Correlation of glomerular mannose-binding lectin deposition with the clinicopathology and prognosis of PLA2R-associated membranous nephropathy

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Abstract

Mannose-binding lectin (MBL) and autoantibody IgG4 staining against the phospholipase A2 receptor (PLA2R)were correlated. To enquire into the pathogenic effect of MBL in IMN as well as its relevance to clinicopathology and prognosis.Patients with IMN in 2021–2022 at our hospital were divided into positive and negative groups based on glomerular MBL immunofluorescence results and anti-PLA2R antibody characterization, and their clinical, pathological and follow-up data were evaluated.Among 39 patients with IMN, the positive rates of glomerular MBL and IgG4 deposition and serum anti-PLA2R antibodies were 31 (79.5%), 37 (100%) and 26 (70.3%), respectively. There were no notable differences in clinical and pathological features between the MBL-positive and negative groups of patients, but there were differences in IgG4 expression in the renal tissues ($p < 0.05$). There were no notable differences in MBL deposition between IMN patients grouped qualitatively by blood PLA2R antibodies. Renal tissue MBL was highly expressed (79.5%) and C1q was lowly expressed (15.38%). Kaplan-Meier analysis of clinical remission was similar in both groups. In multivariate COX regression analysis adjusted for sex, age, serum anti-PLA2R antibody concentration and blood pressure, MBL deposition (HR, 0.776; 95% CI, 0.311–1.939; $p = 0.587$) was not associated with IMN remission in the MBL-negative compared with the positive group.Renal tissue MBL characterization correlates with IgG4 and anti-PLA2R antibodies are involved in the pathogenesis of IMN through the induction of complement activation by the complement agglutinin pathway. No significant clinical, pathological or prognostic differences between patients with positive and negative MBL deposits were found in the study.

Introduction

IMN(Idiopathic membranous nephropathy) is Ithe most frequently occurring cause of nephrotic syndrome,with a trend towards a progressive rejuvenation and increase in recent years1. The renal pathology of IMN is characterised by subepithelial immune deposits, mainly composed of immunoglobulins and complement2, suggesting that the complement system plays a vital role in iMN. However, the activation pathway is unknown. C4d deposition is detectable in almost 100% of patients with IMN3 and is involved in the CP(classical pathway) and the LP lectin pathway)

. However, because C1q expression is weak or absent in IMN and the major immunoglobulin IgG4 does not bind to C1q, activation of the CP is not considered 4; whereas MBL and C4d staining is usually positive in IMN, which is consistent with LP activation 5,6, but the exact mechanism of MBL complement activation is still not clear. PLA2R is now widely used in the clinical management of IMN as a target antigen for IMN7. There is evidence that the staining intensity of MBL correlates with the intensity of IgG48 and that anti-PLA2R antibodies may activate the lectin complement pathway by binding to MBL 9,10. However, there are relatively few relevant studies. Referring to the experience of previous studies, we hypothesized that complement activation of the MBL pathway is involved in the pathogenesis of IMN. This study explored the role of MBL in the pathogenesis of IMN, the relationship between MBL and anti-pla2r antibodies in clinicopathology, and the prognosis of patients with IMN.

Objectives

The aim of the study was to investigate the role of the MBL pathway in the pathogenesis of PLA2R-associated membranous nephropathy and the relationship with the clinicopathology and prognosis of IMN.

Materials & Methods

Study population

Patients with a primary diagnosis of IMN on renal pathology biopsy at our hospital between January 2021 and February 2022 (120 patients in total). Exclusion criteria: patients with MN in combination with other pathological types were also excluded, e.g. diabetic nephropathy, IgA nephropathy. Additionally, we restricted age, gender and EGFR matching.The final sample included 39 patients with IMN. The study was approved by the Ethics Committee of the First Affiliated Hospital of Bengbu Medical College (Lunke Approval [2020] No. 117).This study was conducted in accordance with the declaration of Helsinki. and all patients were informed and signed an informed consent form.

Clinical features data collection

Demographic data were collected from patients: including gender, age, weight, history of hypertension and hyperglycaemia; blood and body fluid indicators: including albumin, blood creatinine, uric acid, lipids, eGFR (calculated by CKD-EPI formula), immunoglobulins A, G, M, complement C3, C4, 24h urine protein quantification; Anti-PLA2R antibody was detected by ELISA with double-layered antibodies. Serum PLA2R antibody kit $\leq$ 14 RU/ml is considered negative.

Renal pathology

Renal penetration tissue was observed by light microscopy (HE, Masson, PASM staining), electron microscopy and immunofluorescence. Pathological staging was based on the criteria of Ehrenreich and Churg11 in stages I, II, III and IV, looking at glomeruli, tubules, interstitium and small renal arteries. A semi-quantitative method was used for interstitial renal fibrosis. It is divided into 4 grades according to the extent of the lesion, defined as follows: grade 1: 0 < lesion area < 5%; grade 2: lesion area 5%-24%; grade 3: lesion area 25%-49%; grade 4: lesion area $\geq$ 50%. Immunofluorescence, direct immunofluorescence for IgG (IgG1; IgG2; IgG3; IgG4); IgA; IgM; C3; C1q; PLA2R; C3a, C5a, C5b-9, Recombinant Mannose Associated Serine Protease 1 (MASP1). The fluorescence intensity can be graded from 0 to 4 + under microscopy. In formalin-fixed and paraffin-embedded kidney biopsies, C4D staining was performed using the IP method

Treatment and follow-up

All patients were treated and followed up according to the 2012 KDIGO guidelines12. Clinical remission, including complete and partial remission, was determined using the 2012 Kidney Disease Improvement

Global Prognosis Organization (KDIGO) guidelines: (1) complete remission: 24-h urine protein quantification < 0.3 g, serum albumin > 35 g/L, and normal blood creatinine; (2) partial remission: 24-h urine protein quantification of 0.3 (2) Partial remission: 24-h urine protein quantification of 0.3 ~ 3.5 g/d and > 50% decrease, improvement in serum albumin, and stable creatinine; (3) No remission means that the above criteria are not met. The primary endpoint was defined as a clinical outcome such as remission; renal failure: defined as a 1.5-fold and 2-fold increase in serum creatinine from baseline and a 30% reduction in EGFR.

