Journal of Nephrology (2021) 34:581–587
https://doi.org/10.1007/s40620-020-00915-w

ORIGINAL ARTICLE

Clinical significance of glomerular C3 deposition in primary membranous nephropathy

Ozgur Akin Oto[1] · Erol Demir[1] · Safak Mirioglu[1] · Ahmet Burak Dirim[1] · Yasemin Ozluk[2] · Egemen Cebeci[3] · Taner Basturk[4] · Ali Riza Ucar[1] · Lala Soltanova[1] · Kanan Nuriyev[1] · Isin Kilicaslan[2] · Halil Yazici[1] · Yasar Caliskan[1,5]

Received: 21 August 2020 / Accepted: 11 November 2020 / Published online: 2 January 2021

Abstract

Background We aimed to investigate the effects of glomerular C3 deposition on clinical, histopathological features, and outcomes of patients with primary membranous nephropathy (MN).

Methods A total of 261 patients with biopsy-proven primary MN, who were on follow up for at least 6 months, were included in the study. The patients were grouped according to their C3 immunostaining in kidney biopsy samples at the time of diagnosis: Low intensity [LI; (C3 1 +)] and high intensity [HI; (C3 2 + or C3 3 +)]. The primary outcome was the development of kidney failure. Complete (CR) or partial remission (PR) was defined as secondary outcome.

Results Sixteen patients reached the primary outcome after a median follow-up of 33.8 months. Patients in the high intensity group (119 cases) had lower eGFR and higher proteinuria at admission and last follow-up compared to patients in the low intensity group (142 cases). Also, more patients in the high intensity group reached the primary outcome compared to patients in the low intensity group: twelve patients (10.1%) in the high intensity group and four patients (2.8%) in the low intensity group reached the primary outcome ($p = 0.015$). Kaplan–Meier analysis demonstrated that patients in the high intensity group had a higher risk for kidney failure ($p = 0.02$). In multivariate logistic regression analysis, high intensity C3 deposition and initial estimated glomerular filtration rate (eGFR) indepenently predicted primary outcome.

Conclusion Extensive glomerular C3 deposition is a predictor of kidney failure in patients with MN.

Keywords Complement system · C3 · Kidney failure · Membranous nephropathy

Introduction

Membranous nephropathy (MN) is a glomerular disease mediated primarily by autoreactive antibodies, being the main cause of nephrotic syndrome in non-diabetic white adults [1]. Primary MN is the most common type seen in nearly 75% of patients with MN, while the remaining 25% is manifested as a disease secondary to other conditions, such as infections, drugs, and cancer [2–4]. M-type phospholipase A2 receptor (PLA2R) and thrombospondin type-1 domain-containing 7A (THSD7A) identified as target antigens in primary MN over the last decade [5–7].

The natural course of primary MN varies considerably. Approximately one-third of patients undergo spontaneous remission, another third show persistent proteinuria, and the remaining third progress to kidney failure over ten years [8]. Therefore, understanding the factors that affect prognosis is crucial for treatment options in patients with primary MN. Several risk factors have been identified, such as male

Halil Yazici and Yasar Caliskan co-senior authors.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s40620-020-00915-w) contains supplementary material, which is available to authorized users.

✉ Yasar Caliskan
yasar.caliskan@health.slu.edu

1 Division of Nephrology, Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

2 Department of Pathology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

3 Division of Nephrology, Haseki Training and Research Hospital, Istanbul, Turkey

4 Division of Nephrology, Sisli Hamidiye Etfal Training and Research Hospital, Istanbul, Turkey

5 Division of Nephrology and Hypertension, Saint Louis University School of Medicine, Saint Louis, MO, USA

gender, advanced age, decreased glomerular filtration rate (GFR) at presentation, and persistent severe proteinuria [9–11].

