Research Article

Effect of Glomerular Mannose-Binding Lectin Deposition on the Prognosis of Idiopathic Membranous Nephropathy

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Keywords

Mannose-binding lectin · Anti-phospholipase A2 receptor · Idiopathic membranous nephropathy · Complement · Prognosis

Abstract

Objective: Co-deposition of mannose-binding lectin (MBL) and IgG4 anti-phospholipase A2 receptor (anti-PLA2R) autoantibodies under subepithelial cells has been observed in patients with idiopathic membranous nephropathy (iMN), but the relationships of MBL deposition to iMN severity and progression remain unclear. **Methods:** Patients diagnosed with iMN who underwent renal puncture were enrolled and followed up for a median of 17 months (interquartile range [IQR], 9–25 months). Serum anti-PLA2R and anti-thrombospondin type-1 domain-containing 7A antibodies and MBL were detected by ELISA. Glomerular MBL and anti-PLA2R antibodies were detected by immunofluorescence. Proteinuria remission, including complete remission (CR), was defined as a clinical event. Clinicopathological characteristics and kidney outcomes were compared between patients with and without MBL deposition. **Results:** In 67 prevalent patients with biopsy-proven iMN, serum anti-PLA2R antibodies and anti-THSD7A antibodies were present in 37 (55.3%) and 1 (1.4%) patient with iMN. The positivity of glomerular MBL deposition and tissue anti-PLA2R antibody was 53 (79.1%) and 49 (73.1%), respectively. No significant difference was found between the MBL-positive and negative groups in the albumin level (26.5 ± 6.6 and 28.6 ± 6.1 g/L), eGFR (104.8 ± 17.4 and 114.6

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± 16.1 mL/min/1.73 m²), 24-h proteinuria (5.35 and 4.25 g/day), or serum MBL level corrected by serum Cr 4.92 (IQR, 0.86, 8.90) and 2.28 (IQR, 0.4, 5.62). In a Cox proportional hazards regression model adjusted for sex, age, systolic blood pressure, eGFR, immunosuppressive agent use, 24-h proteinuria, and anti-PLA2R antibody concentration, glomerular MBL deposition was independently associated with ICR of proteinuria (HR, 6.31; 95% CI, 1.1–36.1; $p = 0.039$). **Conclusions:** The MBL pathway of complement activation is commonly initiated in patients with iMN, and patients with MBL deposition reach ICR faster than patients without MBL deposition.

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Introduction

Membranous nephropathy (MN) is the most common nephrotic syndrome in adults. Its typical histological manifestation consists of immunocomplex deposition in the glomerular subepithelium with basement membrane thickening. In 20–25% of patients, MN develops secondary to immune system disease, viral infection, tumor development, drug use, or heavy metal poisoning [1–4]. The remaining 75–80% of cases have no known cause, and this subtype is known as idiopathic membranous nephropathy (iMN) or primary MN. Research on the pathogenesis of iMN has gone through 3 stages. The earliest study, of Heymann nephritis, laid the foundation for our understanding of the pathogenesis of MN in the context of kidney disease [5]. In the 1980s, megalin was confirmed to be the target antigen in the Heymann model in rats [6, 7]. Human podocytes, however, do not express this antigen [8]. In 2002, the anti-neutral endopeptidase antibody was reported to induce MN in infants, confirming that autoimmune antibodies can form in situ immune complexes on podocytes, causing podocyte damage and consequent disease; this discovery spurred research on human podocyte antigens [9]. The phospholipase A2 receptor (PLA2R) and thrombospondin type-1 domain-containing 7A (THSD7A) were found on glomerular podocytes [10, 11]. The relevant antibodies can be detected in the circulation of patients with iMN, and they form in situ immune complexes in the glomerular subepithelium, damaging podocytes via membrane attack complexes to induce proteinuria [12]. Given its high sensitivity and specificity, the presence of PLA2R in the glomerulus has guiding significance in the diagnosis and evaluation of iMN [13–15].