Statistical Analysis

The measured data are represented by (x ± s), and the comparison between groups is by t-test; the count data were expressed as the number of cases (percentage), and the $\chi 2$ test was used for comparison between groups, and the rank data were described as number of cases (percentage) and rank sum test was used for comparison between groups. The COX risk proportional regress-

ion model was used for univariate and multifactorial survival analysis, and the KM survival cur-

ve and Logrank test were used to assess the differences in prognosis between different subgroups of categorical indicators. The statistical software was SPSS 25.0. $p < 0.05$ was considered a statis-

tically significant difference.

Results

Clinical features and pathological characteristics of MBL deposition positive and negative groups

Thirty-nine patients with IMN were randomly selected using statistical software and divided into an MBL-positive group (31 patients) and a negative group (8 patients) according to MBL depo-sits in the renal tissue.There was no big difference between the two groups in terms of gender structure, age and weight ($p = 0.16$;$p = 0.186$;$p = 0.239$); 11 patients (35.5%) in the positive group had hypertension compared to 3 patients (37.5%) in the negative group, all without diabetes mellitus, with no statistical difference between the two groups; 20 patients (64.5%) in the positive group presented with nephrotic syndrome compared to 5 patients (62.5%) in the negative group, regardless of urinary protein There were no notably significant differences between the two groups in terms of urine protein quantification, serum albumin and creatinine, uric acid and glomerular filtration rate; there were also no significant differences in terms of anti-PLA2R antibodies and blood IgG, IgA, IgM and C3 and C4 expression.

On the other hand, when comparing the pathological features, we found that there was no significant difference between the two groups in terms of the degree of glomerular thylakoid cell proliferation or acute and chronic tubulointerstitial lesions; there was no difference in immuno-fluorescence IgG (IgG1;

IgG2; IgG3); IgA; IgM; C3; PLA2R; however, there was a difference in IgG4 ($p = 0.033$). Electron microscopic angles showed no difference in MN staging between the two groups. See Table 1 for details and Fig. 1.

Conclusion: There were no significant differences in clinical and pathological features between the MBL-positive and negative groups, but there were differences in the expression of IgG4 in the renal tissues, as detailed in Fig. 1.

MBL expression in anti-PLA2R antibody-positive and negative groups

The titer of anti-PLA2R antibodies has been shown to assess the clinical and pathological manifestations of patients, as well as the prognosis.

In our study we grouped IMN patients according to antibody characterisation and compared MBL and other fluorescence results between the positive and negative groups and found no differences in C1q, MBL, MASP1 (100%), C4d (100%), IgG4 (100%) and C5b-9 expression between the two groups. There was no significant difference in complement component deposition, regardless of whether the anti-PLA2R antibody was positive or negative. See Table 2 for details.

Tab.1 Comparison of clinical and pathological features between two groups of IMN patients with positive and roticnegative MBL deposits

Projects		Positive MBL deposition	Negative for MBL deposition	T/c2/Z	P
Sex, male	Male	22 (71%)	8 (100%)	-	0.16
Age years		45.63±10.501	51.26±10.558	-1.347	0.186
Body weight Kg		77.5±16.562	71.25±11.904	1.199	0.239
Hypertension	Yes	11 (35.5%)	3 (37.5%)	-	1
Nephrotic syndrome	Yes	20 (64.5%)	5 (62.5%)	-	1
Urinary protein,g/24 h		4.665±1.495	4.981±2.258	-0.373	0.711
Albumin, g/L		25.113±4.492	26.706±4.778	-0.851	0.4
Creatinine umol/l		80.38±23.04	69.03±18.375	1.479	0.148
UA μmol/L		404.75±80.827	341.9±91.631	1.767	0.085
eGFR (mL/min/1.73 m2)		105.255±28.711	114.09±29.327	-0.763	0.45
Cholesterol mg/dl		7.99±2.481	6.652±1.93	1.559	0.128
Triglycerides, mg/dl		1.84(1.39-2.5)	2.38(1.63-3.085)	0.993	0.359
Serum anti-PLA2R antibody RU/ml		22.8(7.97-62.46)	39.8(8.63-87.41)	-0.035	0.972
Serum IgG, g/L		4.805±2.558	5.927±2.822	-1.017	0.316
Serum IgA, g/L		1.788±0.567	2.004±0.815	-0.702	0.487
Serum IgM, g/L		0.905±0.322	1.227±0.555	-1.562	0.127
C3		0.965±0.369	0.862±0.225	1.004	0.322
C4		0.299±0.129	0.256±0.071	1.288	0.206
Serum anti-PLA2R antibody positive	Positive	21 (67.7%)	5 (62.5%)	-	1
Serum THSD7A antibody	Negative	31 (100%)	8 (100%)	-	-
Number of glomeruli		22.13±8.061	18.13±7.864	1.275	0.21
Grading of glomerular thylakoid hyperplasia	0	24 (77.4%)	3 (37.5%)	-	0.079
	1	7 (22.6%)	5 (62.5%)		
Segmental sclerosis	0	30 (96.8%)	7 (87.5%)	3.58	0.372
	1	1 (3.2%)	0 (0%)		

	2	0 (0%)	1 (12.5%)		
Collaterals necrotic [n(%)]	0	31 (100%)	8 (100%)	-	-
Infiltration of interstitial inflammatory cells	Yes	31 (100%)	8 (100%)	-	-
Interstitial inflammatory cell infiltration Scattered	0	25 (80.6%)	4 (50%)	-	0.167
	1	6 (19.4%)	4 (50%)		
Interstitial inflammatory cell infiltration Focal	0	8 (25.8%)	4 (50%)	-	0.221
	1	23 (74.2%)	4 (50%)		
Interstitial inflammatory cell infiltration Large patch	0	29 (93.5%)	8 (100%)	-	1
	1	2 (6.5%)	0 (0%)		
Interstitial inflammatory cell infiltration Diffuse	0	31 (100%)	8 (100%)	-	-
Small arterial lesions [n(%)]	0	6 (19.4%)	2 (25%)	-	0.658
	1	25 (80.6%)	6 (75%)		
Chronic lesions of the renal tubules and interstitium (atrophy of the tubules and interstitial fibrosis)	None	0 (0%)	1 (12.5%)	-	0.205
	Yes	31 (100%)	7 (87.5%)		
Chronic lesions of the renal tubules and interstitium	0	0 (0%)	1 (12.5%)	-	0.334
	1	13 (41.9%)	4 (50%)		
	2	17 (54.8%)	3 (37.5%)		
	3	1 (3.2%)	0 (0%)		
MN stages 1, 2, 3 n		1/22/6	0 /6/2	1.013	1
IF intensity score					
PLA2R	1+	7 (22.6%)	2 (25%)	0.253	1
	2+	19 (61.3%)	5 (62.5%)		
	3+	5 (16.1%)	1 (12.5%)		
THSD7A	Negative	31 (100%)	8 (100%)	-	-

