RENAL FAILURE
2023, VOL. 45, NO. 1, 2179335
https://doi.org/10.1080/0886022X.2023.2179335

Taylor & Francis
Taylor & Francis Group

ARTICLE

The thickness of glomerular basement membrane predicts complete remission in primary membranous nephropathy

Suyan Duan[a]*, Lianqin Sun[a]*, Chengning Zhang[a], Ming Zeng[a], Bin Sun[a], Yanggang Yuan[a], Huijuan Mao[a], Changying Xing[a]† and Bo Zhang[a,b]†

[a]Department of Nephrology, the First Affiliated Hospital of Nanjing Medical University, Nanjing Medical University, Nanjing, P.R. China; [b]Department of Nephrology, Pukou Branch of JiangSu Province Hospital (Nanjing Pukou Central Hospital), Nanjing, P.R. China

ABSTRACT

Objective: Glomerular basement membrane (GBM) thickening is a typical and essential histopathological characteristic for the diagnosis of primary membranous nephropathy (PMN). The present study aimed to explore the relationship between GBM thickness and treatment response in PMN patients.

Methods: A total of 128 patients with nephrotic syndrome concurrent with PMN were studied. The highest GBM thickness was measured from at least five glomerular capillary loops using an electron microscope, and the mean value was obtained. Patients were categorized into three groups according to the tertiles of GBM thickness as follows: Group 1 (GBM thickness $\leq$ 1100 nm, $n = 48$), Group 2 (1100 nm $<$ GBM thickness $\leq$ 1300 nm, $n = 40$), Group 3 (GBM thickness $>$1300 nm, $n = 40$). Clinicopathological features and treatment response were compared among the three groups. The associations of GBM thickness with complete remission (CR) were assessed by Cox proportional hazard analyses and a cubic spline curve.

Results: During a median follow-up period of 25.80 months, 69 (53.9%) patients achieved CR. Kaplan–Meier analysis showed that the non-CR probability was significantly higher in the highest tertile of GBM thickness (p<0.001). Univariate Cox proportional hazard analysis indicated that GBM thickness was associated with CR (HR per SD 0.617, 95% CI [0.471–0.809], p<0.001). After adjusting for age, duration of PMN, estimated glomerular filtration rate (eGFR), urinary protein excretion, grade of C3 deposition, and titer of serum anti-phospholipase A2 receptor (PLA2R) antibody, GBM thickness remained an independent predictor of CR (HR per SD 0.580, 95% CI [0.436–0.772], p<0.001). Further multivariable-adjusted restricted cubic spline analysis confirmed a significant reverse linear association between GBM thickness and CR (p for nonlinear = 0.1261).

Conclusions: GBM thickness is an independent risk factor of CR. PMN patients with an increased level of GBM thickening at diagnosis have a lower probability of achieving CR.

ARTICLE HISTORY
Received 22 August 2022
Revised 3 February 2023
Accepted 4 February 2023

KEYWORDS
GBM thickness; primary membranous nephropathy; complete remission; proteinuria

Introduction

Membranous nephropathy (MN), a common cause of nephrotic syndrome in adults, is histologically characterized by thickening of the glomerular basement membrane (GBM) on light microscopy, deposition of anti-podocyte targeted immunoglobulin G (IgG) and complement 3 (C3) on immunofluorescence, and deposition of subepithelial electron-dense material on electron microscopy [1]. The deposition of subepithelial immune complexes and the subsequent complement activation is responsible for the functional impairment of the glomerular capillary wall, which results in observed proteinuria [2,3]. Among patients with MN, approximately 20% are classified as secondary MN, which is associated with systemic disease, infection, drug exposure, and malignancy [4]. The remaining 80% of patients have primary membranous nephropathy (PMN), which is characterized by a uniform and diffuse thickening of the wall of the glomerular capillaries [4,5]. The initial pathogenetic event is the binding of circulating autoantibodies to proteins or autoantigens in

CONTACT Bo Zhang zhangbo@jsph.org.cn; Changying Xing, xingchangying@jsph.org.cn Department of Nephrology, The First Affiliated Hospital of Nanjing Medical University, Jiangsu Province Hospital, 300 Guangzhou Road, Nanjing, 210029, Jiangsu Province, P. R. China

Footnotes

*Co-first authors of this study

†Senior authors with equal contribution in this study

Supplemental data for this article can be accessed online at https://doi.org/10.1080/0886022X.2023.2179335.

glomerular podocytes. This leads to the formation of immune complexes in GBM [1,6–10]. The GBM-the crucial component of the glomerular filtration barrier-consists of type IV collagen, laminin, nidogen, and heparin sulfate proteoglycans [11]. This membrane is also adjacent to glomerular endothelial cells and podocytes [12]. The diffuse thickening of GBM is not only a typical and essential histopathological characteristic for the diagnosis of MN, but also the major basis for the current understanding of the pathogenesis of MN [6,13]. GBM has a "spike" to enfold the deposits. The immune complex deposits encompass the in-situ antigens, with IgG binding to antigens, and membrane attack complexes (MAC) formed by complement activation. MACs are the traces left by the antibody-dependent immune response [6,13]. Tremendous progress has been made in understanding the components that cause the thickening of GBM. However, it remains unclear whether GBM thickness is associated with disease severity and treatment response.

The natural course of PMN varies considerably. Therefore, understanding the factors of the prognostic assessment is crucial for treatment options and risk stratification in patients with PMN [4,6,13]. The current indicators applied to evaluate disease activity and predict the remission time of PMN patients mainly focus on serum and urinary parameters that are easily detected and readily available for wide clinical application. The 24-h urinary protein and serum PLA2R titer remains well-standardized and are widely used biomarkers in clinical practice. Moreover, accumulating data have been pointing to the important role of biomarkers for evaluating immune status such as a proliferation-inducing ligand, serum complement 4, Interleukin-35 levels [14–16], and for reflecting tubular injury in PMN [19]. Besides, risk factors of metabolic syndrome such as body mass index (BMI), total cholesterol (TC), non-high-density lipoprotein cholesterol (non-HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides (TG) are also demonstrated to be associated with renal outcomes in PMN [17,18]. However, the relationship between renal histological features and renal prognosis is controversial to some extent due to the lack of sufficient attention and data support [20]. The advanced stage of glomerular lesions, glomerulosclerosis or focal segmental glomerular sclerosis, and chronic tubulointerstitial injury have been linked to worse renal survival in some but not all studies [20–24]. The diffuse thickness of GBM, which involves the formation of subepithelial immunocomplex deposits, is the main pathological change in PMN. Nevertheless, it remains largely unclear whether GBM thickness could predict the likelihood of achieving complete remission (CR) in PMN patients. Furthermore, factors associated with GBM thickness are unknown.

In the current study, we aimed to explore the association of GBM thickness with clinicopathological features and treatment response of PMN patients with nephrotic syndrome, providing substantiated clinical guidance for risk stratification and more optimal individualized therapy.

Materials and methods

Subjects

A total of 178 patients with biopsy-proven MN with nephrotic syndrome from the time period between January 2017 to August 2019 were retrospectively reviewed. The patients were diagnosed and followed up at the Department of Nephrology, the First Affiliated Hospital of Nanjing Medical University. Patients who fulfilled the following criteria were included in this study: (1) MN diagnosis based on renal biopsy, with concurrent nephrotic syndrome; (2) treatment-naïve at the time of renal biopsy, i.e. without a drug history of immunosuppressive therapy or corticosteroids; (3) the follow-up time since biopsy-proven diagnosis more than 6 months; (4) age $>$ 18 years. Patients with secondary MN, such as those with autoimmune diseases (e.g. lupus nephritis and Sjogren syndrome), infection-related MN resulting from hepatitis B and C virus infection, or MN related to malignancies, medications, or heavy metal poisoning, were excluded. Patients with an incomplete investigation, such as those without electron micrographs and anti-PLA2R antibody testing, were also excluded from this study. In total, 128 patients were recruited (Figure 1). This study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (approval number: No. 2018-SR-250).