Studies on MN have suggested a prominent role of the complement system in human MN, such as C3, and C5b-9 are consistently found within immune deposits [12, 13]. Immunofluorescence (IF) staining for C3 and C4d are characteristic findings of primary MN, with the absence of C1q deposition [13]. Also, the development of proteinuria requires the formation of a membrane attack complex (MAC) at the location of glomerular damage [13]. Anti-PLA2R IgG, which is predominantly of the IgG4 subclass, is thought to activate the alternative complement pathway (AP) or mannose-binding lectin (MBL) pathway [12]. Previous studies point to the accumulation of glomerular MBL and C4b in MN [12]. In secondary MN, C1q, C3, C4, complement factor B (CFB), MBL, and C5b-9 accumulation typically occur with IgG deposition, which may indicate that AP and MBL play a role in disease pathogenesis [12]. Despite the improvement in our understanding of the role of the complement system in MN, the effect of C3 deposition in glomeruli on disease course and prognosis of MN has not been clarified yet.

Therefore, we aimed to investigate the effect of glomerular C3 deposition on clinical, histopathological features, and outcomes of patients with primary MN in this retrospective multicenter study.

Materials and methods

Patients

Patients diagnosed with biopsy-proven primary MN between 1996 and 2019 and on clinical follow up at three centers for at least six months were included in this study. All relevant information regarding patients was retrieved from their medical records. Patients with systemic diseases such as malignancies (n = 4), rheumatic diseases (n = 3), infections with hepatitis B or C virus (n = 7), or other conditions associated with secondary MN including diabetes mellitus (n = 2), graft versus host disease (n = 1) were excluded, as were patients who had insufficient glomerular sampling for histopathological evaluation (n = 12). Patients with an estimated GFR (eGFR) < 15 ml/min/1.73 m^2) (n = 5) at the time of diagnosis were also excluded.

During follow-up, blood pressure (BP) was measured twice in the sitting position after 5 min of rest with an ERKA sphygmomanometer (PMS Instruments Ltd., Berkshire, UK) with an appropriately sized cuff on the right upper arm, and the average of two measurements were recorded. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [14]. Laboratory values including complete blood cell count and serum biochemical parameters were measured by standard enzymatic procedures. Urinary protein-to-creatinine ratio (uPCR, g/g) in the first-morning urine specimen was used to measure the level of proteinuria.

Study design

We classified 261 patients into two groups according to their glomerular C3 immunostaining in kidney biopsy samples at the time of diagnosis: Low intensity [LI; (C3 1 +)] and high intensity [HI; (C3 2 + or C3 3 +)]. Unless there were contraindications, angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) were given to all patients. During the first 6 months, low and medium risk patients were treated with supportive therapies. Cyclophosphamide or calcineurin inhibitors (CNIs) and corticosteroids were used as first-line immunosuppressive treatment [15]. Other immunosuppressive agents were used in patients who did not respond to initial treatment. After 2012, patients were treated according to the Kidney Disease Improving Global Outcomes (KDIGO) Clinical Practice Guideline for Glomerulonephritis [16].

Study outcomes

The primary outcome of the study was defined as the development of kidney failure, which was described as a category G5 CKD (eGFR < 15 mL/min/1.73 m^2) [17, 18]. Estimated GFRs were calculated at least by two different laboratory results to determine a category G5 CKD. Complete and partial remissions were defined as secondary outcomes. Complete remission (CR) was determined as proteinuria < 0.3 g/g and an eGFR of ≥ 60 mL/min/1.73 m^2 (or a return of ± 15% of baseline values in patients with eGFR < 60 mL/min/1.73 m^2). Partial remission (PR) was defined as a proteinuria reduction of > 50% (and a proteinuria value of < 3.5 g/g in patients with nephrotic-range proteinuria at baseline) and stabilization (± 25%) or improvement in eGFR. The follow-up period was considered as the time interval between kidney biopsy and last outpatient visit or development of kidney failure. Effects of demographic, clinical, and histopathological parameters (tubular atrophy, interstitial fibrosis, intensity, and pattern of staining for IgG and C3) on primary and secondary outcomes were analyzed.

Histopathological evaluation

All renal biopsy samples from the three participating centers included in the study were initially evaluated at Istanbul School of Medicine Pathology Department by two nephropathologists. All samples had 7 or more glomeruli for routine pathological examination, which included histochemical

(hematoxylin and eosin, periodic acid-Schiff, Congo red, Masson trichrome, methenamine silver-periodic acid) and IF stains (IgG, IgM, IgA, C1q, C3, kappa, lambda, and fibrinogen). Renal biopsy specimens were fixed in Hollande's fixative, embedded in paraffin wax, and processed routinely. All histochemical and immunohistochemical stains were prepared using 3-µm paraffin sections.