IgA	Positive	16(51.6)	6(75%)	1.414	0.426
	Negative	1(3.2%)	1(12.5%)		
IgM	Positive	24(77.4%)	6(87.5%)	0.021	1
	Negative	7(22.6)	2(25)		
C3	1+	1(3.2%0	1(12.5%)	1.125	0.57
	2+	13(41.9%)	3(37.5%)		
C1q	1+	14 45.2%	3 37.5%	0.152	0.697
	Positive	17(54.8%)	5(62.5%)		
IgG	2+	3(9.7%)	0(0%)	0.839	1
	3+	28(90.3%)	8(100%)		
IgG1	1+	2(6.5%)	1(12.5%)	0.328	0.508
	2+	29(93.5)	7(87.5%)		
IgG2	1+	1(3.2%)	1(12.5)	1.124	0.372
	2+	30(96.8%)	7(87.5%)		
IgG3	Negative	11 35.5%	3(37.5%	0.869	0.844
	Positive	18(57.1%)	4(50%)		
IgG4	1+/2+	4(12.9%)	2(25%)	0.715	0.583
	3+/4+	27(87.1%)	6(75%)		

Table 2
Expression of complement components in the glomeruli of IMN patients in the anti-PLA2R antibody positive and negative groups

Projects	Fluorescence results	Group		Total	p
		Anti-PLA2R antibody Positive	Anti-PLA2R antibody Negative		
C1q[n(%)]	Positive	5(19.23)	1(7.69)	6(15.38)	0.643
	Negative	21 (80.77)	12 (92.31)	33 (84.62)	
MBL[n(%)]	Positive	21 (80.77)	10 (76.92)	31 (79.49)	1
	Negative	5(19.23)	3(23.08)	8 (20.51)	
C3a[n(%)]	Positive	22 (84.62)	11 (84.62)	33 (84.62)	1.000
	Negative	4(15.38)	2(15.38)	6(15.38)	
C5b-9[n(%)]	Positive	18 (69.23)	12 (92.31)	30 (76.92)	0.225
	Negative	8 (30.77)	1(7.69)	9(23.08)	

Expression of complement components in glomeruli

We performed immunofluorescence examination of complement components in 39 patients with IMN. Positive MBL staining (+) in kidney tissue specimens from one patient with IgA nephro-pathy (M1 E0 S1 T0 C1) was used as a positive control.

In the study we found that.

C1q represents the classical pathway of complement activation. linear deposition of C1q along glomerular capillary loops was seen in the glomeruli of 6 patients (15.38%). Positive staining intensity was at 1+.

MBL represents the complement-activated lectin pathway. 31 cases (79.5%) were positive for MBL deposition, which was deposited in the glomerular basement membrane and stained positively at an intensity of 1+-3+.

C4d represents both the CP and LP. The results of immunohistochemical staining indicated that C4D was expressed in granular form along the glomerular capillary wall in 39 patients (100%). This suggests that both the classical and MBL pathways may be involved in IMN injury.

Activation of the C3a complement pathway resulted in the conversion of C3 to C3a and C3b. 100% of C3 kidney tissue was detected by immunofluorescence. 33 patients (84.62%) had glomeruli in which C3a was visible along the glomerular capillary wall, in clusters or comma-like patterns, with positive staining intensity of 1+-3+. As a common component of the 3 complement pathways, it suggests that all 3 complement pathways may be involved in IMN injury.

C5b-9 is the end product of complement activation. 30 patients (76.92%) with IMN had glome-rular deposits of C5b-9, which were found in the capillary walls of the glomeruli, expressed in clusters or comma-like patterns, with a positive staining intensity of 1 + to 3+.

It is important to note that areas of glomerulosclerosis, tubulointerstitial damage, vascular vitreous changes and sclerosis were also evident in the study.

MASP-1 mannan-binding serine peptidase (MASP)-1, a downstream product of the MBL-activated complement agglutinin pathway, was deposited in the renal tubules of 39 patients (100%), mainly in the tubules, as a fine-grained deposit, staining lightly at 3+.

MASP-2 mannan-binding serine peptidase (MASP)-2, only one patient (2.56%) had MASP-2 deposition in the renal tubules as fine granular deposits, staining lightly at +. For details see Table 3, Fig. 2

Table 3
Expression of complement components in the glomeruli of IMN patients

Complement composition	Deposition site and morphology	Immunodeposited fluorescence intensity				
		Negative	1+	2+	3+	4+
C1q	Capillarycollaterals,deposited in lines	33 (84.62)	6 (15.38)	0	0	0
MBL	Basement membrane of the glomerulus, deposited in clumps or comma	8 (20.51)	18 (46.15)	8 (20.51)	5 (12.82)	0
C4d	Glomerular capillary wall with granular deposits Total positive expression	-	-	-	-	-
C3a	Glomerular capillary wall, clumped or comma-shaped.	6 (15.38)	20 (51.28)	12 (30.77)	1 (2.56)	0
C5b-9	Capillary wall of glomerulus, lumpy or comma-like	9 (23.08)	21 (53.85)	7 (17.95)	2 (5.13)	0
MASP1	Renal tubules, in fine granular deposits	0	0	0	39 (100)	0
MSAP2	Renal tubules, in fine granular deposits	38 (97.44)	1 (2.56)	0	0	0

In the present study, we examined seven complement components containing three complement activation pathways in renal tissue sections from IMN patients. RESULTS: MBL was positive in 79.5% of patients and was similar to C4d, C3a and C5b-9 in terms of deposition site and mor-phology in the glomeruli. Only a minority (15.38%) of patients had deposition of C1q, and the deposition was weak.

