Clinical relevance of glomerular IgM deposition in patients with lupus nephritis

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Abstract

Background

The aim of the study was to investigate the clinical relevance of IgM deposition in patients with LN in a large cohort.

Results

217 patients with renal biopsy–proven lupus nephritis were enrolled. The associations between glomerular IgM deposition and clinicopathological parameters were further analyzed. IgM deposition was positively correlated with glomerular C1q and C3 deposition moderately (r = 0.436, $P < 0.001$; r = 0.408, $P < 0.001$, respectively), and inversely correlated with plasma levels of C3 and CFH mildly (r=-0.138, $P = 0.043$; r=-0.147, $P = 0.037$, respectively). By multivariate analysis, we found that glomerular IgM deposition independently contributes to glomerular C3 deposition in patients with lupus nephritis (OR = 2.002, 95% CI: 1.295–3.094, $P = 0.002$). In addition, we also found that patients with IgM 0+-2 + had similar plasma CFH levels, but in patients with IgM3+-4+, plasma CFH levels were significantly lower (300.4 ± 155.8μg/ml vs. 429.9 ± 187.5μg/ml, $P < 0.001$). Furthermore, patients with high density of glomerular IgM and low levels of CFH had heavier proteinuria, higher serum creatinine and lower plasma C3 levels (5.7 ± 3.1g/d *vs.* 4.7 ± 3.5g/d, $P = 0.037$;150.1 ± 121.0μmol/L *vs.* 105.6 ± 97.1μmol/L, $P = 0.005$; 0.3 ± 0.2μg/L vs. 0.4 ± 0.2μg/L, $P = 0.04$, respectively), comparing with those with low density of glomerular IgM and low levels of CFH.

Conclusions

Our results suggested IgM might bind to injury-associated epitopes and be involved in disease progression and provided a possible relevance of CFH and IgM in the process of alternative pathway (AP) activation.

Background

Lupus nephritis (LN) is the most common complication of systemic lupus erythematosus (SLE). The pathogenesis of LN involves initiation by immune complexes, activation of the immune system in the kidney, and the responses of renal parenchymal cells to these insults. Although LN is characterized by a “full-house” pattern of immune deposits, it is mostly suggested to be initiated by the glomerular deposition of nephritogenic IgG type autoantibodies at present [1–4].

Glomerular IgM deposition occurs in a wide range of glomerular diseases. It was previously considered to be passively trapped in areas of glomerulosclerosis. However, recent studies found that IgM specifically binds insulted glomeruli and exacerbates renal injury. In mice deficient in the complement factor H (CFH),

a model of non-sclerotic and nonimmune-complex glomerular disease, IgM was identified as binding to glomerular epitopes and contributing to the progression of glomerular damage [5]. In another animal model of adriamycin-induced focal segmental glomerulosclerosis (FSGS), IgM deposition activated the complement system and mediated glomerular injury [6]. In the subsequent clinical studies, IgM deposition independently associated with worse renal outcomes in patients with various glomerular diseases, including FSGS, IgA nephropathy (IgAN), and diabetic nephropathy (DN) [7–9].

Since natural antibody IgM is suggested to bind to endogenous neoepitopes that are exposed after injury and exacerbates damage [10–13], whether it is involved in the pathogenesis of LN presented with various types of kidney injury deserves to be investigated. To our knowledge, this is the first study designed to investigate the clinical relevance of IgM deposition in patients with LN in a large cohort. Our findings are helpful for understanding the mechanism of IgM deposition and its indication for clinical practice in patients with LN.

Results

Glomerular IgM deposition and correlations with clinicopathological parameters in patients with LN

In total, 217 consecutive patients with renal biopsy–proven primary LN were enrolled. The median age of the patients was 32.7 years and ranged from 14 to 70 years. The female-to-male ratio was 5.2:1. The clinical and pathological data of the 217 patients with lupus nephritis were summarized in Table 1. Among the 217 patients, 33 (15.3%) patients had no IgM deposition in glomeruli, whereas 184 patients had IgM deposition in glomeruli, including 72 (33.2%) patients with IgM 1+, 81 (37.3%) patients with IgM 2+, 31 (14.3%) patients with IgM 3+.