Clinical data collection

Data from each patient's record encompassed clinical data such as age, gender, comorbidities, blood pressure, BMI, and laboratory test results (urinary protein, serum creatinine, serum albumin, immunoglobulin G, and complement 3 (C3)). Serum samples collected from each patient at the time of renal biopsy were stored at $-80\,^{\circ}C$ and thawed simultaneously for the measurement of anti-PLA2R antibodies. Serum level of anti-PLA2R antibodies was determined using commercially available ELISA kits (EUROIMMUN AG, Lubeck, Germany). Briefly, PLA2R-coated microplates were

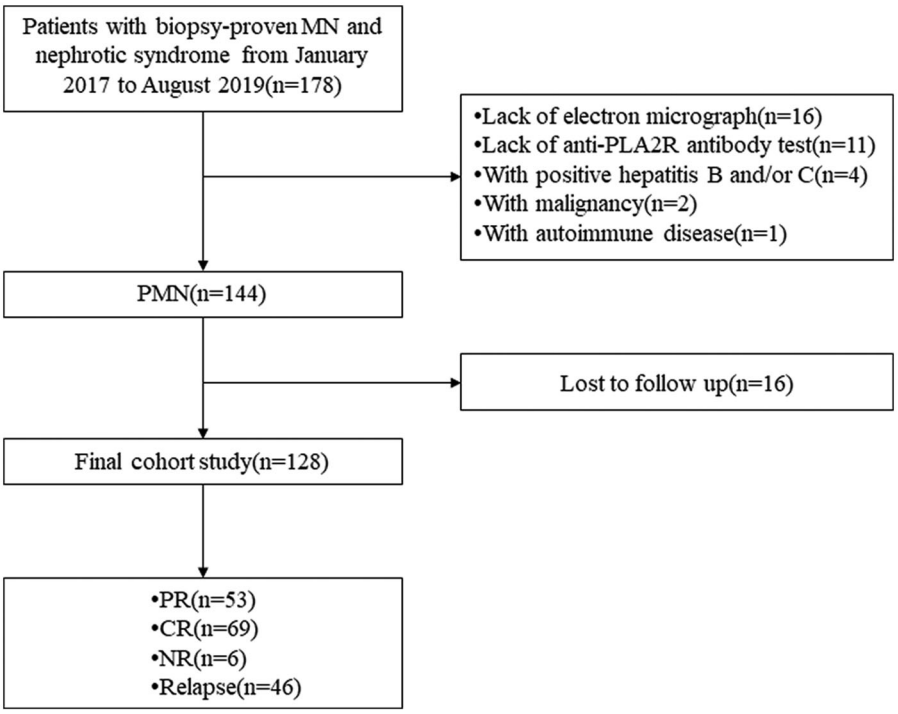

Figure 1. Flowchart of study participants. MN, membranous nephropathy; PLA2R, phospholipase A2 receptor; PMN, primary membranous nephropathy; PR, partial remission; CR, complete remission; NR, no remission.

incubated with human sera diluted 1:100 in sample buffer for 30 min and were visualized after incubation with anti–human-IgG HRP conjugate for 30 min. The optical density was measured at 450 nm using a microplate absorbance reader (Model 550; BioRad, Hercules, CA, USA). In accordance with the manufacturer's guidelines, values $\geq$ 20 RU/mL were considered positive. In addition, clinical response to treatment, progression to end-stage kidney disease (ESKD), and death were recorded for each patient. The patients were followed up for at least 6 months. The clinical data were collected at the time of renal biopsy, and at the time points of 3, 6, and 12 months after treatment. BMI was calculated as the ratio of weight in kilograms divided by the square of the height in meters. Estimated glomerular filtration rate (eGFR) was determined by the CKD-EPI^{2009} equation [25].

Renal biopsy

All of the patients underwent a renal biopsy before treatment. Each biopsy specimen was evaluated by light microscopy, direct immunofluorescence, and electron microscopy. Paraffin-embedded sections (3 μm in thickness) stained with Periodic acid–Schiff (PAS), Periodic acid–silver metheramine (PASM), and Masson were observed by light microscopy. The number of sclerotic glomeruli and crescents was presented as the percentage of the total number of glomeruli in the biopsy specimen. The chronic tubulointerstitial injury was graded as follows: grade 0 (none), grade 1 (below 25% of tubulointerstitial tissue affected), grade 2 (25% to 50% of tubulointerstitial tissue affected), grade 3 (above 50% of tubulointerstitial tissue affected). In addition, the acute tubulointerstitial injury was graded 0 (absent) or 1 (present). Glomerular lesions were assigned one of four stages, from I to IV, in accordance with Ehrenreich and Churg's classification criteria [26]. Frozen sections (4 μm in thickness) were used for direct immunofluorescence to detect IgG, IgA, IgM, C3, C1q, and PLA2R. The fluorescence intensity was described by a semiquantitative method as follows: 0 (none), 1 (+), 2 (2+), 3 (3+ to 4+). Transmission electron microscopy was performed using ultrathin sections embedded in resin and stained with lead citrate and uranium acetate in order to observe the ultrastructure. In each measurement, GBM thickness was defined by the length between the endothelial cell and podocyte membrane. This included intramembranous immune deposits. The highest GBM thickness was routinely measured on at least five glomerular capillary loops and reviewed, and the mean value was obtained. Pathologists who measured GBM thickness were blinded to the patient's clinical data and outcome. Iteratively, a review of any

scoring differences between the two pathologists was performed.

Treatment methods and response

The enrolled patients received calcineurin inhibitors including cyclosporine and tacrolimus, with or without corticosteroids. The regimen choice was decided by renal physicians, considering the patient's preferences and potential drug side effects.

The therapeutic responses were in compliance with the KDIGO (Kidney Disease: Improving Global Outcomes) guideline for glomerulonephritis [27]. Clinical outcome was evaluated based on proteinuria levels at diagnosis compared with assessment after 3, 6, and 12 months of follow-up. CR was defined as urinary protein excretion $<$ 0.3 g/day with a stable GFR. Partial remission (PR) was defined as urinary protein excretion $<$ 3.5 g/day and a reduction of at least 50% from baseline values with a stable GFR. Relapse was defined as urinary protein excretion $>$ 3.5 g/day after reaching CR or PR. Treatment failure was defined as the absence of CR or PR during the follow-up period. ESKD was defined as the initiation of maintenance dialysis or kidney transplantation. The endpoints were defined as CR and a composite of PR and CR. Outcomes of patients who did not reach the endpoints were recorded using the information from their last follow-up visit. Survival time was calculated from the enrollment to the occurrence of the end-point events or the last follow-up. The duration of PMN was calculated from the occurrence of nephropathy-related symptoms to treatment initiation.