CONCLUSION: Immune complexes are involved in the pathogenesis of IMN through the indu-ction of complement activation by the complement agglutinin pathway; the classical pathway may also be involved in IMN injury. See Fig. 3 for details

Treatment response and renal outcomes in patients with IMN and MBL deposition

Three patients were lost to follow-up, and the remaining 36 patients were treated with either an angiotensin-converting enzyme inhibitor or an angiotensin receptor blocker (ACEI/ARB); 21 (58.3%) patients chose hormone combined with cyclophosphamide and 5 (13.9%) patients chose a hormone combined with a calcineurin phosphatase inhibitor regimen. During the follow-up period, a total of 29 (80.6%) patients in the IMN group achieved remission, including complete and partial remission, and 7 (19.4%) patients were treatment-naïve; no patient had renal failure. Details are shown in Fig. 4.

At follow-up, we grouped partial remission and complete remission together as dependent variables for survival analysis. the Kaplan-Meier analysis showed that the mean survival time to remission for MBL-negative patients was 6.952 months (95% CI: 4.094–9.811); the mean survival time to remission for MBL-positive patients was 7.815 months (95% CI. 6.743–8.886), with a chi-squared value of 0.388 and a p-value of 0.534 for the Log-rank test, indicating that there was no statistical difference in the time to remission between MBL-negative and MBL-positive patients. See Fig. 5 for details

In univariate and multifactorial survival analyses using COX risk proportional regression models, MBL deposition (HR, 0.776; 95% CI, 0.311–1.939; p = 0.587) was not associated with remission of IMN in the MBL-negative compared to the positive group,adjusting for sex, age, serum anti-PLA2R antibody concentration and blood pressure in multivariate COX regression analyses. However, the probability of achieving remission in patients with MBL deposition was only 77.6% (95% CI: 0.311–1.939, p = 0.587)of negative patients.:See Table 4for details.

Table 4
Univariate and multifactorial survival analyses affecting patient prognosis (dependent variables are remission and non-remission, HR > 1 represents an elevated indicator or a higher probability of remission compared to dummy variables

	Single factor analysis			Multi-factor analysis		
Characteristics	HR	CI	p	HR	CI	P
Age	0.97	0.93–1.01	0.113			
Body weight	0.96	0.92–1.004	0.077			
Gender (Female vs Male)	1.19	0.51–2.76	0.691			
Hypertension (yes vs. no)	0.79	0.35–1.77	0.571			
Nephrotic syndrome (no vs. yes)	1.27	0.58–2.77	0.555			
eGFR, mLmin173m2	1	0.99–1.01	0.799			
Serum albumin	0.97	0.9–1.05	0.461			
Urine protein quantification	0.88	0.73–1.06	0.239			
Pre + CTX/CNI vs ACEI/ARB	0.838	0.352–1.995	0.69			
Final eGFR, mLmin173m2	1.05	1.02–1.08	0.004	1.047	1.008–1.087	0.016
Final follow-up serum albumin	0.99	0.96–1.03	0.584			
Urine protein quantification at last follow-up	1	0.95–1.04	0.887			
Anti-PLA2R antibody (negative vs. positive)	0.94	0.43–2.04	0.873			
IgG	0.98	0.84–1.14	0.804			
IgA	0.63	0.36–1.13	0.125			
IgM	1.07	0.5–2.28	0.862			
C3	1.07	0.19–6.09	0.938			

	Single factor analysis			Multi-factor analysis		
C4	2.84	0.05–169.72	0.617			
Thylakoid cell hyperplasia	2.46	0.97–6.26	0.059			
Spherical hardening	0.93	0.78–1.12	0.442			
Segmental sclerosis	3.39	1.1–10.39	0.033	1.392	0.412–4.698	0.594
Small arterial lesions	0.88	0.35–2.21	0.781			
Atrophy and interstitial fibrosis of the renal tubules (yes vs no)	0.14	0.02–1.21	0.074			
PLA2R (3 vs 1)	0.98	0.41–2.36	0.971			
PLA2R (3 vs 2)	0.69	0.2–2.37	0.559			
MNstages (1 vs 0)	0.43	0.04–4.89	0.498			
MNstages (2 vs 0)	0.51	0.12–2.26	0.375			
MNstages (3 vs 0)	0.34	0.06–1.81	0.204			
IgA (2 + vs 1+)	1.43	0.31–6.59	0.646			
IgA (negative vs 1+)	1.01	0.46–2.24	0.974			
IgM (negative vs 1+)	1.28	0.46–3.53	0.635			
C3 (2 + vs 1+)	1.46	0.19–11.29	0.717			
C3 (3 + vs. 1+)	1.37	0.18–10.62	0.765			
C1q (positive vs. negative)	0.93	0.41–2.09	0.852			
IgG (3 + vs 2+)	0.87	0.2–3.74	0.849			
IgG1 (positive vs. negative)	1.352	0.313–5.843	0.687			

	Single factor analysis		Multi-factor analysis
IgG2 (positive vs. negative)	1.348	0.18–10.07	0.771
IgG3(positive vs. negative)	1.798	0.814–3.973	0.147
IgG4(2/2 + vs1/1+)	1.359	0.491–4.006	0.579
C3a (positive vs. negative)	1.667	0.959–2.899	0.07
C5a (positive vs. negative)	2.658	0.346–20.388	0.347
C5b9 (positive vs. negative)	1.371	0.733–2.562	0.323
MBL (positive vs. negative)	1.135	0.718–1.794	0.587

Discussion

Research into IMN has progressed from the Heymann animal model to the discovery of anti-PLA2R antibodies, but the pathogenesis of IMN is still being investigated. Anti-PLA2R antibodies have been found to assess the condition and prognosis of patients, but how they are involved in the pathogenesis of IMN remains unclear. Previous studies have demonstrated the involvement of complement in the pathogenesis of IMN13, and deposition of C3, C4 and their breakdown products in the glomerulus can be seen in most IMN patients 6. Almost all of these patients had C5b-9 deposition, which further elucidates the involvement of complement in the pathogenesis of MN. In our study, 79.5% of the patients had positive renal tissue immuno-fluorescence for MBL, 100% were positive for MASP-1 and C4d, with a high rate of positive expression of C3a and C5b-9, and similar sites and patterns of deposition of MBL, C4d, C3a and C5b-9 in the glomeruli. Only a minority (15.38%) of patients had C1q deposition, and this was weak. Our results suggest that IgG4 is involved in the pathogenesis of IMN by binding to MBL to form immune complexes that induce complement activation, but the classical pathway cannot be completely excluded. We also provide a comprehensive assessment of glomerular MBL depo-sition in relation to IMN clinicopathology and prognosis.