The complement system plays important roles in the regulation of immunity and combating of pathogenic microorganisms in humans. It is activated primarily through 3 pathways: the classic pathway (CP), alternative pathway (AP), and mannose-binding lectin (MBL) pathway (LP). Glomerular deposition of C3, C4, and their decomposition products can be seen in most patients with iMN [16]. C5b–9 are also deposited in almost all of these patients, which further elucidates involvement of components in the pathogenesis of MN. C4d is activated via the LP or CP. Val-Bernal et al. [16] and Espinosa-Hernandez et al. [17] found C4d deposition in the glomerular basement membrane in all examined patients with iMN. The absence of C1q in the glomeruli of most such patients suggests that the CP plays a nondominant role in the pathogenesis of iMN. Most antibodies against PLA2R and THSD7A are IgG4 subtypes [11, 18], and IgG4 does not activate the CP due to its low affinity to C1q and Fcγ receptors [19–21]. Yang et al. [22] reported that serum levels of MBL and MBL-associated serine proteases 1 and 2 were significantly increased in patients with PLA2R antibody positivity.

Based on this evidence, we hypothesized that the MBL-mediated LP is involved in the pathogenesis of iMN through activation via the anti-PLA2R IgG4 subtype. Additionally, as the N-glycation modification site in the constant region Cγ2 of the heavy chain of the IgG4 molecule

is of great significance for the properties of the Fc segment of this molecule [23], we hypothesized that IgG4 activates the LP via abnormal glycation modification. Hence, in this study, we analyzed the glomerular deposition of MBL in patients with iMN and evaluated its correlation with the renal prognosis and treatment response.

Materials and Methods

Patients and Samples

Patients who underwent percutaneous renal biopsy and were diagnosed with iMN were enrolled in this study and followed up for a median of 17 months (range 6–60 months) in the period 2013–2018 via our registration system. Clinical and laboratory examinations were performed to rule out secondary factors, such as the presence of a tumor, immune deficiency, drug use, and viral infection. The exclusion criteria were age <18 or >75 years, presence of a complicated tumor, and use of immunosuppressive therapy prior to admission. Baseline patient data, including blood pressure, routine blood parameters, renal and liver function, blood lipids, and routine urine and urinary protein levels, were obtained from the registration system. Blood and urine samples were collected from patients on the day of admission, centrifuged, and stored at -80°C .

Pathological Examination

Pathological specimens from percutaneous renal biopsies were examined by light microscopy, electron microscopy, and immunofluorescence. Microscopic assessment involved hematoxylin and eosin, periodic acid–Schiff, and periodic acid–silver methenamine staining. Immunofluorescence analysis included the assessment of IgG, IgA, IgM, C1q, and C3 expression (all antibodies were obtained from DAKO).

Detection of Serum Anti-PLA2R and Anti-THSD7A Antibodies

Serum anti-PLA2R antibodies were detected with commercial ELISA kits (EUROIMMUN AG, Germany). Patients' sera were diluted in PBST at a ratio of 1:100, then added to the wells of a reaction plate, and incubated at room temperature for 30 min. The samples were then washed, and the enzyme-labeled secondary antibody was added at room temperature for 30 min. Net optical absorbance at 450 nm was recorded (Bio-Rad 550, Tokyo, Japan), and antibody positivity was defined as >20 U/mL.

Serum THSD7A antibodies were detected with commercial cell-coated immunofluorescence kits (EUROIMMUN AG). Patients' sera were diluted in PBST at a ratio of 1:10 and then added to the reaction field of a reagent tray. A BIOCHIP was covered at room temperature for 30 min, after which the slides were removed and washed. Then, 25 μL of fluorescein-labeled antihuman globulin was added at room temperature and incubated on the slides for 30 min. The slides were washed again and then mounted in medium on glass slides, and the fluorescence intensity of the encapsulated cells was observed by fluorescence microscopy (LEICA).