The fluorescence intensity of IgG, IgM, IgA, C1q, C3, kappa, and lambda was determined using a semi-quantitative scale of 0–3: 0, negative; 1, weak; 2, moderate; 3, strong staining. Glomerular MN lesions were classified into four stages according to Ehrenreich and Churg's criteria [19]. Interstitial fibrosis and tubular atrophy were also graded using a semi-quantitative scale from 0 to 3: 0, normal; 1 (mild), < 25% of interstitium; 2 (moderate), 25–50% and 3 (severe), > 50%. A nephropathologist (Y.O.) who was blinded to all clinical data confirmed the diagnoses by reviewing the biopsy samples (in the same attempt) for histologic staging (based on Ehrenreich and Churg' classification criteria), interstitial fibrosis and tubular atrophy grading. Concordance rates of these re-evaluations with initial assessments were almost 90–95% for staging and interstitial fibrosis and tubular atrophy grading.

Statistical analyses

Categorical variables were summarized with counts and percentages. Quantitative variables were summarized with means and standard deviations, or medians and interquartile range (IQR) statistics, where appropriate. The chi-square and Fisher's exact tests were performed for qualitative variables, whereas the Mann–Whitney U test was used for quantitative variables with the non-parametric distribution. Kidney survival times were analyzed using the Kaplan–Meier method and kidney survival time for each patient was computed from baseline evaluation to the last follow-up or the development of kidney failure. Since the covariates did not satisfy the assumption of proportionality, logistic regression analysis was used to identify kidney failure and the associated risk in terms of odds ratio (OR) and 95% confidence intervals (CI). Variables were selected by backward elimination using likelihood ratio tests. Calculations were performed using SPSS statistical software (SPSS version 21.0, IBM Corp., Armonk, NY, USA). A p-value of 0.05 or less was considered to be statistically significant. The research complied with the Declaration of Helsinki [20] and was approved by the local ethical committee of Istanbul University.

Results

In total, 261 patients [104 (39.8%) females] with primary MN were followed up for a median of 31 (IQR 14–64) months. The median age was 57 (IQR 45–66) years. The LI and HI groups included 142 (54.4%) and 119 (45.6%) patients, respectively. The median age of the patients was 55 (45–64) in the LI group and 59 (45–70) in the HI group ($p = 0.171$). Follow-up time, systolic, and diastolic BPs were similar between study groups. Patients in the LI group were characterized by a higher level of eGFR [96 (72–119) vs. 86 (58–118) mL/min/1.73m^2, $p = 0.015$], serum albumin (2.9 ± 0.8 vs. 2.5 ± 0.7 g/dL, $p < 0.001$), and hemoglobin (13.1 ± 1.8 vs. 12.4 ± 1.9 g/dL, $p = 0.007$) as compared to patients in the HI group at the time of diagnosis. Baseline demographic, clinical, and laboratory characteristics of patients during kidney biopsy regarding C3 deposition intensity are shown in Table 1.

Histopathological and therapeutic features

There were no significant differences between study groups in terms of Ehrenreich and Churg's histological stage ($p = 0.154$). The intensity of interstitial fibrosis and tubular atrophy was similar between the two groups ($p = 0.291$). However, strong IgG intensity was higher in patients with HI-C3 as compared to patients with LI-C3 ($p < 0.001$). Histopathological characteristics of the patients with regard to degree of C3 deposition are summarized in Table 2.

Therapeutic regimens (CNIs, antiproliferative drugs, rituximab) were generally comparable among the groups except for cyclophosphamide. The percentage of patients who did not receive immunosuppressive therapy did not differ between study groups (Table 3).

Study outcomes

Sixteen (6.1%) of 261 patients reached the primary outcome after a median follow-up of 30.6 (IQR 13.8–63.8) months. Twelve patients (10.1%) in the HI group and four patients (2.8%) in the LI group reached the primary outcome ($p = 0.015$). Kaplan–Meier analysis revealed that overall kidney survival was lower in the HI group compared to the LI group (Fig. 1) ($p = 0.02$). Although CR [33 (23.2%) vs. 18 (15.1%)] and PR [64 (45.1%) vs. 49 (41.2%)] rates were higher in the LI group compared to HI group, these differences did not achieve statistical significance ($p = 0.1$ and $p = 0.527$, respectively).