The current study confirmed the involvement of MBL in the pathogenesis of a variety of nephri-tis. Guo et a14 found that MBL deficiency and excess may contribute to the progression of IgA nephritis, including haematuria, proteinuria and crescentic proportions in renal pathology. Zhang et al15 measured circulating complement composition in 134 IMN patients and found that serum MBL levels correlated positively with 24 h urinary protein in anti-PLA2R antibody-positive patients quantification was positively correlated with 24-h urine protein in antibody-negative patients, but not in antibody-negative patients. In

contrast, Yuchao Zhao16 found that serum MBL levels were not associated with clinical presentation or prognosis in patients with IMN. Ying Zhang 17 also found no significant differences in expression between the positive and negative glomerular MBL deposition groups in terms of albumin, urinary protein excretion rate, eGFR and anti-PLA2R antibodies. In our study, we found no significant differential expression of clinical and pathological features between the MBL deposition positive and negative groups. Considering that our previous study has demonstrated that anti-PLA2R antibody titers correlate with hypoalbumin and proteinuria levels in IMN patients, with pathological staging and C3 and IgG4 immunodeposition, it can be used as an indicator to assess clinical and pathological features and prognosis. We noted no difference in serum anti-PLA2R antibody expression levels between the two groups and propose the hypothesis that this may account for the lack of significant differences in clinical features between the two groups of IMN patients.

In our study we found no significant differences in tubular and interstitial chronic lesions between the two groups, but the incidence was higher in the MBL-positive group (100% vs 87.5%) and to a greater extent. In IgA nephropathy, patients with glomerular MBL deposits showed more severe histological damage and more proteinuria18,19 In membranous nephro-pathy, deposition of C5b-9 in the kidney is associated with interstitial inflammation and interstitial fibrosis 20. A Japanese study showed that deposition of MBL in glomeruli and excretion of MBL and C5b-9 in the urine of IMN patients may lead to tubulointerstitial damage8. Our study also found a higher rate of C5b-9 immunofluorescence positivity and stronger fluore-scence intensity in the renal tissues of the MBL-positive group; C5b-9 was also found to be significantly deposited by immunofluorescence in areas of tubulointerstitial damage. This may explain to some extent why the MBL-positive group had more chronic tubulointerstitial damage than the negative group.

We observed glomerular C4d deposition in all IMN patients, suggesting that LP and/or CP were activated. We found a high rate of positive MBL staining in IMN patients and the same sites of IgG4 deposition in renal tissue, and some studies have also found co-localization of MBL with IgG48,17; also renal tissue IgG4 immunofluorescence expression was stronger in the MBL-positive group. All evidence suggests a correlation between MBL and IgG4. Previous studies have reported that membranous nephropathy is an immune response triggered by antigens expressed by podocytes, in most cases PLA2R. IgG4, an autoantibody to PLA2R, does not activate the CP due to its low affinity for C1q or Fcγ receptors4, and researchers have found that MBL can bind to IgG terminated by N-acetyl-d-glucosamine Fc fragment, thereby activating the LP. In IMN associated with PLA2R, MBL may bind to terminal galactose-deficient anti-PLA2R-IgG4 antibodies and activate the lectin pathway. C4d, a product of MASP-induced C4 cleavage, was expressed in 100% of IMN kidney tissue C4d in the experiment; also MBL and IgG4 were expressed at the same sites, supporting our hypothesis to some extent. It should be noted that we found differential expression of IgG4 between the MBL negative and positive groups, but no significant differences in serum PLA2R antibodies, similar to the findings of Zhang et al 17. Previous studies have also shown that the positive rate of anti-pla2r antibodies in renal tissue is higher than that of serum antibodies.This phenomenon may be due to the fact that antibodies are cleared from the blood faster than from glomerular deposits.

In our study, trace C1q deposition was found in 15.38% of IMN patients, all in the MBL-positive group, and the lack of C1q in the glomeruli of most IMN patients suggests that CP is not a major pathway of complement response in IMN renal tissue. We found no difference in C1q positivity or intensity between the anti-PLA2R antibody positive and negative groups, which is consistent with the low affinity of IgG4 for C1q and Fcγ receptors 4 without activation of CP. It has been found that MBL activation is not the only way to develop IMN, as patients with complete MBL deficiency can also develop IMN21. Recent advances in detection methods have revealed that IMN also has trace amounts of C1q deposition22,23. The presence of C1q suggests that the pathogenesis of IMN may include a classical complement pathway in addition to the IgG4-mediated pathway. One study identified IgG1 as the major subclass in the early stages of IMN (stage 1 in the Ehrenreich and Churg classification)23, while IgG4 predominates in the later stages. It is hypothesized that IgG1 may activate CP at low levels in the early stages and IgG4 activates the MBL pathway in the later stages. The high rate of positive MBL and IgG4 expression in renal tissues of our IMN patients and conversely the low expression of C1q and IgG1 may correlate with the fact that our IMN patients are mostly stage II-III patients. However, we need more longitudinal studies to address the assessment of IgG subclass conversion during the clinical course.