Glomerular MBL and Anti-PLA2R Antibody Staining

Fresh renal tissue specimens embedded in optimal cutting temperature compound and frozen in a liquid nitrogen tank were sliced on a cryostat at 4 μm thickness. The sections were blocked with 5% bovine serum albumin diluted with 0.01 mol/L PBS for 30 min. Mouse monoclonal anti-MBL antibody (1:300; Abcam, Cambridge, UK) and rabbit anti-PLA2R antibody (1:500; Sigma, St. Louis, MO, USA) were incubated with the slides overnight at 4°C . After washing, fluorescein-labeled secondary goat anti-mouse and goat anti-rabbit antibodies were added. Fluorescence intensity was observed by fluorescence microscopy (LEICA) and

was graded from 0 to 3 (0, negative; 1, weak; 2, moderate; 3, strong). Two observers separately performed the assessment and were blinded to the background data.

Detection of Serum MBL Concentrations

Serum MBL concentrations were detected with a commercial ELISA kit (Elabscience, China). Patients' sera and samples of standard diluent were diluted at a ratio of 1:400. Then, 100 μ L of diluent was added to an enzyme-labeled plate and incubated at 37°C for 90 min. Biotinylated antibody was added directly to the wells, followed by incubation at 37°C for 60 min and washing 3 times with concentrated detergent. Next, enzyme-binding reaction solution was added, followed by incubation at 37°C for 30 min and 5 washes. Substrate color solution was added and incubated at 37°C for 15 min, and reaction termination solution was then added to terminate the chromogenic reaction. Absorbance at 450 nm was recorded. The sample concentration was calculated according to the standard curve.

Treatment and Kidney Outcomes

All patients were treated and followed up according to the 2012 KDIGO guidelines [24]. Clinical endpoint definitions included proteinuria remission and renal function deterioration. Proteinuria status was reported as follows: no response to treatment, no improvement in the urinary protein level at 24 h; incomplete remission (ICR) 1, urinary protein 0.3–1.0 g/day; and ICR2, urinary protein 1.0–3.5 g/day or decrease to less than half of baseline [25]. Complete remission (CR) was defined as urinary protein <0.3 g/day. Renal function was assessed according to the eGFR, and renal dysfunction was defined as a 30% decline in the eGFR from baseline.

Statistical Analysis

The normality of the distributions of continuous variables was tested. Normally distributed data were expressed as means $\pm$ SD and compared between the groups using Student's *t* test. Non-normally distributed data were expressed in quartiles and compared between the groups using the Mann-Whitney U test. Categorical variables were summarized as frequencies and percentages and compared using Fisher's exact test and the χ^2 test. A Cox regression model was used to evaluate risk factors for clinical outcomes. Univariate Cox regression was used to analyze the correlation between MBL deposition and renal dysfunction (defined as a clinical endpoint). Multivariate Cox regression was used to analyze the correlation between MBL deposition and urinary protein remission. The Kaplan-Meier method was used to analyze the cumulative remission rate. The results are expressed as HRs with 95% CIs, and *p* < 0.05 was considered to indicate significance. Analyses were performed with the SPSS statistical software package (ver. 24.0).

Results

Patients' Clinical and Pathological Features

Sixty-seven patients (62.7% male, mean age 43 $\pm$ 14.4 years) with definite pathological diagnoses of iMN were included in this study. The median follow-up duration was 17 months (range 6–60 months). Twenty (29.9%) patients had hypertension, 10 (14.9%) had diabetes, and 2 (3.0%) had thrombosis. The mean serum albumin concentration was 26.9 $\pm$ 6.5 g/L, the median urinary protein level was 5.1 g/day (interquartile range [IQR], 2.3–7.3 g/day), the mean eGFR was 106.9 $\pm$ 17.5 mL/min/1.73 m², the median serum anti-PLA2R concentration was 39.2 IU/mL (IQR, 2.0–94.7 IU/mL), and the serum anti-PLA2R antibody positivity rate was 55.3% (Table 1). One (1.4%) patient was positive for both serum anti-THSD7A and anti-