Statistical analysis

Continuous variables were presented as either the mean ± standard deviation (SD) or the median and interquartile range (IQR). Categorical variables were presented as counts (n) and percentages (%). We divided the participants into tertiles according to the GBM thickness. Comparisons between the groups were performed using one-way analysis of variance (ANOVA), Kruskal–Wallis test, or χ^2 test, as appropriate. Spearman correlation analysis was used to explore the relationship between GBM thickness and laboratory parameters. The Kaplan–Meier curve was generated to assess the incidence of non-CR among the different groups of GBM thickness. Hazard ratios for the associations of GBM thickness with CR and a composite of PR and CR were estimated using Cox proportional hazards models with follow-up time. The assumption of proportionality was tested using Schoenfeld residuals and interaction terms with time for each exposure variable and covariate. Multiple covariates were adjusted for age, duration of PMN, baseline eGFR, grade of C3 deposition, baseline urinary protein excretion and the titer of serum anti-PLA2R antibodies. A cubic spline curve was used to explore the association between GBM thickness and CR rate. The same multivariate model was applied to the cubic spline curve. A two-tailed p-Value $<$ 0.05 was considered statistically significant. All data were analyzed using IBM SPSS v.20.0 and R v.4.1.0 software.

Results

Clinical and laboratory characteristics of the enrolled patients

A total of 128 biopsy-proven PMN patients with nephrotic syndrome were recruited. The median follow-up period was 25.80 months (IQR 14.32–42.21). During the follow-up time, 122 (95.3%) patients achieved remission. The median time of achieving remission was 2.22 months. Among the patients with remission, 53 (43.4%) patients had PR, 69 (56.6%) patients achieved CR, and 46 (37.7%) patients experienced a relapse after achieving remission. In addition, one (0.8%) patient progressed to ESKD and two (1.6%) patients died. At baseline, 90 (70.3%) patients were men, and the median age was 52.7 ± 14.3 years. Hypertension and diabetes were present in 53.1% and 10.9% of patients, respectively. In the enrolled patients, the median GBM thickness was 1200 nm (IQR 1100–1400) with a median 55.78 RU/mL (IQR 15.68–168.77) anti-PLA2R Abs level. Urinary protein excretion was 7.25 g/day (IQR 5.15–10.10), and eGFR was 91.79 ± 20.16 mL/min/1.73 m^2 (Table 1).

Comparison of clinical and pathological features according to the tertiles of GBM thickness

The enrolled patients were then categorized into three groups according to the tertiles of GBM thickness: Group 1 (GBM thickness $\leq$ 1100 nm, n = 48), Group 2 (GBM thickness between 1100 and 1300 nm, n = 40), and Group 3 (GBM thickness $>$ 1300 nm, n = 40). Representative electron microscopy images of the three groups were presented in Figure 2. And the corresponding figures representing the process of GBM sampling were displayed in Supplementary Figure 1. As displayed in Table 2, patients with the highest tertile of GBM thickness had the most advanced Ehrenreich and Churg's stages (p = 0.003), and the most severe C3 and PLA2R antigen deposition (p = 0.048, p = 0.036, respectively), along with the lowest hemoglobin levels (p = 0.032). Moreover, patients with the lowest tertile of

Table 1. General clinical parameters of patients with PMN.

Parameter	$n = 128$
Follow-up time (months)	25.80(14.32, 42.21)
Duration of PMN (months)	2.00(0.62, 6.00)
Gender (male/female)	90/38
Age (years)	52.73 ± 14.30
Body mass index (kg/m2)	25.51 ± 3.72
SBP (mmHg)	136.95 ± 17.34
DBP (mmHg)	85.55 ± 12.08
Hypertension (%)	68(53.1)
RAAS inhibitor receivers (%)	103(80.5)
Diabetes (%)	14(10.9)
Level of anti-PLA2R Abs (RU/mL)	55.78(15.68, 168.77)
Positive rate of anti-PLA2R Abs (%)	92(71.9)
Urinary protein (g/d)	7.25(5.15, 10.10)
eGFR (ml/min/1.73 m^2)	91.79 ± 20.16
Serum albumin (g/L)	20.10(17.23, 24.15)
Scr (μmol/L)	76.10(62.80, 90.33)
Hemoglobin (g/L)	134.00(120.00, 146.00)
Pathological characteristics	
GBM thickness (nm)	1200.00(1100.00, 1400.00)
Churg's stages (I/II/III/IV)	18/83/26/1
Grade of IgG deposition (0/1/2/3)	0/2/22/104
Grade of C3 deposition (0/1/2/3)	17/69/20/22
Grade of PLA2R antigen deposition (0/1/2/3)	17/44/38/29
Global sclerosis (%)	5.64(0.00, 14.29)
Crescent (%)	0.00(0.00, 7.87)
Chronic tubulointerstitial injury (0/1/2/3)	46/35/41/6
Acute tubulointerstitial injury (0/1)	48/80
Remission and progression	
Complete remission (%)	69(53.9)
Partial remission (%)	53(41.4)
No remission (%)	6(4.7)
Relapse (%)	46/122(37.7)
ESKD (%)	1(0.8)
Death (%)	2(1.6)
Time to remission (months)	2.22(1.178, 4.87)
Time to CR (months)	10.65(6.90, 18.67)
Time to relapse (months)	13.40(8.78, 22.22)

PMN primary membranous nephropathy; GBM glomerular basement membrane; PLA2R phospholipase A2 receptor; SBP systolic blood pressure; DBP diastolic blood pressure; eGFR estimated glomerular filtration rate; Scr serum creatinine; IgG immunoglobulin G; C3 complement 3; RAAS renin-angiotensin-aldosterone system; ESKD end stage kidney disease. Data were presented as the mean ± standard, the median with interquartile range or counts and percentages.

GBM thickness had the lowest level of anti-PLA2R Abs and the lowest grade of C3 and PLA2R antigen deposition, along with the highest hemoglobin level (all $p < 0.05$). With respect to renal function (urinary protein, eGFR, serum creatinine, etc.) and remaining pathological features (such as global sclerosis, chronic tubulointerstitial injury, acute tubulointerstitial injury, and grade of IgG deposition), there were no differences among the three groups (all $p > 0.05$). During the follow-up, CR was the highest in the first tertile and the lowest in the third tertile, but the significance level was not achieved ($p = 0.055$). The incidence rates of relapse of proteinuria, ESKD, and death were comparable between the three groups (all $p > 0.05$, Table 2).

Correlations between GBM thickness and laboratory parameters

As shown in Table 3, GBM thickness positively correlated with the level of anti-PLA2R Abs ($rs = 0.240$, $p = 0.006$), Churg's stages ($rs = 0.222$, $p = 0.012$), grade of IgG deposition ($rs = 0.186$, $p = 0.035$), grade of C3 deposition ($rs = 0.190$, $p = 0.032$), and grade of PLA2R antigen deposition (rs $=0.271$, $p = 0.002$). However, the correlations of GBM thickness with age, duration of PMN, baseline eGFR, urinary protein excretion, and serum IgG were not significant (all $p > 0.05$).