Last visit eGFR [84 (64–110) vs. 71 (44–91) mL/min/1.73 m^2, $p < 0.001$] was significantly higher, and last visit proteinuria [1.7 (0.3–3.5) vs. 2.4 (1–4.3) g/g, $p = 0.009$] was significantly lower in the LI compared to the HI group. Details of treatment modalities and study outcomes in study groups are shown in Table 3.

Table 1 Baseline demographic, clinical, and laboratory characteristics of patients with regard to C3 deposition intensity

	LI group (N = 142)	HI group (N = 119)	p-value
Age (mean ± SD, year)	55 (45–64)	59 (45–70)	0.171
Sex (N, %)			
Male	85 (59.9)	72 (60.5)	0.916
Female	57 (40.1)	47 (39.5)	
Blood Pressure (median-IQR 25–75, mmHg)			
Systolic	130 (120–140)	120 (120–140)	0.859
Diastolic	80 (70–90)	80 (70–90)	0.839
Initial proteinuria (median-IQR 25–75, g/g)	4.8 (3.2–8)	6.1 (4–8.8)	0.031
Severity of proteinuria (N, %)			
Non-nephrotic range	43 (30.3)	20 (16.8)	0.011
Nephrotic range	99 (69.7)	99 (83.2)	
Initial serum albumin (mean ± SD, g/dL)	2.9 ± 0.8	2.5 ± 0.7	< 0.001
Initial hemoglobin (mean ± SD, g/dL)	13.1 ± 1.8	12.4 ± 1.9	0.007
Initial eGFR (median-IQR 25–75, mL/min/1.73 m^2)	96 (72–119)	86 (58–118)	0.015

Abbreviations: eGFR, estimated glomerular filtration rate; HI, high intensity; IQR, interquartile range; LI, low intensity; SD, standard deviation

Note: P-values compared low intensity and high intensity, obtained from the Chi-Square test, Fisher's exact test, or Mann–Whitney U test

P-values in bold show statistically significant differences

Table 2 Histopathological characteristics of patients with regard to C3 deposition

	LI group (N = 142)	HI group (N = 119)	p-value
Histological stage (N, %)			
Stage I	38 (26.8)	23 (19.3)	0.154
Stage II	68 (47.9)	71 (59.7)	
Stage III	36 (25.3)	25 (27.8)	
IgG intensity (N, %)			
II +	50 (35.2)	18 (15.1)	< 0.001
III +	92 (64.8)	101 (84.9)	
The intensity of IFTA (N, %)			
Mild	69 (48.6)	44 (37)	0.291
Moderate	2 (1.4)	3 (2.5)	

Abbreviations: HI, high intensity; IFTA, interstitial fibrosis tubular atrophy; LI, low intensity

Note: P-values compared low intensity and high intensity, obtained from the Chi-Square test,

Fisher's exact test, or Mann–Whitney U test

P-values in bold show statistically significant differences

Predictors of primary outcome

In univariate logistic regression analysis, initial hemoglobin levels (OR 0.646, 95% CI 0.482–0.866, p = 0.004), initial eGFR (OR 0.957, 95% CI 0.938–0.976, p < 0.0001), and HI staining (OR 3.869, 95% CI 1.214–12.335, p = 0.022) were associated with primary outcome. However male gender (OR 2.030, 95% CI 0.732–5.633, p = 0.174), age (OR 1.028, 95% CI 0.991–1.067, p = 0.140), initial serum albumin levels (OR 0.893, 95% CI 0.458–1.741, p = 0.740), IgG staining (OR 0.762, 95% CI 0.255–2.277, p = 0.626), histological stage (OR 1.153, 95% CI 0.550–2.420, p = 0.706) and initial proteinuria levels (OR 1.000, 95% CI 0.999–1.000, p = 0.790) were not identified as risk factors for primary outcome.

In multivariate logistic regression analysis, HI staining (OR 4.557, 95% CI 1.112–18.672, p = 0.035), and initial eGFR (OR 0.954, 95% CI 0.929–0.980, p = 0.001) predicted primary outcome, whereas, male gender (OR 2.562, 95% CI 0.664–9.892, p = 0.172), age (OR 0.975, 95% CI 0.933–1.020, p = 0.270), initial hemoglobin (OR 1.033, 95% CI 0.733–1.456, p = 0.852), and initial proteinuria (OR 0.999, 95% CI 0.999–1.000, p = 0.391) levels did not.