In our analysis of MBL deposition in glomeruli, we did not find MBL deposition to be a prog-nostic factor in patients with IMN. A Japanese study contradicted our results by finding that MBL deposition was a detrimental factor in the deterioration of renal function8, but their subjects were older (mean age 62.5 years) and had lower baseline eGFR levels (mean baseline eGFR, 71.9 mL/min/1.73 m2) compared to our subjects. These factors may produce differences in patient prognosis. Our studies in IMN patients have also shown that the complement agglutinin pathway is associated with the development of IMN and that serum MBL levels are not associated with prognosis 16. However, some studies have also found better remission rates in IMN patients with MBL deposition. Reason for consideration: There was no difference in expression of anti-PLA2R antibody titres in our MBL subgroup, as it has been demonstrated in our previous studies or in other relevant studies24,25 that anti-PLA2R antibody positive patients have more severe proteinuria than negative patients, and if there was no differential expression of antibody titres between the two MBL positive and negative groups, this could lead to no significant difference in the analysis of MBL on prognosis. However, further studies are needed to explore the interaction between glomerular MBL and anti-PLA2R antibodies.

Limitations

Limitations of this study include the short follow-up period and the lack of measurement of renal tissue complement composition in normal controls. We plan to follow up with a multicentre study to include more patients with IMN, as well as extend the follow-up period and increase the longitudinal study in order to make our study more convincing.

Conclusions

This study focused on the analysis of the association between glomerular MBL deposition and clinicopathology and prognosis in Chinese IMN patients. The expression of MBL in renal tissue supports the LP as the main pathway of complement activation in IMN patients; also assessing that there is no clear correlation between MBL deposition and treatment response and renal outcome in IMN patients, but further studies are needed to explore the interaction between renal tissue MBL and anti-PLA2R antibodies. Current treatment of IMN includes initial treatment with ACEI/ARB and the use of immunosuppressive agents in the absence of remission after optimal treatment, but inevitably with side effects. Blockade of the lectin pathway may give us new ideas for treatment and may serve as a subsequent emerging therapeutic option.

Declarations

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Author Contribution

Yan Pan and Lei Lu: Design, and co-wrote the paper.Jiqiang Zhang: Analyzed the data. Yan Pan and Ruiping Zhao: Performed experiments.Weidong Chen: Supervision and revised final manuscript and confirmed; All authors read and approved the final manuscript.

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Data Availability

The data that support the findings of this study are available on request from the corresponding author.

Ethics approval and consent to participate

The written informed consent was signed by all the participants and the study was performed according to the Declaration of Helsinki and approved by the Ethics Committee of the First Affiliated Hospital of Bengbu Medical College (Lunke Approval [2020] No. 117).

Consent for publication

No applicable.

Competing interests

The authors declare no competing interests

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Figures

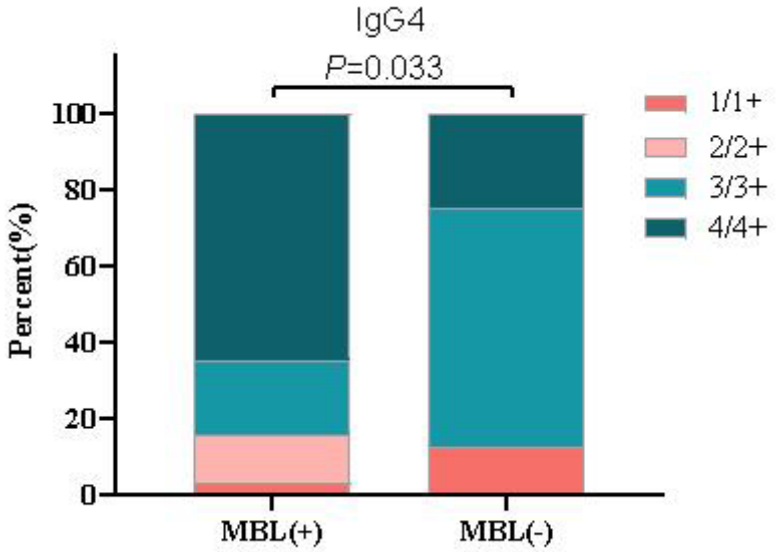

Figure 1

Comparison of glomerular IgG4 immunofluorescence in MBL deposition positive group and negative group

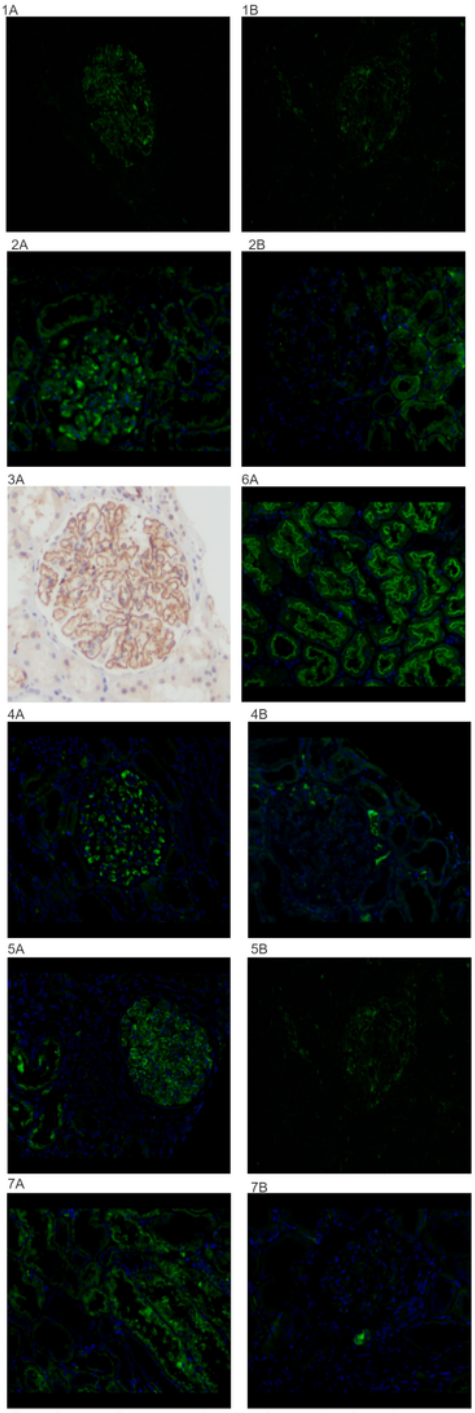

Figure 2:Immunofluorescence detection of expression of complement components in renal tissues of IMN patients. 1A:C1(3+);1B:C1q(-);2A:MBL(3+);2B:MBL(-);3A: Immunohistochemical detection of C4d full positive expression; 4A: C3a(3+); 4B: C3a(-); 5A: C5b-9(3+); 5B: C5b-9(-); 6A. MASP1 (3+); 7A: MASP2 (+); 7B: MASP2 (-).Magnification, ×400