Relationships of GBM thickness with remission rate

As shown in Figure 3, Kaplan–Meier analysis showed that the non-CR probability was significantly higher in the highest tertile of GBM thickness than in the middle and lowest tertiles ($p<0.001$). The risk for CR in PMN patients was determined by Cox proportional hazards model (Table 4). The univariate analyses identified that GBM thickness (HR per standard deviation (SD) 0.617, 95% CI [0.471–0.809], $p<0.001$) was significantly associated with CR. In the multivariate analysis, after adjusting for important clinicopathological factors including age, duration of PMN, baseline eGFR, grade of C3

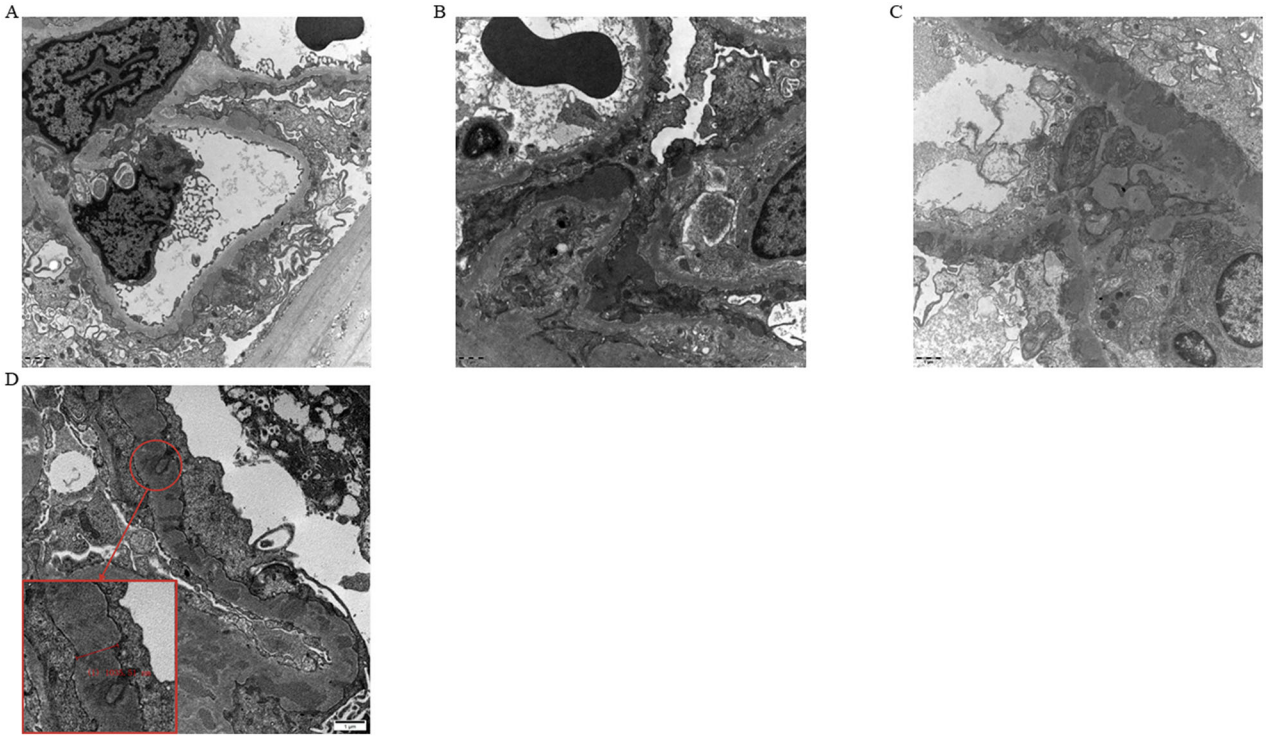

Figure 2. Representative examples according to the tertiles of GBM thickness in PMN patients. A. Electron microscopy of a patient in Group 1 (GBM thickness $\leq$ 1100 nm). B. Electron microscopy of a patient in Group 2 (GBM thickness 1100–1300 nm). C. Electron microscopy of a patient in Group 3 (GBM thickness $>$ 1300 nm) (original print magnifications $\times$8000. Scale bar = 1.0 μm). D. Representative image of measuring the GBM thickness (Scale bar = 1.0 μm).

deposition, baseline urinary protein excretion, and the titer of serum anti-PLA2R Abs, the GBM thickness was still independently associated with CR (HR per SD 0.580, 95% CI [0.436–0.772], $p<0.001$). Likewise, the multivariate-adjusted restricted cubic spline analyses indicated a significant reverse linear association between GBM thickness and CR rate (p for nonlinear = 0.1261, Figure 4). Additionally, age was also independently associated with CR (HR 1.028, 95% CI [1.005–1.051], $p = 0.018$, Table 4).

As shown in Table 5, the risk for a composite of PR and CR in PMN patients was also determined by a Cox proportional hazards model. The univariate analyses revealed that GBM thickness was not a significant risk factor for predicting the composite remission of PMN. In the multivariate analysis, the GBM thickness remained not significantly associated with the composite remission of PMN patients (HR per SD 0.895, 95% CI [0.730–1.098], $p = 0.288$).

Discussion

In the present study, we compared clinical and pathological features among PMN patients with different thicknesses of GBM, which is an essential diagnostic pathological feature of MN. In addition, the relationship between GBM thickness and CR rate in PMN patients was assessed. By stratifying the enrolled patients into three groups according to the tertiles of GBM thickness, we found that serum levels of anti-PLA2R Abs and grades of C3 and PLA2R antigen deposition were significantly elevated. Meanwhile, hemoglobin levels significantly decreased with the increasing tertile of GBM thickness. Most importantly, the multivariate Cox regression analysis suggested that GBM thickness was an independent risk factor of CR after adjusting for other risk factors. In addition, further multivariate-adjusted restricted cubic spline analyses showed a significant reverse linear association between GBM thickness and CR rate.

MN is one of the major glomerular diseases in adults, accounting for 20–30% of glomerulopathies [13,28]. Although it is associated with significant morbidity and mortality [29,30], the course of the renal function impairment in patients with PMN typically worsens over several years. For patients who do not reach a reduction of proteinuria to a subnephrotic range, the risk of progression to ESKD is about 25% over 8 years and about 50% over 10 to 15 years [31]. Due to the relatively slow rate of disease development, it is necessary to use a surrogate endpoint in clinical practice to complete clinical trials for drug licensing within a realistic time

Table 2. Clinical and pathological features of patients with PMN according to tertiles of GBM thickness.

