk, Garip Sahin, Sedat Ustundag, Zulfukar Yilmaz, Ozkan Gungor, Erhan Tatar, Feyza Bora, Murvet Yilmaz, Murat Hayri Sipahioglu, Sena Ulu, Ramazan Ozturk, Meltem Gursu, Bulent Kaya, Omer Faruk Akcay, Yelda Deligoz Bildaci, Cuma Bulent Gul, Hakki Arikan, Sebnem Karakan, Dilek Torun, Haci Hasan Yeter, Mansur Kayatas, Can Sevinc, Yavuz Ayar, Kultigin Turkmen, Saide Elif Gullulu Boz.

Data Analysis: Savas Ozturk, Engin Onan, Sevinc Puren Yucel Karakaya, Nazli Totik Dogan, Gulsah Seydaoglu.

Manuscript Drafting: Engin Onan, Aysegul Oruc, Muge Doksan, Ayse Serra Artan, Savas Ozturk, Necmi Eren, Serhat Karadag, Gulizar Sahin.

Data Acquisition: All authors contributed to data acquisition through the TSN-GOLD Working Group.

Critical Revision of the Manuscript: Savas Ozturk, Nurhan Seyahi, Sim Kutlay, Serap Yadigar, Sedat Ustundag, Engin Onan, Kenan Turgutalp, Dilek Torun, Musa Pinar, Hakki Arikan.

Data Interpretation: Engin Onan, Savas Ozturk, Ayse Serra Artan, Kenan Turgutalp, Belda Dursun, Serap Yadigar, Taner Basturk, Sebnem Karakan.

Literature Review: Engin Onan, Ayse Serra Artan, Aysegul Oruc, Muge Doksan, Serhat Karadag, Simal Koksal Cevher.

All authors read and approved the final version of the manuscript.

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Statements and declarations

Ethics statement: This study was conducted in accordance with the principles outlined in the Declaration of Helsinki as revised in 2024. Ethical approval was obtained from the Istanbul University, Istanbul Faculty of Medicine Ethics Committee (Chairperson: Prof. Dr. A. Yagız Üresin; Approval No: 2011/1131-614; Approval date: 08 July 2011). Informed consent was waived due to the study's retrospective nature; all patient data were anonymized and

de-identified before analysis to ensure confidentiality.

Informed Consent to Participate: Since the study involved retrospective analysis of existing data, patient consent was waived. However, all patient data were anonymized and de-identified before analysis to ensure confidentiality.

Competing Interests: The authors have no relevant financial or non-financial interests to disclose. On behalf of all authors, the corresponding author states that there is no conflict of interest.

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