Figure 2

Immunofluorescence detection of expression of complement components in renal tissues of IMN patients. 1A:C1q(3+);1B:C1q(-);2A:MBL(3+);2B:MBL(-);3A: Immunohistochemical detection of C4d full positive expression; 4A: C3a(3+); 4B: C3a(-); 5A: C5b-9(3+); 5B: C5b-9(-); 6A. MASP1 (3+); 7A: MASP2 (+); 7B: MASP2 (-).Magnification, ×400

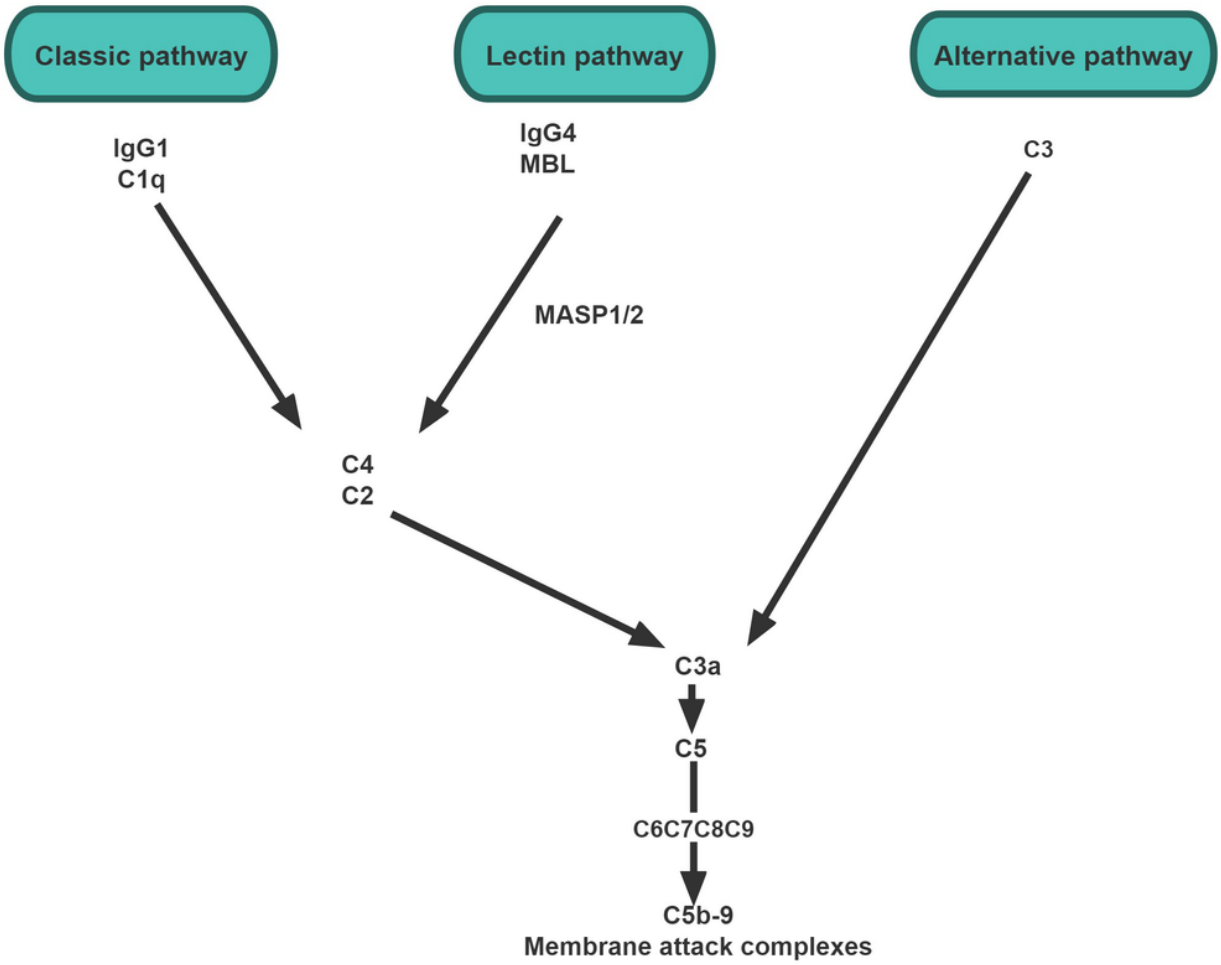

Figure 3

Roadmap for simulating the complement mechanism in PLA2R-related membrane nephropathy