	Tertiles of GBM thickness			
Parameter	T1(≤1100) ($n = 48$)	T2(1100–1300) ($n = 40$)	T3(>1300) ($n = 40$)	p-Value
Demographic characteristics				
Age (years)	53.75 ± 13.25	50.38 ± 15.16	53.88 ± 14.72	0.456
Gender (male/female)	35/13	28/12	27/13	0.857
Comorbid disease				
Hypertension (%)	24(50.0)	23(57.5)	21(52.5)	0.778
Diabetes (%)	5(10.4)	4(10.0)	5(12.5)	1.000
Cardiovascular diseases (%)	2(4.2)	5(12.5)	5(12.5)	0.256
Clinical characteristics				
Body mass index (kg/m^2)	25.51 ± 3.66	25.61 ± 3.64	25.41 ± 3.94	0.973
SBP (mmHg)	135.92 ± 19.23	135.18 ± 14.84	139.95 ± 17.29	0.412
DBP (mmHg)	84.58 ± 12.20	86.08 ± 11.87	86.18 ± 12.37	0.785
Duration of PMN (months)	2.00(0.62, 6.00)	2.00(0.50, 6.00)	2.00(1.00, 9.00)	0.533
Follow up time (months)	22.99(13.31, 35.15)	25.92(14.40, 39.25)	31.33(16.48, 48.65)	0.139
Laboratory characteristics				
Urinary protein (g/d)	7.49(4.85, 10.22)	7.46(5.30, 10.08)	6.40(5.19, 9.69)	0.790
eGFR (ml/min/1.73 m^2)	89.70 ± 17.87	95.17 ± 21.56	90.93 ± 21.35	0.428
BUN (mmol/L)	5.07(3.95, 6.95)	5.12(4.00, 5.98)	5.10(3.84, 6.68)	0.823
Scr (μmol/L)	75.35(62.18, 93.00)	76.70(63.50, 85.18)	77.70(60.35, 93.20)	0.929
Uric acid(mmol/L)	394.46 ± 112.22	381.63 ± 85.31	384.39 ± 76.59	0.794
Serum albumin (g/L)	21.24 ± 5.56	20.26 ± 4.07	20.89 ± 5.04	0.654
Level of anti-PLA2R Abs (RU/mL)	23.62(1.71, 81.18)	87.77(31.89, 209.65)	71.14(20.89, 249.19)	**0.001**
Triglyceride (mmol/L)	2.52(1.66, 3.66)	2.06(1.46, 2.84)	2.43(1.74, 3.53)	0.156
Total cholesterol (mmol/L)	8.16 ± 2.69	8.25 ± 2.59	8.06 ± 2.38	0.948
LDL-C (mmol/L)	4.80(3.79, 6.52)	5.26(3.99, 6.64)	5.06(4.18, 5.94)	0.654
HDL-C (mmol/L)	1.28(1.09, 1.81)	1.38(1.09, 1.75)	1.31(1.16, 1.49)	0.844
Hemoglobin(g/L)	138.94 ± 19.38	134.38 ± 18.43	128.13 ± 19.09	**0.032**
IgA(g/L)	1.99(1.62, 2.84)	2.09(1.76, 2.71)	1.99(1.34, 2.60)	0.555
IgG(g/L)	5.83(3.83, 7.71)	5.26(3.83, 6.52)	5.22(3.13, 6.56)	0.282
C3(g/L)	1.07(0.93, 1.24)	1.09(0.92, 1.23)	1.04(0.90, 1.18)	0.670
C4(g/L)	0.29(0.23, 0.34)	0.27(0.22, 0.34)	0.29(0.23, 0.34)	0.966
Pathological characteristics				
GBM thickness (nm)	1000.00(900.00, 1100.00)	1200.00(1200.00, 1300.00)	1500.00(1400.00, 1600.00)	**<0.001**
Churg's stages (I/II/III/IV)	8/32/7/1	8/28/4/0	2/23/15/0	**0.003**
Grade of IgG deposition (0/1/2/3)	0/1/12/35	0/1/6/33	0/0/4/36	0.120
Grade of C3 deposition (0/1/2/3)	10/26/6/6	5/23/5/7	2/20/9/9	**0.048**
Grade of PLA2R antigen deposition (0/1/2/3)	9/18/17/4	4/14/12/10	4/12/9/15	**0.036**
Global sclerosis (%)	6.07(0.00, 12.01)	4.19(0.00, 12.50)	6.19(0.00, 17.91)	0.563
Crescent (%)				
Chronic tubulointerstitial injury (0/1/2/3)	14/13/18/3	19/7/14/0	13/15/9/3	0.267
Acute tubulointerstitial injury (0/1)	14/34	16/24	18/22	0.291
Remission and progression				
Complete remission (%)	31(64.6)	23(57.5)	15(37.5)	0.055
Partial remission (%)	14(29.2)	16(40.0)	23(57.5)	
No remission (%)	3(6.3)	1(2.5)	2(5.0)	
Relapse (%)	15/45(33.3)	13/39(33.3)	18/38(47.4)	0.334
ESKD (%)	1(2.1)	0(0.0)	0(0.0)	1.000
Death (%)	0(0.0)	0(0.0)	2(5.0)	0.192

PMN: primary membranous nephropathy; GBM: glomerular basement membrane; ESRKD: end stage kidney disease; SBP: systolic blood pressure; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; BUN: blood urea nitrogen; Scr: serum creatinine; PLA2R: phospholipase A2 receptor; LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; IgA: immunoglobulin A; IgG: immunoglobulin G; C3: complement 3; C4: complement 4.

Data were presented as the mean ± standard, the median with interquartile range or counts and percentages. A two-tailed $p < 0.05$ was considered statistically significant.

frame. Also, it would be helpful to promote risk stratification and more appropriate risk-based treatments in clinical practice. CR has already been considered valuable and suitable for clinical use as a disease activity monitor and surrogate endpoint [32]. Previous studies have demonstrated that achieving CR of proteinuria indicates an excellent long-term prognosis in PMN patients [32–35]. The key finding of our study is that GBM thickness is independently associated with CR. The finding persists in the multivariable-adjusted restricted cubic spline analyses which suggest a significant reverse linear association between GBM thickness and CR. However, one previous study indicated that GBM thickness could be used as an additional method for risk stratification but not independently associated with renal failure or death in PMN patients [36]. Our results that GBM thickness did not correlate with baseline proteinuria may at least partially explain the observation suggested by Horvatic et al. On the other hand, 62.3% of the enrolled patients achieved remission without relapse in our study, which is

higher than the percentage of patients reported by Horvatic et al. [36] and should be taken into account when considering long-term impact.

Another important finding is that the patients with severe GBM thickening had higher titers of anti-PLA2R antibodies and severe PLA2R antigen deposition, which reinforces the evidence that autoimmune disorders play a crucial role in the pathogenesis of PMN [37,38]. PLA2R, a major target antigen located on podocytes, is taken up by antigen-presenting cells. Then, it is presented to helper T cells, which ultimately stimulate B cells to generate IgG anti-PLA2R antibodies [3,6,39]. Early evidence hints that IgG anti-PLA2R antibodies not only can combine with specific antigen and form immune complexes that are deposited in the subepithelial layer, but can also bind mannan-binding lectin to activate the lectin complement pathway and thus participate in both innate and adaptive immunity [3,39–42]. C5b-9, the final step of complement activation, increases the extracellular matrix by promoting the production of TGF-β by podocytes, which may also contribute to GBM thickening [43]. In addition, Dettmar et al. reported that intracellular autoantibodies, circulating anti-SOD2, and anti-aENO autoantibodies were independently associated with poor outcomes in terms of PR and CR of proteinuria, and eGFR. These antibodies have an added value over anti-PLA2R for refinement of association with clinical outcome [44]. Furthermore, except for cytoplasmic antigen binding its circulating autoantibodies as the first podocyte-targeted antigen–antibody system found in adult PMN, inflammation, PM2.5, and oxidative microenvironments may alter the microenvironment of PLA2R1-expressing cells [45,46]. Consequently, anti-PLA2R1 antibodies exogenous to glomeruli induce subepithelial immune complex deposition into the space between podocytes and GBM [45]. This also suggests that GBM thickening is likely caused by different pathogenic mechanisms. The formation and pathogenesis of GBM thickening involving various antigen deposition will be the subject of future studies by mass spectrometric analysis.

Many studies have focused on the prognostic significance of C3 deposition for PMN, but a large diversity has been found. A study by Oto et al. showed that C3 deposition was the predictor of ESKD in PMN patients

Table 3. Correlations between GBM thickness and laboratory parameters in PMN.

	GBM thickness (nm)	
	rs	*p*-Value
Age (years)	−0.008	0.929
Duration of PMN (months)	0.099	0.267
Baseline eGFR (ml/min/1.73 m^2)	0.028	0.752
urinary protein excretion (g/d)	−0.092	0.302
Level of anti-PLA2R Abs (RU/mL)	0.240	0.006
IgG(g/L)	−0.117	0.189
Churg's stages (I/II/III/IV)	0.222	0.012
Grade of IgG deposition (0/1/2/3)	0.186	0.035
Grade of C3 deposition (0/1/2/3)	0.190	0.032
Grade of PLA2R antigen deposition (0/1/2/3)	0.271	0.002

PMN: primary membranous nephropathy; GBM: glomerular basement membrane; PLA2R: phospholipase A2 receptor; IgG: immunoglobulin G; eGFR: estimated glomerular filtration rate; C3: complement 3. A two-tailed $p < 0.05$ was considered statistically significant.