Table 1. Baseline clinical data for patients with iMN

	Total	MBL positive	MBL negative	p value
Number	67	53 (79.1)	14 (20.9)	
Age, years	43±14.4	43.7±14.4	41.9±14.7	0.69
Sex, male	42 (62.7%)	34 (64.2%)	8 (57.1%)	0.63
Albumin, g/L	26.9±6.5	26.5±6.6	28.6±6.1	0.28
Serum CHO, mmol/L	8.2±2.6	8.1±2.2	8.3±3.6	0.83
LDL, mmol/L	5.0±1.9	4.9±1.8	5.5±2.7	0.45
Serum IgG, g/L	6.4±2.9	6.3±3.0	6.8±2.6	0.64
Serum IgA, g/L	2.2±0.8	2.2±0.7	2.3±1.12	0.62
Serum IgM, g/L, median (IQR)	0.9 (0.6–1.2)	0.88 (0.6–1.1)	1.05 (0.6–1.7)	0.64
Proteinuria, g/24 h, median (IQR)	5.1 (2.3–7.3)	5.35 (3.0–7.4)	4.25 (1.9–6.7)	0.26
eGFR, mL/min/1.73 m ²	106.9±17.5	104.8±17.4	114.6±16.1	0.06
<i>Renal function</i>				
Normal	13	8	5	0.315
CKD stage 1, 2, 3, 4, 5	43/10/1/0/0	35/9/1/0/0	8/1/0/0/0	
Serum MBL/Cr, median (IQR)	4.40 (0.70, 8.20)	4.92 (0.86, 8.90)	2.28 (0.4, 5.62)	0.29
Serum anti-PLA2R antibody, IU/mL, median (IQR)	39.2 (2.0–94.7)	39.2 (2.0–79.5)	40.5 (2.0–140.1)	0.77
Serum anti-PLA2R antibody positive	37 (55.3%)	32 (60.4%)	5 (35.7%)	0.10
Tissue anti-PLA2R positive	49 (73.1%)	45 (84.9%)	4 (28.6%)	<0.001*
Double positive ^a	1 (1.4%)	0	1 (7.1%)	0.05
<i>IgG IF intensity score</i>				
1+	8 (11.9%)	8 (15.1%)	0	0.26
2+	30 (45.8%)	22 (41.5%)	8 (57.1%)	
3+	29 (43.3%)	23 (43.4%)	6 (42.9%)	
<i>C3 IF intensity score</i>				
Negative	13 (19.4%)	10 (18.9%)	3 (21.4%)	0.56
1+	17 (25.4%)	15 (28.3%)	2 (14.3%)	
2+	37 (55.2%)	28 (52.8%)	9 (64.3%)	
<i>IF staining</i>				
IgA	7 (10.4%)	5 (9.4%)	2 (14.3%)	0.24
IgM	11 (16.4%)	9 (17%)	2 (14.3%)	0.21
C1q	15 (22.4%)	12 (22.6%)	3 (21.4%)	0.89
<i>Immunosuppressive</i>				
Agents	48 (71.6%)	40 (75.5%)	8 (57.1%)	0.18
<i>Renal function, n</i>				
Normal	4	3	1	0.835
CKD stage 1, 2, 3, 4, 5	42/18/3/0/0	33/14/3/0/0	9/4/0/0/0/)	

Data are presented as n (%) or mean±SD, unless indicated otherwise. MBL, mannose-binding lectin; CHO, cholesterol; IF, immunofluorescence; IQR, interquartile range; PLA2R, phospholipase A2 receptor; iMN, idiopathic membranous nephropathy. **p* < 0.05. ^a Serum anti-PLA2R and anti-THSD7A antibody positivity.

PLA2R antibodies. Renal histology yielded a 100% IgG positivity rate; the numbers of patients with C3, IgA, IgM, and C1q positivity were 54 (80.6%), 7 (10.4%), 11 (16.4%), and 15 (22.4%), respectively. Forty-eight (71.6%) patients were treated with immunosuppressive agents.