Predictors of secondary outcome

In univariate logistic regression analysis, initial serum albumin (OR 1.508, 95% CI 1.071–2.123, p = 0.019), initial hemoglobin (OR 1.180, 95% CI 1.028–1.354, p = 0.018) levels and HI staining (OR 1.673, 95% CI 1.008–2.775, p = 0.046) predicted the secondary outcome. However, age (OR 0.985, 95% CI 0.968–1.002, p = 0.084), initial proteinuria (OR 1.000, 95% CI 1.000–1.000, p = 0.144), initial eGFR (OR 1.006, 95% 0.998–1.015, p = 0.165), and IgG staining (OR 0.628, 95% CI 0.346–1.139, p = 0.126) were not identified as risk factors for secondary outcome.

In multivariate logistic regression analysis, only initial hemoglobin (HR 1.179, 95% CI 1.022–1.359, p = 0.024)

Table 3 Treatment modalities and study outcomes in study groups

	LI group (N = 142)	HI group (N = 119)	p-value
Follow-up time (median-IQR 25–75, months)	28 (13–67)	36 (16–61)	0.31
Last visit eGFR (median-IQR 25–75, mL/min/1.73)	84 (64–110)	71 (44–91)	< 0.001
Last visit proteinuria (median-IQR 25–75, g/g)	1.7 (0.3–3.5)	2.4 (1–4.3)	0.009
Treatment agent (N, %)			
Calcineurin inhibitors	54 (38)	46 (38.7)	0.917
Cyclophosphamide	20 (14.1)	5 (4.2)	0.007
Antiproliferative drugs	15 (10.6)	24 (20.2)	0.416
Rituximab	8 (5.6)	10 (8.4)	0.379
No immunosuppression	50 (35.2)	41 (34.5)	0.898
Primary outcome (N, %)			
Kidney failure	4 (2.8)	12 (10.1)	0.015
Secondary outcome (N, %)	97 (68.3)	67 (56.3)	
Complete remission	33 (23.2)	18 (15.1)	0.1
Partial remission	64 (45.1)	49 (41.2)	0.527

Abbreviations: eGFR, estimated glomerular filtration rate; HI, high intensity; IQR, interquartile range; LI, low intensity; SD, standard deviation

Note: P-values compared low intensity and high intensity, obtained from the Chi-Square test, Fisher's exact test, or Mann–Whitney U test