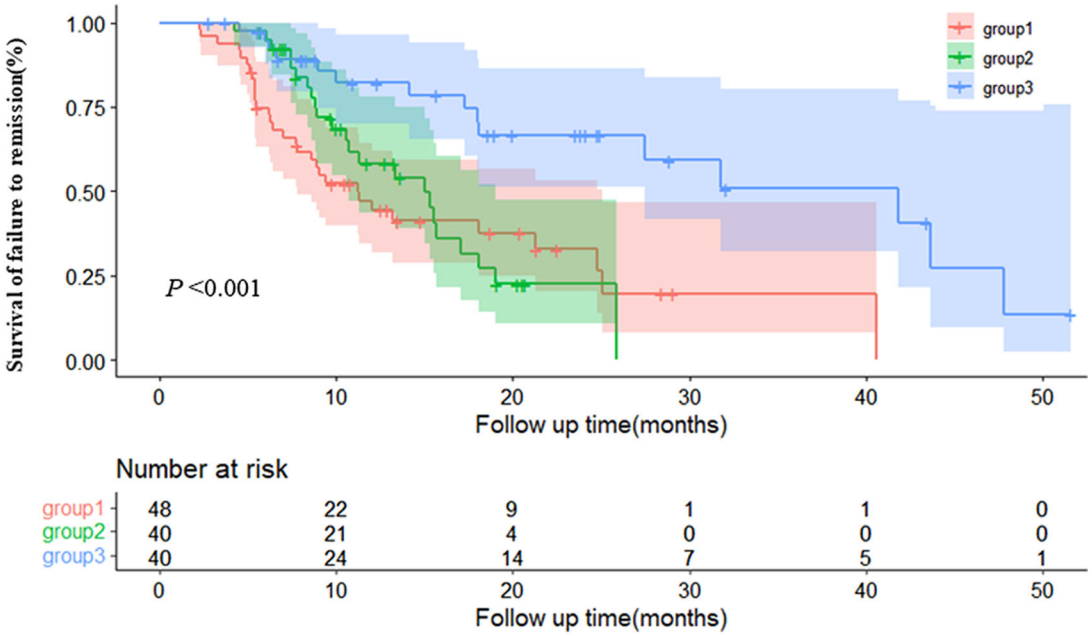

Figure 3. Kaplan–Meier curves of non-CR probability in PMN patients with different GBM thickness. Comparison of non-CR probability between patients in Group 1 (GBM thickness ≤ 1100 nm), Group 2 (GBM thickness 1100–1300 nm), and Group 3 (GBM thickness > 1300 nm); CR, complete remission.

Table 4. Risk factors of CR in PMN determined by univariate and multivariate Cox regression.

	Univariate		Multivariate	
Variables	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Age (years)	1.016(0.999, 1.034)	0.065	1.028(1.005, 1.051)	0.018
Duration of PMN (months)	0.997(0.976, 1.018)	0.745	0.994(0.974, 1.015)	0.596
Baseline eGFR (ml/min/1.73 m^2)	1.001(0.989, 1.013)	0.875	1.010(0.993, 1.027)	0.235
Baseline urinary protein excretion (g/d)	0.965(0.917, 1.015)	0.171	0.946(0.892, 1.002)	0.060
Level of anti-PLA2R Abs (RU/mL)	1.000(0.999, 1.001)	0.452	1.000(0.999, 1.000)	0.549
Grade of C3 deposition (0/1/2/3)	0.807(0.616, 1.057)	0.119	0.840(0.639, 1.103)	0.209
GBM thickness (per SD nm)	0.617(0.471, 0.809)	<0.001	0.580(0.436, 0.772)	<0.001

PMN: primary membranous nephropathy; GBM: glomerular basement membrane; eGFR: estimated glomerular filtration rate; PLA2R: phospholipase A2 receptor; CR: complete remission; C3 complement 3.

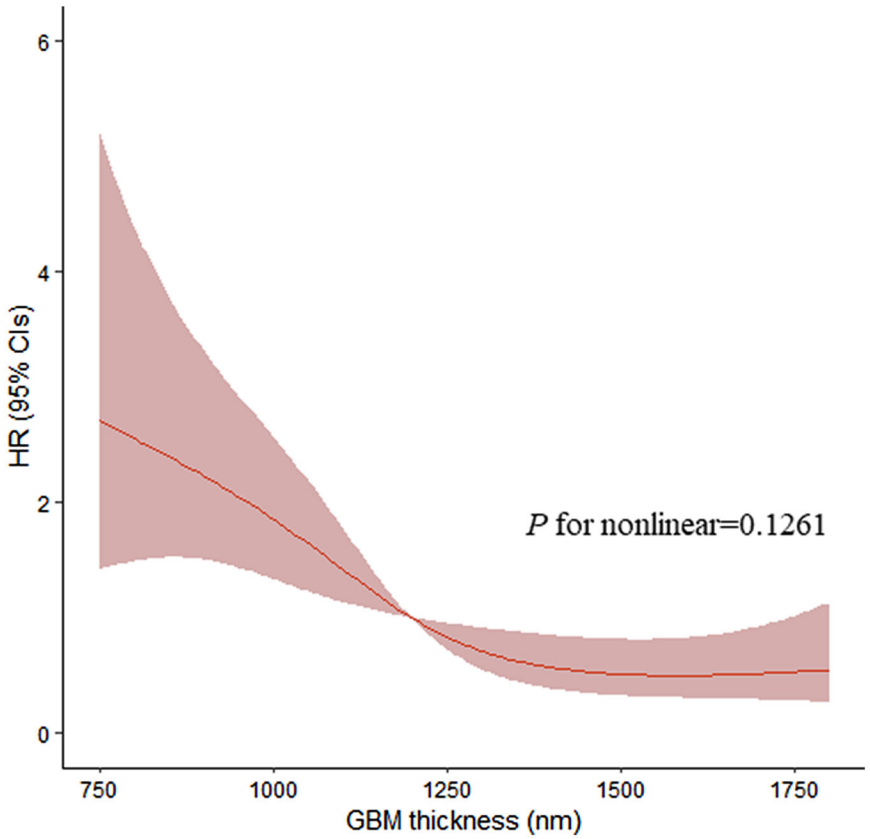

Figure 4. Association of GBM thickness with HR of complete remission rate by restricted cubic splines. Restricted cubic splines were plotted using three default knots. Hazard ratios were adjusted for age, duration of PMN, baseline eGFR, baseline urinary protein excretion, and level of anti-PLA2R Abs. p value for nonlinear association was 0.1261. HR, hazard ratio; PMN, primary membranous nephropathy; eGFR, estimated glomerular filtration rate; PLA2R, phospholipase A2 receptor.

Table 5. Risk factors of a composite of PR and CR in PMN determined by univariate and multivariate Cox regression.

	Univariate		Multivariate	
Variables	HR (95%CI)	*p*-Value	HR (95%CI)	*p*-Value
Age (years)	1.006(0.994, 1.019)	0.328	1.015 (0.999, 1.032)	0.068
Duration of PMN (months)	0.993(0.975, 1.012)	0.483	0.989(0.969, 1.011)	0.327
Baseline eGFR (ml/min/1.73 m^2)	1.004(0.996, 1.013)	0.330	1.009 (0.998, 1.019)	0.119
Baseline urinary protein excretion (g/d)	0.968(0.931, 1.007)	0.102	0.973(0.936, 1.012)	0.172
Level of anti-PLA2R Abs (RU/mL)	1.000(1.000, 1.000)	0.465	1.000 (1.000, 1.000)	0.521
Grade of C3 deposition (0/1/2/3)	1.040 (0.856, 1.264)	0.692	1.038 (0.851, 1.265)	0.714
GBM thickness (per SD nm)	0.934(0.769, 1.135)	0.490	0.895(0.730, 1.098)	0.288

PMN: primary membranous nephropathy; GBM: glomerular basement membrane; eGFR: estimated glomerular filtration rate; PLA2R: phospholipase A2 receptor; PR: partial remission; CR: complete remission; C3 complement 3.