Glomerular MBL Deposition

Among the 67 patients with iMN, 53 (79.1%) showed MBL deposition (Table 1); scores were 1 for 11 patients, 2 for 22 patients, and 3 for 20 patients. Figure 1 shows the co-localization of MBL with anti-PLA2R antibodies. In the MBL-positive group, 45 (84.9%) patients were positive for glomerular anti-PLA2R antibody and 21 (39.6%) patients were positive for serum anti-PLA2R antibody. In the MBL-negative group, 4 (28.6%) patients were positive for

Fig. 1. Immunofluorescence of anti-PLA2R antibody (**A**) and MBL (**B**) in renal tissues from patients with iMN. **C** Co-localization of MBL and PLA2R in immune deposits. **A1, B1, C1** Double positivity. **A2, B2, C2** Double negativity. **A3, B3, C3** Anti-PLA2R antibody negativity and MBL positivity. **A4, B4, C4** Anti-PLA2R antibody positivity and MBL negativity. Magnification, $\times 400$. MBL, mannose-binding lectin; PLA2R, phospholipase A2 receptor; iMN, idiopathic membranous nephropathy.

glomerular anti-PLA2R antibody and 9 (64.3%) patients were positive for serum anti-PLA2R antibody. The median serum MBL concentrations corrected by serum Cr in the MBL-positive and negative groups were 4.92 (IQR, 0.86–8.90) and 2.28 (IQR, 0.4–5.62), respectively. No significant difference was detected between these groups in the albumin level (26.5 ± 6.6 and

Table 2. Clinicopathological characteristics of patients with iMN according to serum anti-PLA2R status

	Serum anti-PLA2R positive	Serum anti-PLA2R negative	<i>p</i> value
<i>n</i> (%)	37 (55.3)	30 (44.7)	
Age, years	42.04±14.36	43.22±14.27	0.76
Sex, male	27 (73%)	15 (50%)	0.053
Total protein, g/L	44.75±7.4	49.97±9.76	0.046*
Serum albumin, g/L	25.08±5.45	29.04±7.13	0.033*
Proteinuria, g/24 h, median (IQR)	5.60 (3.13–7.82)	5.09 (2.97–6.8)	0.000*
Serum MBL, ng/mL, median (IQR)	311.28 (66.4–670.5)	206.47 (28.12–434.66)	0.408
Serum MBL/Cr, median (IQR)	4.99 (1.11–10.36)	3.88 (0.40–7.11)	0.673
eGFR, mL/min/1.73 m ²	107.69±18.5	108.30±17.4	0.906
Serum IgG, g/L	5.01±2.61	7.39±3.15	0.024
Serum IgM, g/L	0.88 (0.60–1.095)	0.93 (0.61–1.185)	0.019*
Serum IgA, g/L	2.55±0.75	1.88±0.67	0.012*
Double positive ^a (<i>n</i> , %) IF staining (score)	1	0	0.050
MBLIF (score)	2 (1, 3)	2 (1, 3)	0.62
Anti-PLA2R antibody (score)	2 (1, 3)	2 (1, 3)	0.73

Data are presented as *n* (%) or mean±SD unless indicated otherwise. PLA2R, phospholipase A2 receptor; MBL, mannose-binding lectin; IF, immunofluorescence; IQR, interquartile range; iMN, idiopathic membranous nephropathy. * *p* < 0.05. ^a Serum anti-PLA2R and anti-THSD7A antibody positivity.

28.6 ± 6.1 g/L), eGFR (104.8 ± 17.4 and 114.6 ± 16.1 mL/min/1.73 m²), urinary protein level (5.35 and 4.25 g/day), or any other clinical baseline data (Table 1).

Serum Anti-PLA2R Antibody Positivity

Significant differences were observed between the serum anti-PLA2R antibody-positive and negative groups in the serum albumin level (*p* = 0.033), 24-h proteinuria quantification (*p* < 0.001), and serum IgG (*p* = 0.024), IgA (*p* = 0.012), and IgM (*p* = 0.019) levels (Table 2). The urinary protein level and IgA level were lower in the anti-PLA2R antibody-negative group, while levels of the other clinical parameters were lower in the anti-PLA2R antibody-positive group (Table 2). The median serum MBL concentration corrected by serum Cr did not differ between these groups (4.99; IQR 1.11, 10.36 and 3.88; IQR 0.40, 7.11), respectively. MBL positivity rates were 82.5% in the anti-PLA2R and THSD7A antibody-positive group and 70.9% in the anti-PLA2R antibody-negative group. One patient had serum anti-THSD7A antibody positivity and no glomerular MBL deposition.