P-values in bold show statistically significant differences

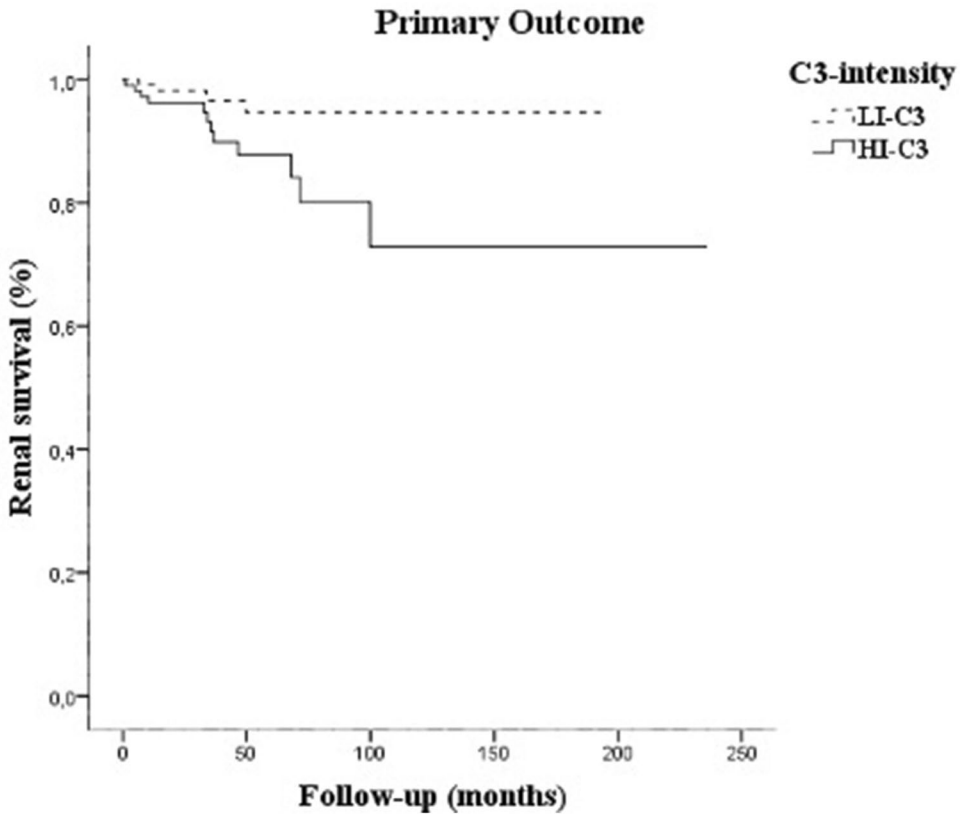

Fig. 1 Kaplan–Meier analysis revealed that kidney survival was lower in the HI-C3 group compared to the LI-C3 group (p = 0.020)

and initial albumin levels (HR 1.543, 95% CI 1.071–2.223, p = 0.02) predicted secondary outcome, whereas HI staining (HR 1.329, 95% CI 0.771–2.291, p = 0.306) did not.

Discussion

In this multicenter, observational study, we investigated glomerular C3 deposition and its clinical importance in patients with primary MN. Patients with extensive C3 deposition were found to have lower hemoglobin and serum albumin levels at baseline compared to patients with mild C3 deposition. Also, patients with extensive C3 deposition had lower eGFR and higher proteinuria at admission and at the end of the follow-up period compared to patients with mild C3 deposition. In addition, extensive C3 deposition was associated with the development of kidney failure.

Glomerular C3 deposits are usually demonstrated in patients with primary MN. These deposits are considered to be the result of primary immune mechanisms. Also, there is strong evidence to support complement activation as a prerequisite for the development of tissue injury and subsequent proteinuria [21–23]. Previous studies demonstrated that MBL, C4b, and Bb residues were associated with the lectin pathway (LP), and, the deposition of C3b, MAC, and urinary excretion of C3dg have been linked with the AP pathway in the pathogenesis of MN [24–26]. The exact underlying mechanism of complement activation in MN still needs to be clarified.

Many studies investigated the prognostic significance of histopathological parameters in primary MN, but their results differ widely [27, 28]. Also, the data on the extensive complement deposition and clinicopathological findings are conflicting. Zhang et al. found that patients with extensive complement deposition had a higher positive rate of serum anti-PLA2R antibodies, higher levels of urinary protein excretion and serum creatinine, and lower levels of serum albumin compared to patients with mild complement deposition. However, they found that C3 intensity was not predictive of unfavorable outcomes [29]. Horvatic et al. reported that there was no relationship between C3 intensity and negative outcomes [30]. On the other hand, Troyanov et al. demonstrated a strong relationship between quantitative complement deposition and progression. However, they could not make certain judgments about achieving definitive results because of the semiquantitative and unverified grading system and the variations in reagent specificity used to estimate complement deposition [28]. In our study, patients with extensive C3 accumulation had a worse kidney outcome compared to patients with mild C3 accumulation, and extensive C3 accumulation predicted the development of kidney failure. The main reasons for the differences between the study results can be explained by the differences in follow-up time and classification of C3 accumulation, and the changes in the course of the disease in different populations.

Less use of cyclophosphamide in patients in HI may have affected the study outcome. This point can be explained by the differences in treatment protocols between centers and the lack of a standard treatment protocol before glomerulonephritis guidelines [16]. On the other hand, our study was not conducted to investigate the efficacy of these drugs.

Interestingly, in this study, male gender was not a statistically significant predictor of primary and secondary outcomes. Although male gender was associated with poor clinical prognosis in many studies [10, 11], this finding has not been confirmed by all [31]. In general, the degree of proteinuria and/or hypoalbuminemia is considered the major determinant of disease severity [32], and this determinant was not associated with the primary outcome in our study. Similar results have been published by Spranger et al. [33]. This finding may be influenced by the use of nonspecific supportive measures such as renin-angiotensin system blockade, and a relatively limited number of patients included in our study.