Table 1
General clinical and renal histopathological profiles of patients with lupus nephritis

Characteristic	Value	Characteristic	Value	Characteristic	Value
Age, mean ± SD, years	32.7 ± 11.9	Anti-Smith antibodies (+), n (%)	51 (23.5)	2	75 (34.6)
Gender, female/male	182/35	Anti-SSA antibodies (+), n (%)	101 (46.5)	3	107 (49.3)
Duration of follow-up (months), median; IQR	42 (8–78)	Anti-SSB antibodies (+), n (%)	24 (11.1)	4	10 (4.6)
		Anti-C1q antibodies (+), n (%)	90 (41.5)		
SLEDAI mean ± SD	17.5 ± 5.7	Scr(μmol/l)	127.3 ± 140.0	C1q	
Fever (non-infectious), n (%)	66 (30.4)	C3(g/l)	0.46 ± 0.23	0	15 (6.5)
Eruption, n (%)	120 (55.3)	C4(g/l)	0.23 ± 0.17	1	52 (24.0)
Photosensitivity, n (%)	50 (23)	**Pathological parameters**		2	83 (38.2)
Oral ulcer, n (%)	64 (29.5)	IgG		3	66 (30.4)
Arthralgia, n (%)	118 (54.4)	0	8 (3.7)	4	2 (0.9)
Neurological disorder, n (%)	12 (5.5)	1	45 (20.7)	**Activity indices score (median, range)**	8 (0, 19)
Anemia, n (%)	143 (65.9)	2	78 (35.9)	Endocapillary hypercellularity (median, range)	3 (0, 3)
Thrombocytopenia, n (%)	66 (30.4)	3	78 (35.9)	Cellular crescents (median, range)	0 (0, 6)
Leukocytopenia, n (%)	101 (46.5)	4	8 (3.7)	Karyorrhexis/fibrinoid necrosis (median, range)	0 (0, 6)
Hematuria, n (%)	166 (76.5)	IgM		Subendothelial hyaline deposits (median, range)	1 (0, 3)

Abbreviations: dsDNA: double—stranded DNA; IQR: interquartile range; SSA: Sjogren's syndrome A antigen; SSB: Sjogren's syndrome B antigen; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index

Characteristic	Value	Characteristic	Value	Characteristic	Value
Leukocyturia (non-infection), n (%)	113 (52.1)	0	33 (15.2)	Interstitial inflammatory cell infiltration (median, range)	1 (0, 3)
Acute renal failure, n (%)	40 (18.4)	1	72 (33.2)	Glomerular leukocyte infiltration (median, range)	1 (0, 12)
Hemoglobin (g/l), mean ± SD	101.9 ± 25.8	2	81 (37.3)	**Chronicity indices score** (median, range)	2 (0, 10)
Urine protein (g/24h), mean ± SD	4.3 ± 3.2	3	31 (14.3)	Glomerular sclerosis (median, range)	0 (0, 3)
Serum creatinine (μmol/l), median; IQR	84(68–129)	C3		Fibrous crescents (median, range)	0 (0, 3)
Antinuclear antibody (+), n (%)	214 (98.6)	0	4 (1.8)	Tubular atrophy (median, range)	1 (0, 3)
Anti-dsDNA antibodies (+), n (%)	152 (70)	1	21 (9.7)	Interstitial fibrosis (median, range)	1 (0, 3)

Abbreviations: dsDNA: double—stranded DNA; IQR: interquartile range; SSA: Sjogren's syndrome A antigen; SSB: Sjogren's syndrome B antigen; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index