Treatment Responses and Kidney Outcomes of Patients with iMN and MBL Deposits

All 67 patients received angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. Thirty-seven (53.2%) patients were given calcineurin inhibitors and 11 (16.4%) patients were given cyclophosphamide.

In the univariate Cox regression analysis, MBL deposition was a protective factor for ICR1 (HR, 2.78; 95% CI, 1.07–7.19; *p* = 0.035) but not for ICR2 or CR. In the multivariate Cox regression model adjusted for sex, age, serum anti-PLA2R antibody concentration, baseline eGFR, treatment regimen, and systolic blood pressure, MBL deposition was a protective factor for ICR1 (HR, 6.31; 95% CI, 1.10–36.14; *p* = 0.039; Table 3). And a significant difference was observed between the groups. Table 4 shows a Cox regression analysis for complete proteinuria remission (<0.3 g/day) in patients with iMN. After adjusting for sex, age, serum anti-PLA2R antibody concentration, baseline eGFR, treatment regimen, and systolic blood

Table 3. Univariate and multivariate Cox regression analysis for partial proteinuria remission (0.3–1.0 g/day) in patients with iMN

Factor	Univariate model		Multivariate model	
	HR (95% CI)	<i>p</i> value	HR (95% CI)	<i>p</i> value
Sex	0.81 (0.42, 1.57)	0.53	0.697 (0.26, 1.91)	0.48
Age	1.00 (0.99, 1.02)	0.82	1.01 (0.96, 1.07)	0.66
Serum anti-PLA2R antibody	0.99 (0.99, 1.00)	0.44	0.99 (0.98, 1.00)	0.13
eGFR, mL/min/1.73 m ²	0.99 (0.97, 1.01)	0.30	0.99 (0.95, 1.05)	0.89
Systolic blood pressure	0.99 (0.98, 1.01)	0.47	0.99 (0.96, 1.02)	0.47
Immunosuppressive agent use	1.48 (0.73, 3.03)	0.28	3.10 (0.76, 12.73)	0.12
MBL deposition	2.78 (1.08, 7.19)	0.035*	6.31 (1.10, 36.14)	0.039*

PLA2R, phospholipase A2 receptor; iMN, idiopathic membranous nephropathy; MBL, mannose-binding lectin. * *p* < 0.05.

Table 4. Univariate and multivariate Cox regression analysis for complete proteinuria remission (<0.3 g/day) in patients with iMN

Factor	Univariate model		Multivariate model	
	HR (95% CI)	<i>p</i> value	HR (95% CI)	<i>p</i> value
Sex	0.79 (0.36, 1.73)	0.56	0.39 (0.10, 1.51)	0.176
Age	1.00 (0.98, 1.03)	0.74	1.08 (0.99, 1.19)	0.07
Serum anti-PLA2R antibody	0.99 (0.99, 1.01)	0.73	0.99 (0.98, 1.01)	0.56
eGFR, mL/min/1.73 m ²	0.99 (0.97, 1.01)	0.51	1.06 (0.99, 1.14)	0.09
Systolic blood pressure	1.01 (0.98, 1.06)	0.56	1.02 (0.98, 1.06)	0.39
Immunosuppressive agent use	1.80 (0.77, 4.6)	0.17	4.05 (0.76, 21.58)	0.10
MBL deposition	1.08 (0.57, 4.68)	0.358	8.00 (0.65, 98.23)	0.10

MBL, mannose-binding lectin; PLA2R, phospholipase A2 receptor; iMN, idiopathic membranous nephropathy.

pressure variables in the multivariate Cox regression, there was no statistical difference between the 2 groups (HR, 8.00; 95% CI, 0.65–98.23; *p* = 0.10).

Kaplan-Meier analysis of the proteinuria remission rates in the 2 groups yielded similar results (Fig. 2). Thirty-eight MBL-positive and 6 MBL-negative patients achieved ICR; 43 and 11 patients in these respective groups achieved ICR2, and 28 and 4 respective patients achieved CR.

Univariate Cox regression was used to analyze the correlation between tissue MBL deposition and renal dysfunction in patients. No significant difference in renal function deterioration was observed between the groups (Table 5).