[47]. However, the intensity of C3 deposition was not a risk factor for ESKD or kidney dysfunction in a retrospective study of 371 Chinese PMN patients and a prospective study of 60 Caucasian PMN patients [20,36]. The current study demonstrated that patients with the highest tertile of GBM thickness had the highest grade of C3 deposition, and a significant but weak association between them was observed in correlation analysis (rs =0.190, $p = 0.032$, Table 3). Nevertheless, C3 deposition was not a significant predictor of CR or a composite of CR and PR in either univariate or multivariate analysis. Troyanov et al. explained the possible reason for the discrepancy the amount of C3 deposition did not predict renal survival but did correlate with progression rate [24]. Moreover, animal studies of the typical pathological lesion of MN have indicated that the glomerular subepithelial deposition of C3 can only occur after immunization with a cationized antigen [48,49]. Consistent with this, injection of preimmune IgG or anti-THSD7A IgG in mice induced proteinuria within 3 days, but C3 deposits were not seen in the initial heterologous phase [50]. Combined with our results, it seems that the complement activation may not be essential for the initiation of GBM thickening, which may result from a complex multifactorial process. The underlying mechanism of GBM thickening beyond the complement activation needs to be further explored.

Moreover, our results indicated that GBM thickness was independently associated with CR; however, it was not significantly associated with the composite remission outcome (CR and PR). One of the possible explanations for the potential mechanism may rely on the differences in circulating serum PLA2R Abs and PLA2R antigen deposition. Luo et al. found that among those who received immunosuppressive treatment, patients with positive serum PLA2R Abs concurrent with negative PLA2R antigen deposition had a lower probability of remission than those with both positive circulating Abs and antigen deposits (57% vs 92.7% at 12 months after treatment) [51]. In the current study, patients in the second but not the third tertile of GBM thickness had the highest level of sero-PLA2R Abs. Meanwhile, 10% of patients in the third tertile had no PLA2R staining on kidney biopsy. And correlation analysis indicated a weak but significant association between GBM thickness and anti-PLAR Ab levels. These findings may indicate that different antigen-antibody constituents in the in-situ formation of nephritogenic immune complexes may play diverse roles in disease severity and treatment response. When GBM thickness is higher than 1300 nm, especially in patients with circulating PLA2R autoantibodies but without detected PLA2R antigen in glomerular deposits, there may be a more urgent need for the fine details of autoepitope expression, recognition, and autoantibody formation, and therefore more optimized individual treatments. However, the GBM thickening was likely caused by different pathogenic mechanisms on the basis of the result that GBM thickness was associated with MN stages, grade of IgG, C3, PLA2R deposition in the correlation analysis. It implied that GBM thickness may not only be the result of "Ab burden" but also of "long-term immune process," and both of them are bad and portend poorer outcomes in PMN. Based on this hypothesis, to achieve a high probability of composite remission outcome, a more sustained or combined initial immunosuppressive treatment, for example, calcineurin inhibitor (CNIs) with RTX (recommended for high-risk cases by 2021 KDIGO guidelines) [27], should receive attention in patients with thicker GBM. Further large, randomized controlled, prospective studies are still needed to ascertain the short- and long-term effects of GBM thickening on prognosis of PMN patients.

GBM thickening is a unique and classic pathological feature in PMN. On this basis, our results provide evidence for its wider clinical use to predict CR rate. However, this study has several limitations. First, it has all the limitations of observational, single-center studies. The follow-up period was short given that only three patients progressed to ESKD or death in this study. Further multicenter, long-term follow-up and controlled prospective studies on large groups of patients are needed. Second, the simultaneous measurement of other membrane-bound antigens such as THSD7A, or the new extracellular autoantigens, in particular, NELL-1, or intracellular autoantibodies such as anti-SOD2 and anti-aENO autoantibodies was missing. These may be potentially important confounders to the renal prognosis. Third, there were scarce previous data on glomerular IgG subtypes (especially IgG1and IgG4), filtrated slit length density per glomerulus and number of podocytes per glomerulus, and foot process width to analyze their associations with GBM thickness. Fourth, to minimize the effect of different therapeutic strategies on treatment response, patients who received treatment of CNI ± glucocorticoids were selected in the current study. Continuing research with a more extensive study population receiving different immunosuppressive agents is still warranted in the future.

Conclusions

GBM thickness is an independent risk factor of CR. PMN patients with an increased level of GBM thickening at diagnosis have a relatively unfavourable therapeutic response and a lower probability of achieving CR.

Acknowledgments

None.

Ethical approval

The research was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University (approval number: No.2018-SR-250).

Informed consent

Informed consent was obtained from all patients included in the study.

Consent for publication

All authors gave consent for publication.

Author contributions

SD designed and conducted the research and analyzed the data. LS contributed substantially to the writing and critical review of the manuscript. CZ, MZ, BS, YY, and HM reviewed the manuscript. CX coordinated and conceived the study as well as revised the manuscript. BZ was the guarantor of this work and had complete access to all the data in the study. All authors have read the final paper and approved the submission.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by grants from the National Natural Science Foundation of China (82100767), the Natural Science Foundation of Jiangsu Province (BK20191075), "PRO•Run" Fund of the Nephrology Group of CEBM, Project of clinical capability improvement of the First Affiliated Hospital of Nanjing Medical University (JSPH-MC-2021-14), Project of Bethune PuAi Medical Research Fund (PAYJ-058), and the Priority Academic Program Development (PAPD) of Jiangsu Higher Education Institution.

Data availability

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

References

[1] Ronco P, Debiec H. Pathophysiological advances in membranous nephropathy: time for a shift in patient's care. Lancet. 2015;385(9981):1983–1992.

[2] Debiec H, Ronco P. Immunopathogenesis of membranous nephropathy: an update. Seminars Immunopathology. 2014;36(4):381–397.

[3] Ronco P, Debiec H. Molecular pathogenesis of membranous nephropathy. Annual Rev Pathol. 2020;15:287–313.

[4] Huang L, Zhao YJ, Dong QR, et al. Immune-mediated membranous nephropathy: long term fluconazole usage caused podocyte autophagy. J Biochem Mol Toxicol. 2022;36(1):e22935.

[5] Nieto-Gañán I, Iturrieta-Zuazo I, Rita C, et al. Revisiting immunological and clinical aspects of membranous nephropathy. Clin Immunol. 2022;237:108976.

[6] Couser WG. Primary membranous nephropathy. Clin J Am Soc Nephrol. 2017;12(6):983–997.

[7] Beck LH, Jr., Bonegio RG, Lambeau G, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009;361(1):11–21.

[8] Tomas NM, Beck LH, Jr., Meyer-Schwesinger C, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. N Engl J Med. 2014;371(24):2277–2287.

[9] Cattran DC, Brenchley PE. Membranous nephropathy: integrating basic science into improved clinical management. Kidney Int. 2017;91(3):566–574.

[10] Reinhard L, Stahl RAK, Hoxha E. Is primary membranous nephropathy a complement mediated disease? Molecular Immunology. 2020;128:195–204.

[11] Miner JH. Renal basement membrane components. Kidney Int. 1999;56(6):2016–2024.

[12] Miner JH. The glomerular basement membrane. Exp Cell Res. 2012;318(9):973–978.