To explore the clinical implications of glomerular IgM deposition in LN, we analyzed the correlations between the intensity of IgM deposition and clinico-histological manifestations of patients at biopsy. It was found that the intensity of IgM deposition was correlated with the intensity of IgG and IgA deposition in glomeruli ($r = 0.297$, $P < 0.001$; $r = 0.456$, $P < 0.001$). IgM deposition was positively correlated with glomerular C1q and C3 deposition moderately ($r = 0.436$, $P < 0.001$; $r = 0.408$, $P < 0.001$, respectively), and inversely correlated with plasma levels of C3 and CFH mildly ($r=-0.138$, $P = 0.043$; $r=-0.147$, $P = 0.037$, respectively), but no correlation with plasma C1q levels ($r=-0.109$, $P = 0.115$). Among the pathologic indices, glomerular IgM deposition was positively correlated with subendothelial hyaline deposits mildly ($r = 0.136$, $P = 0.045$) and inversely correlated with interstitial inflammatory cell infiltration ($r=-0.153$, $P = 0.025$). There was no correlation between IgM deposition and proteinuria levels, serum creatine levels or SLEDAI scores (detailed in Table 2).

Table 2
Correlations between clinicopathological data and glomerular IgM deposition in lupus nephritis

Parameters	r	*P* Value
Clinical parameters		
Age	-0.154	0.024
SLEDAI	0.013	0.847
Hemoglobin	0.008	0.907
24-hour urine protein	0.050	0.466
Serum creatinine	-0.039	0.572
Plasma C1q levels	-0.109	0.115
Plasma C3 levels	-0.138	0.043
Plasma CFH levels	-0.147	0.037
Plasma soluble C5b-9 levels	0.009	0.899
Pathological parameters		
AI	0.003	0.965
Endocapillary hypercellularity	0.050	0.461
Cellular crescents	-0.064	0.345
Karyorrhexis/fibrinoid necrosis	0.024	0.720
Subendothelial hyaline deposits	0.136	0.045
Interstitial inflammatory cell infiltration	-0.153	0.025
Glomerular leukocyte infiltration	-0.005	0.943
CI	-0.022	0.745
Glomerular sclerosis	-0.014	0.843
Fibrous crescents	-0.012	0.860
Tubular atrophy	-0.033	0.626
Interstitial fibrosis	-0.062	0.360
Glomerular IgG deposition	0.293	< 0.001
Glomerular IgA deposition	0.456	< 0.001

Abbreviations: SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; AI: Activity indices; CI: Chronicity indices.

Parameters	r	*P* Value
Glomerular C3 deposition	0.436	< 0.001
Glomerular C1q deposition	0.408	< 0.001

Abbreviations: SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; AI: Activity indices; CI: Chronicity indices.

Glomerular IgM deposition independently contributes to glomerular C3 deposition in patients with LN

Complement activation was one of the mechanisms contributing to renal injury initiated by immune complexes deposition. Since glomerular IgM deposition was not only positively correlated with C3 deposition, but also correlated with glomerular IgG, and IgA deposition, multivariate analysis was used to determine whether IgM contributed to complement activation independently or dependent on IgG.

As shown in Table 1, in our cohort, almost all the patients had C3 deposition in glomeruli.

Among them, 46.1% patients had relatively mild C3 deposition (0+-2+), and 53.9% patients had relatively strong C3 deposition (3+-4+).
Then multivariate binary logistic regression using strong C3 deposition was carried out. We found that glomerular IgM deposition independently contributes to glomerular C3 deposition in patients with LN (OR = 2.002, 95% CI: 1.295–3.094, *P* = 0.002) (shown in Table 3).

Table 3
Risk factors for glomerular C3 deposition in patients with lupus nephritis

	Univariate Analysis		Multivariate Analysis	
Parameter	**OR (95% CI)**	**Pvalue**	**OR (95%CI)**	**Pvalue**
Sex, men	0.557(0.262–1.187)	0.130	—	—
Age, per 10yr	0.870(0.682–1.108)	0.259	—	—
Urinary protein, per 1g/24h	1.050(0.972–1.135)	0.216	—	—
Serum creatinine	1.002(0.999–1.004)	0.171	—	—
Plasma c1q levels	0.993(0.980–1.007)	0.328	—	—
Plasma CFH levels	0.999(0.997-1.000)	0.123	—	—
Plasma C3 levels	0.163(0.047–0.559)	0.004	0.243(0.052–1.138)	0.072
C1q deposition	2.890 (2.890–4.158)	< 0.001	1.940(1.281–2.937)	0.002
IgG	2.238(1.600–3.130)	< 0.001	1.908(1.278–2.848)	0.002
IgA	2.339(1.677–3.263)	< 0.001	1.360(0.915–2.021)	0.129
IgM	2.993(2.073–4.322)	< 0.001	2.002(1.295–3.094)	0.002

Abbreviations: OR: odd ratio; CI, confidence interval; —, not included in the multiple analysis.