Discussion

In this study, glomerular MBL deposition was detected in 79.1% of patients with iMN. Our findings suggest that this factor may be a favorable predictor of proteinuria remission.

MBL has been identified in the glomeruli of various glomerular diseases. In Japanese and Chinese studies of IgA nephropathy, glomerular MBL deposition rates were 25 and 34%,

Fig. 2. Proteinuria remission in patients with iMN with and without MBL deposition. Patients with MBL deposition tended to achieve ICR1 (urinary protein level 0.3–1.0 g/day) more rapidly (**a**), with no significant difference found for ICR2 (urinary protein level 1.0–3.5 g/day) (**b**) or complete response (urinary protein level <0.3 g/day) (**c**). MBL, mannose-binding lectin; iMN, idiopathic membranous nephropathy; ICR, incomplete remission.

respectively [26, 27]. In patients with lupus nephritis, the glomerular MBL deposition rate was 82% [28]. MBL deposition in the glomeruli of iMN patients has also been reported [29]. MBL was detected in the glomeruli of 66.7% patients with iMN in the study of Lhotta [29], albeit the association between MBL deposits and prognosis of iMN was not performed. Shiiki et al. [30] carried out a national survey of the renal survival rate of iMN patients with nephrotic syndrome and found that a remission from heavy proteinuria likely resulted in a favorable

Table 5. Univariate Cox regression results for renal dysfunction risk factors in patients with iMN

Factor	HR (95% CI)	p value
Sex	1.04 (0.38, 2.865)	0.94
Age	1.01 (0.97, 1.03)	0.95
Anti-PLA2R antibody	0.99 (0.98, 1.01)	0.81
Serum albumin	1.04 (0.96, 1.13)	0.35
Immunosuppressive agent use	1.18 (0.41, 3.37)	0.76
Systolic blood pressure	0.99 (0.96, 1.02)	0.36
MBLdeposition	0.48 (0.16, 1.40)	0.18

MBL, mannose-binding lectin; PLA2R, phospholipase A2 receptor; iMN, idiopathic membranous nephropathy.

renal outcome, but the glomerular MBL deposition was not analyzed. Hayashi et al. [31] found that 43.4% of patients with iMN were MBL positive and suggested that complement activation via the LP is an unfavorable predictor for proteinuria remission. This result is incompatible with our findings. Our results suggest that iMN patients with glomerular MBL activation can easily reach proteinuria remission, especially to the target of proteinuria less than 1.0 g/day but not less than 0.3 g/day. A reasonable explanation may be attributed to the not enough endpoints of CR, which needed a longer follow-up time to reach.

Complement activation is generally regarded as a double-edged sword; it causes not only pathogen opsonization but also tissue damage [32]. Complements also bind to apoptotic cells and initiate their absorption and elimination [33]. Thus, local MBL deficiency may lead to reduced autoantigen clearance, thereby promoting the development of autoimmunity, leading to a poor therapeutic response and a low clinical remission rate. In 2001, Saevarsdottir et al. [34] reported that patients with rheumatoid arthritis and low MBL levels had more serious joint damage and worse response to treatment. In 2011, Brazilian researchers found that low to moderate MBL deficiency was associated with a high incidence of lupus nephritis [35]. In recent years, Guo et al. [36] confirmed that the symptoms of hematuria and infection were significantly worse in patients with low MBL levels than in those without MBL deficiency in a sample of 749 patients with IgA nephropathy; in an adjusted analysis, MBL deficiency was an independent risk factor for renal deterioration in these patients. In 2019, Ouyang et al. [37] found that the rs1800450-AA genotype was associated with markedly decreased serum MBL levels, renal MBL negativity, and severe tubulointerstitial damage (OR = 3.38), indicating that LP inactivation independently and adversely affected the renal outcome of IgA nephropathy. In this study, we found that glomerular MBL activation may play a protective role in terms of proteinuria remission. We speculate the glomerular activated MBL may mediate the clearance of local anti-PLA2R antibodies or absorption of damaged podocytes, which needs further in vitro studies to validate.