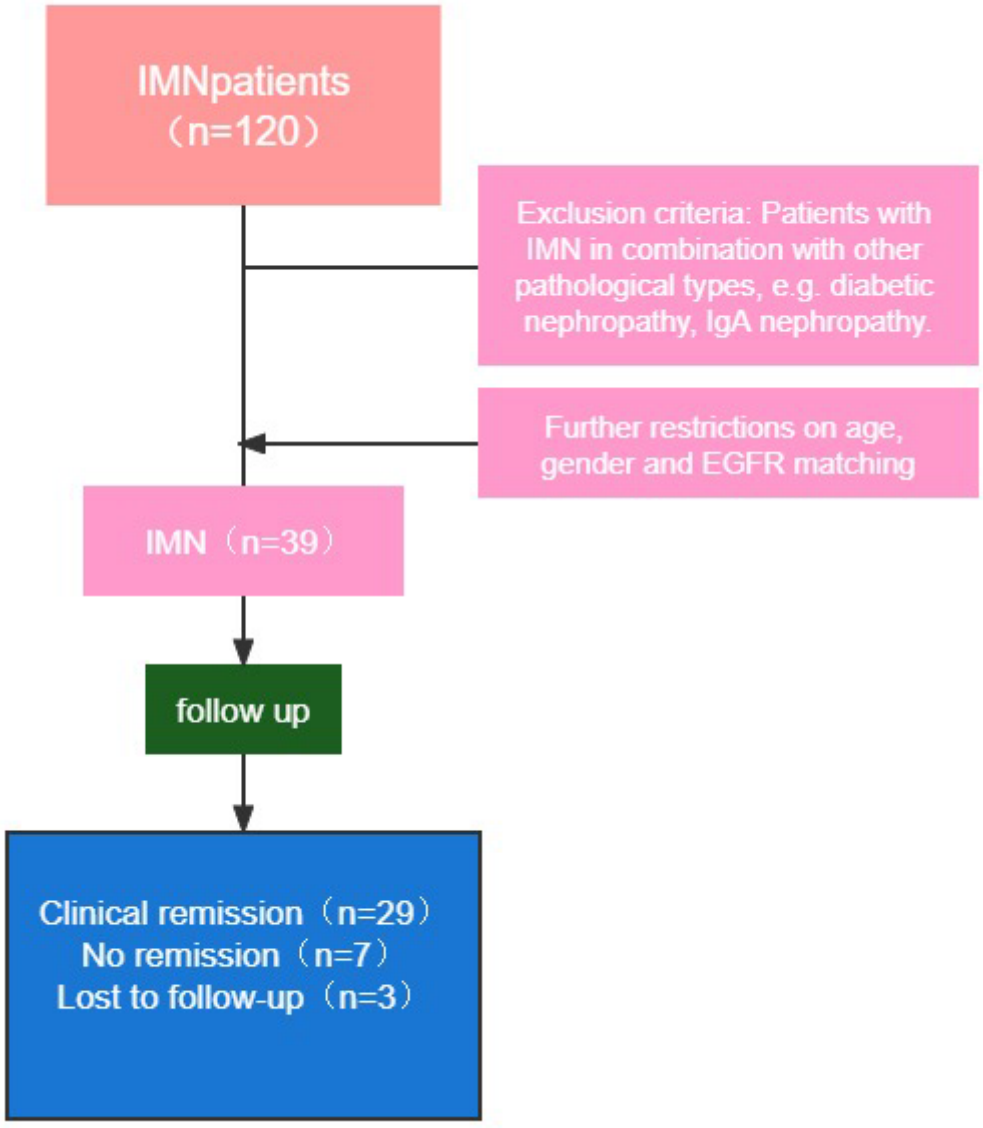

Figure 4

Flow chart of patient inclusion and follow up

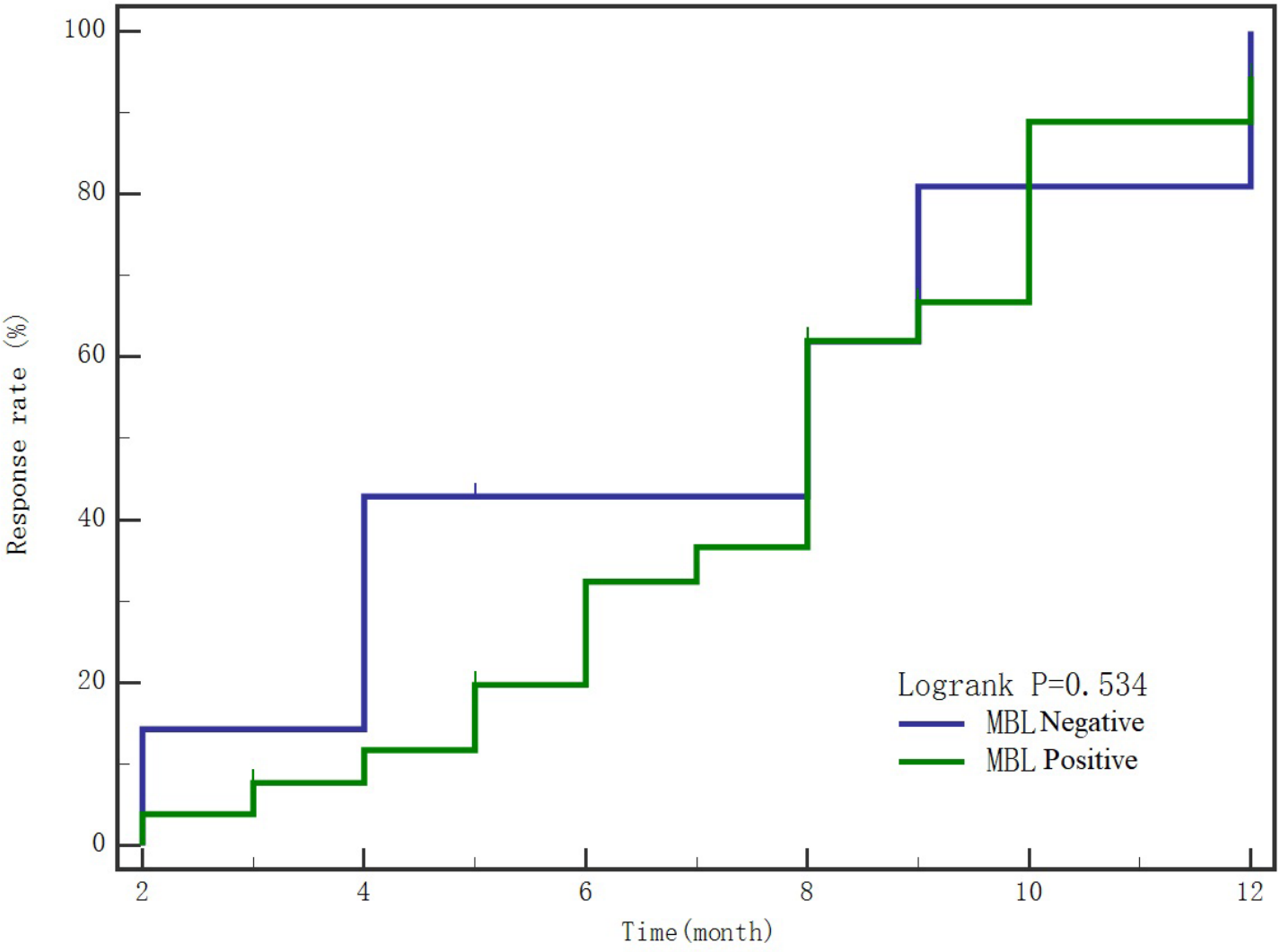

Figure 5

Kaplan-Meier survival curve analysis