This study has limitations inherent to its retrospective design. The study was primarily conducted with chart reviews. Serum anti-PLA2R was not determined and monitored at the time of diagnosis, and the staining of PLA2R in glomeruli was not available. Therefore, we could not examine the relationship between C3 deposits and autoantibody levels. Similarly, the distribution and characterization of the C3 deposits (electron-dense versus electron-lucent deposits) could not be evaluated due to a lack of electron microscopic evaluation in all cases. IgG subgroups were not taken into consideration either. Since data obtained from three centers were used, our results may not be generalizable. Also, we could not exclude the possibility of potential residual confounders.

In conclusion, patients with extensive glomerular C3 deposition had a worse clinical presentation at baseline and poor clinical outcomes during follow-up than patients with mild C3 deposition. This finding suggests that determining and applying specific treatment protocols for these patients might be beneficial.

Author contributions OAO, ED, and YC participated in study design, acquisition of data and regulatory approvals, data analysis, and writing of the paper. SM, ABD, EC, TB, and HY participated in study design, interpretation, and writing of the paper. YS and IK reviewed all biopsy samples and participated in interpretation and writing of the paper. ARU, LS and KN reviewed the patient charts and participated in data interpretation and writing of the paper. All of the authors read and approved the last version of the manuscript.

Compliance with ethical standards

Conflict of interest The authors of this manuscript have no conflicts of interest to disclose.

Ethics approval The research was approved by the local ethical committee of Istanbul University.

Consent to participate The authors declare that the patients reported here have provided authorization for use of their medical records for research.

Consent for publication All authors gave consent for publication.