Based on the high frequency of co-localization of MBL deposition and anti-PLA2R antibody in renal tissue in this study, we speculate that these factors mutually interact. This phenomenon hints at the possibility that anti-PLA2R antibody in the glomeruli activates the local MBL pathway. Previous studies have shown that MBL can bind to terminal carbohydrate residues, especially microbial carbohydrates and apoptotic host cells [38]. Researchers have found that MBL can bind to the IgG Fc fragment terminating with N-acetyl-d-glucosamine, thereby activating the LP. Purified anti-PLA2R antibodies from patients with iMN showed high GalNAC exposure in the terminal position, which may be recognized by MBL, thereby activating the LP [39]. We found more severe proteinuria in anti-PLA2R antibody-positive than anti-PLA2R antibody-negative patients, consistent with other studies [40–42], but also more favorable

clinical endpoints in MBL-positive than MBL-negative patients. Together, these results suggest that other mechanisms underlie the role of the anti-PLA2R antibody and that further studies are needed. The role of the AP in disease progression also needs to be examined in greater depth. Some recent studies have confirmed that factor B and both C3 and C5–9 co-deposit in the glomeruli, suggesting AP activation [43, 44]. Bally et al. [45] reported a case of glomerular MBL deficiency and B factor deposition, and conducted genetic testing in 77 patients, 4 of whom showed MBL gene mutations and AP activation. Another study confirmed that high levels of factor B predicted less proteinuria remission [22]. Consistent with the previous study, we also found a higher positivity rate of tissue anti-PLA2R antibody than that of serum ones [46]. This phenomenon may be due a higher rapid clearance of antibodies from blood than that from deposition in glomeruli.

In contrast to our findings, Japanese scholars found that MBL deposition was an unfavorable factor for renal function deterioration [31]. Compared with our subjects, those enrolled in the Japanese study were older (mean age, 62.5 years) and had more severely impaired renal function (mean baseline eGFR, 71.9 mL/min/1.73 m²). This eGFR corresponds to stage 2 of CKD and is close to the stage 3 cutoff. These factors may partially explain differences in patient prognosis between studies.

In our study, C1q deposition was found in 22.4% of patients and did not differ between the MBL-positive and negative groups, suggesting that the CP and LP were involved in the pathogenesis of kidney disease in some patients. The C1q positivity rate and intensity did not differ between the anti-PLA2R antibody-positive and negative groups. Zhang et al. [47] demonstrated that the CP was implicated in the pathogenesis of iMN in some patients but did not play a key role in the regulation of renal injury.

The limitations of our study include the relatively short follow-up, with a median of 17 months. This reduces the study power, with fewer renal dysfunction points observed during this time period. Other limitations stem from the insufficiency of renal tissue samples for the detection of other complement regulatory proteins, such as C3a, C4d, C5b–9, factor B, and factor D. Finally, this study was retrospective and conducted at a single center; the findings need to be validated in additional longitudinal studies conducted with more diverse patient cohorts.

In conclusion, this study is the first to examine the relationships between glomerular MBL deposition and iMN severity and progression in Chinese patients. The high MBL positivity rate supports the importance of the LP in the pathogenesis of iMN. We also found that patients with MBL deposition reach ICR more rapidly than those without MBL deposition. Further studies are needed to explore the protective role that glomerular MBL plays and the interaction between local MBL and anti-PLA2R antibodies in iMN patients.

Statement of Ethics

This study was conducted according to the principles of the Declaration of Helsinki and approved by the Ethics Committee of Shandong Provincial Qianfoshan Hospital. Written informed consent was obtained from all enrolled patients before the samples were used.

Conflict of Interest Statement

There are no conflicts of interest.

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Author Contributions

Ying Zhang and Yipeng Liu: drafting the work and revising it critically for important intellectual content; final approval of the version to be published; and agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Liming Liang, Liyan Liu, and Qinlan Chen: agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Xueqing Tang, Lijun Tang, Ping Chen, Juan Chen, Zunsong Wang, and Wei Cao: acquisition and analysis of the work. Na Zhao and Dongmei Xu: substantial contributions to the conception or design of the work; interpretation of data for the work; critical revision of the work for important intellectual content; and final approval for the version to be published.

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