[13] Liu W, Gao C, Liu Z, et al. Idiopathic membranous nephropathy: glomerular pathological pattern caused by extrarenal immunity activity. Front Immunol. 2020;11:1846.

[14] Zhang N, Dai H, Dong X, et al. Level of interleukin-35 in patients with idiopathic membranous nephropathy and its predictive value for remission time. Front Immunol. 2022;13:926368.

[15] Netti GS, Infante B, Spadaccino F, et al. Serum levels of BAFF and APRIL predict clinical response in anti-PLA2R-positive primary membranous nephropathy. J Immunol Res. 2019;2019:8483650.

[16] Liu J, Zha Y, Zhang P, et al. The association between serum complement 4 and kidney disease progression in idiopathic membranous nephropathy: a multicenter retrospective cohort study. Front Immunol. 2022;13:896654.

[17] Chen X, Chen S, Li Z, et al. Correlation of body mass index with clinicopathologic parameters in patients with idiopathic membranous nephropathy. DMSO. 2022;ume 15:1897–1909.

[18] Dong L, Li YQ, Guo SM, et al. Hypercholesterolemia correlates with glomerular phospholipase A2 receptor deposit and serum anti-phospholipase A2 receptor antibody and predicts proteinuria outcome in idiopathic membranous nephropathy. Front Immunol. 2022;13:905930.

[19] An C, Akankwasa G, Liu J, et al. Urine markers of renal tubular injury in idiopathic membranous nephropathy:

a cross sectional study. Clin Chim Acta.. 2019;492:7–11.
[20] Zhang XD, Cui Z, Zhang MF, et al. Clinical implications of pathological features of primary membranous nephropathy. BMC Nephrol. 2018;19(1):215.
[21] Wei C, He Y, Li T, et al. Glomerulosclerosis predicts poor renal outcome in patients with idiopathic membranous nephropathy. Int Urol Nephrol. 2021;53(3):505–514.
[22] Ponticelli C, Zucchelli P, Imbasciati E, et al. Controlled trial of methylprednisolone and chlorambucil in idiopathic membranous nephropathy. N Engl J Med. 1984;310(15):946–950.
[23] Wakai S, Magil AB. Focal glomerulosclerosis in idiopathic membranous glomerulonephritis. Kidney Int. 1992;41(2):428–434.
[24] Troyanov S, Roasio L, Pandes M, et al. Renal pathology in idiopathic membranous nephropathy: a new perspective. Kidney Int. 2006;69(9):1641–1648.
[25] Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150(9):604–612.
[26] Churg J, Ehrenreich T. Membranous nephropathy. Persp Nephrol Hypertension. 1973;1 Pt1:443–448.
[27] Rovin BH, Adler SG, Barratt J, et al. Executive summary of the KDIGO 2021 guideline for the management of glomerular diseases. Kidney Int. 2021;100(4):753–779.
[28] Canetta PA, Troost JP, Mahoney S, et al. Health-related quality of life in glomerular disease. Kidney Int. 2019;95(5):1209–1224.
[29] Lee T, Chung Y, Poulton CJ, et al. Serum albumin at partial remission predicts outcomes in membranous nephropathy. Kidney Int Reports. 2020;5(5):706–717.
[30] Lee T, Derebail VK, Kshirsagar AV, et al. Patients with primary membranous nephropathy are at high risk of cardiovascular events. Kidney Int. 2016;89(5):1111–1118.
[31] Polanco N, Gutiérrez E, Covarsí A, et al. Spontaneous remission of nephrotic syndrome in idiopathic membranous nephropathy. J Am Soc Nephrol. 2010;21(4):697–704.
[32] Thompson A, Cattran DC, Blank M, et al. Complete and partial remission as surrogate end points in membranous nephropathy. J Am Soc Nephrol. 2015;26(12):2930–2937.
[33] Cattran D. Management of membranous nephropathy: when and what for treatment. J Am Soc Nephrol. 2005;16(5):1188–1194.
[34] Passerini P, Pasquali S, Cesana B, et al. Long-term outcome of patients with membranous nephropathy after complete remission of proteinuria. Nephrol Dial Transplant 1989;4(6):525–529.
[35] Laluck BJ, Jr., Cattran DC. Prognosis after a complete remission in adult patients with idiopathic membranous nephropathy. Am J Kidney Dis. 1999;33(6):1026–1032.
[36] Horvatic I, Ljubanovic DG, Bulimbasic S, et al. Prognostic significance of glomerular and tubulointerstitial morphometry in idiopathic membranous nephropathy. Pathol Res Pract. 2012;208(11):662–667.
[37] Hoxha E, Thiele I, Zahner G, et al. Phospholipase A2 receptor autoantibodies and clinical outcome in patients with primary membranous nephropathy. J Am Soc Nephrol. 2014;25(6):1357–1366.
[38] Hofstra JM, Beck LH, Jr., Beck DM, et al. Anti-phospholipase A_2 receptor antibodies correlate with clinical status in idiopathic membranous nephropathy. Clin J Am Soc Nephrol. 2011;6(6):1286–1291.
[39] Hoffman W, Lakkis FG, Chalasani G. B cells, antibodies, and more. Clin J Am Soc Nephrol. 2016;11(1):137–154.
[40] Motavalli R, Etemadi J, Kahroba H, et al. Immune system-mediated cellular and molecular mechanisms in idiopathic membranous nephropathy pathogenesis and possible therapeutic targets. Life Sci. 2019;238:116923.
[41] Hayashi N, Okada K, Matsui Y, et al. Glomerular mannose-binding lectin deposition in intrinsic antigen-related membranous nephropathy. Nephrol Dial Transplant. 2018;33(5):832–840.
[42] Ma H, Sandor DG, Beck LH. Jr. The role of complement in membranous nephropathy. Seminars in Nephrology. 2013;33(6):531–542.
[43] Nangaku M, Shankland SJ, Couser WG. Cellular response to injury in membranous nephropathy. J Am Soc Nephrol. 2005;16(5):1195–1204.
[44] Ghiggeri GM, Seitz-Polski B, Justino J, et al. Multi-autoantibody signature and clinical outcome in membranous nephropathy. Clin J Am Soc Nephrol. 2020;15(12):1762–1776.
[45] Liu W, Gao C, Dai H, et al. Immunological pathogenesis of membranous nephropathy: focus on PLA2R1 and its role. Front Immunol. 2019;10:1809.
[46] Xu X, Nie S, Ding H, et al. Environmental pollution and kidney diseases. Nat Rev Nephrol. 2018;14(5):313–324.
[47] Oto OA, Demir E, Mirioglu S, et al. Clinical significance of glomerular C3 deposition in primary membranous nephropathy. J Nephrol. 2021;34(2):581–587.
[48] Batsford S, Oite T, Takamiya H, et al. Anionic binding sites in the glomerular basement membrane: possible role in the pathogenesis of immune complex glomerulonephritis. Renal Physiology. 1980;3(1-6):336–340.
[49] Border WA, Kamil ES, Ward HJ, et al. Antigenic changes as a determinant of immune complex localization in the rat glomerulus. Lab Invest. 1981;45(5):442–449.
[50] Tomas NM, Meyer-Schwesinger C, von Spiegel H, et al. A heterologous model of thrombospondin type 1 domain-containing 7A-associated membranous nephropathy. J Am Soc Nephrol. 2017;28(11):3262–3277.
[51] Luo J, Yuan Y, Tian J, et al. Clinicopathological characteristics and outcomes of PLA(2)R-Associated membranous nephropathy in seropositive patients without PLA(2)R staining on kidney biopsy. Am J Kidney Dis. 2022;80(3):364–372.