References

1. Glassock RJ (2010) The pathogenesis of idiopathic membranous nephropathy: a 50-year odyssey. Am J Kidney Dis 56(1):157–167
2. Feng Z, Wang S, Huang Y, Liang X, Shi W, Zhang B (2016) A follow-up analysis of positron emission tomography/computed tomography in detecting hidden malignancies at the time of diagnosis of membranous nephropathy. Oncotarget 7(9):9645–9651
3. Seitz-Polski B, Dolla G, Payré C, Girard CA, Polidori J, Zorzi K et al (2016) Epitope spreading of autoantibody response to PLA2R associates with poor prognosis in membranous nephropathy. J Am Soc Nephrol 27(5):1517–1533
4. Zeng CH, Chen HM, Wang RS, Chen Y, Zhang SH, Liu L et al (2008) Etiology and clinical characteristics of membranous nephropathy in Chinese patients. Am J Kidney Dis 52(4):691–8
5. Beck LH Jr, Bonegio RG, Lambeau G, Beck DM, Powell DW, Cummins TD et al (2009) M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med 361(1):11–21
6. Sethi S, Madden BJ, Debiec H, Charlesworth MC, Gross L, Ravindran A et al (2019) Exostosin 1/exostosin 2–associated membranous nephropathy. J Am Soc Nephrol 30(6):1123–1136
7. Tomas NM, Beck LH Jr, Meyer-Schwesinger C, Seitz-Polski B, Ma H, Zahner G et al (2014) Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. N Engl J Med 371(24):2277–2287
8. Davison A, Cameron J, Kerr D, Ogg C, Wilkinson R (1984) The natural history of renal function in untreated idiopathic membranous glomerulonephritis in adults. Clin Nephrol 22(2):61–67
9. Kanigicherla D, Gummadova J, McKenzie EA, Roberts SA, Harris S, Nikam M et al (2013) Anti-PLA2R antibodies measured by ELISA predict long-term outcome in a prevalent population of patients with idiopathic membranous nephropathy. Kidney Int 83(5):940–948
10. Pei Y, Cattran D, Greenwood C (1992) Predicting chronic renal insufficiency in idiopathic membranous glomerulonephritis. Kidney Int 42(4):960–966
11. Schieppati A, Mosconi L, Perna A, Mecca G, Bertani T, Garattini S et al (1993) Prognosis of untreated patients with idiopathic membranous nephropathy. N Engl J Med 329(2):85–89
12. Ma H, Sandor DG, Beck LH Jr (2013) The role of complement in membranous nephropathy. Semin Nephrol 33(6):531–542
13. Sethi S, Nasr SH, De Vriese AS, Fervenza FC (2015) C4d as a diagnostic tool in proliferative GN. J Am Soc Nephrol 26(11):2852–2859
14. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI et al (2009) A new equation to estimate glomerular filtration rate. Ann Intern Med 150(9):604–612
15. Cattran DC, Appel GB, Hebert LA, Hunsicker LG, Pohl MA, Hoy WE et al (2001) Cyclosporine in patients with steroid-resistant membranous nephropathy: a randomized trial. Kidney Int 59(4):1484–1490
16. Kidney Disease Improving Global Outcomes (KDIGO) Glomerulonephritis Work Group (2012): KDIGO clinical practice guideline for glomerulonephritis. Kidney Int 2:139–274
17. Levey AS, Eckardt K-U, Dorman NM, Christiansen SL, Hoorn EJ, Ingelfinger JR et al (2020) Nomenclature for kidney function and disease: report of a kidney disease: improving global outcomes (KDIGO) consensus conference. Kidney Int Rep 5(7):965–972
18. Summary of Recommendation Statements (2013) Kidney Int Suppl (2011) 3(1):5–14
19. Ehrenreich T, Porush JG, Churg J, Garfinkel L, Glabman S, Goldstein MH et al (1976) Treatment of idiopathic membranous nephropathy. N Engl J Med 295(14):741–746
20. Association WM (2010) World medical association declaration of Helsinki. http://www.wma net/en/30publications/10policies/b3/index html
21. Abe S, Amagasaki Y, Konishi K, Kato E, Iyori S, Sakaguchi H (1986) Idiopathic membranous glomerulonephritis: aspects of geographical differences. J Clin Pathol 39(11):1193–1198
22. Beregi E, Varga I (1974) Analysis of 260 cases of membranous glomerulonephritis in renal biopsy material. Clin Nephrol 2(6):215–221
23. Erwin DT, Donadio JV Jr, Holley KE (1973) The clinical course of idiopathic membranous nephropathy. Mayo Clin Proc 48:697–712
24. Borza D-B (2016) Alternative pathway dysregulation and the conundrum of complement activation by IgG4 immune complexes in membranous nephropathy. Front Immunol 7:157
25. Brenchley PE, Coupes B, Short CD, O'Donoghue DJ, Ballardie FW, Mallick NP (1992) Urinary C3dg and C5b–9 indicate active immune disease in human membranous nephropathy. Kidney Int 41(4):933–937
26. Segawa Y, Hisano S, Matsushita M, Fujita T, Hirose S, Takeshita M et al (2010) IgG subclasses and complement pathway in segmental and global membranous nephropathy. Pediatric Nephrol 25(6):1091–1099
27. Marx BE, Marx M (1999) Prediction in idiopathic membranous nephropathy. Kidney Int 56(2):666–673
28. Troyanov S, Roasio L, Pandes M, Herzenberg A, Cattran D (2006) Renal pathology in idiopathic membranous nephropathy: a new perspective. Kidney Int 69(9):1641–1648
29. Zhang XD, Cui Z, Zhang MF, Wang J, Zhang YM, Qu Z et al (2018) Clinical implications of pathological features of primary membranous nephropathy. BMC Nephrol 19(1):215
30. Horvatic I, Ljubanovic DG, Bulimbasic S, Knotek M, Prkacin I, Tisljar M et al (2012) Prognostic significance of glomerular and tubulointerstitial morphometry in idiopathic membranous nephropathy. Pathol Res Practice 208(11):662–667
31. Yoshimoto K, Yokoyama H, Wada T, Furuichi K, Sakai N, Iwata Y et al (2004) Pathologic findings of initial biopsies reflect the outcomes of membranous nephropathy. Kidney Int 65(1):148–153
32. Cattran D (2005) Management of membranous nephropathy: when and what for treatment. J Am Soc Nephrol 16(5):1188–1194
33. Sprangers B, Bomback AS, Cohen SD, Radhakrishnan J, Valeri A, Markowitz GS et al (2012) Idiopathic membranous nephropathy: clinical and histologic prognostic features and treatment patterns over time at a tertiary referral center. Am J Nephrol 36(1):78–89