Association of glomerular IgM deposition and circulating CFH levels in patients with LN

In animal models, $CFH^{-/-}$ leads to glomerular IgM deposition. Interestingly, in our cohort, we also found negative correlation between the intensity of glomerular IgM deposition and plasma CFH levels, although the correlation coefficient was low. We further compared circulating CFH levels in patients with different intensity of glomerular IgM deposition. Patients with IgM 0+-2 + had similar plasma CFH levels, but in patients with IgM 3+, plasma CFH levels were significantly lower (300.4 ± 155.8 μg/ml *vs.* 429.9 ± 187.5 μg/ml, $P < 0.001$). While referring to IgG, it was found that plasma CFH levels were also associated with the intensity of glomerular IgG deposition, but with an opposite tendency compared with the deposition of IgM (Fig. 1).

Comparisons of clinicopathological data in patients with low plasma CFH with and without high density of glomerular IgM deposition

In our previous study, we found that plasma CFH levels in patients with LN at active phase were significantly lower than in non-renal SLE patients or in normal controls, while in the remission phase of LN, plasma CFH levels returned to the normal control levels, and plasma CFH levels were negatively associated with SLEDAI scores and positively associated with serum C3 levels. These results indicated that active LN might consume factor H, and the consumed factor H might lead to extra glomerular IgM

deposition besides deposition from circulating immune complex due to autoimmunity. Then we compared clinicopathological data in patients with similar levels of plasma CFH with and without high density of glomerular IgM deposition.

The studied patients were divided according to the median plasma CFH level into 2 groups: patients with low plasma CFH (n = 165) and patients with high plasma CFH.
In patients with low plasma CFH, 30 patients were with glomerular IgM 3+ (only 1 patient with glomerular IgM 3 + in this cohort was with high plasma CFH), while 135 patients without strong glomerular IgM deposition. Then the difference of clinicopathological characteristic in patients with low plasma CFH with and without strong glomerular IgM deposition were analyzed. We found that patients with strong intensity of glomerular IgM deposition had heavier proteinuria, higher serum creatinine and lower plasma C3 levels (5.7 ± 3.1g/d *vs.* 4.6 ± 3.3g/d, $P = 0.026$;150.1 ± 121.0μmol/L *vs.* 124.7 ± 140.6μmol/L, $P = 0.015$; 0.3 ± 0.2 μg/L *vs.* 0.44 ± 0.22 μg/L, $P = 0.023$, respectively). There was no significant difference in other indices (detailed in Table 4).

Table 4
Comparisons of clinical, laboratory and pathological data between different groups

	Total patients			Patients with low plasma CFH levels		
	Strong IgM deposition N = 31	less IgM deposition N = 186	P	Strong IgM deposition N = 30	less IgM deposition N = 135	P
Clinical evaluation						
Gender (male %)	6(19.4%)	25(15.6%)	0.601	6 (20.0%)	17 (12.6%)	0.380
Age (mean ± S.D.) (years)	30.8 ± 10.5	33.1 ± 11.2	0.297	31.1 ± 10.5	33.3 ± 11.8	0.411
SLEDAI (mean ± S.D.)	17.3 ± 5.1	17.5 ± 5.8	0.763	17.2 ± 5.2	18.0 ± 5.8	0.408
Anemia no. (%)	19(61.3%)	124(66.7%)	0.543	18 (60.0%)	94 (69.6%)	0.286
Thrombocytopenia no. (%)	8(25.8%)	58(31.2%)	0.674	8 (26.7%)	48 (35.6%)	0.399
Leukocytopenia no. (%)	14(45.2%)	87(46.8%)	1.000	14 (46.7%)	67 (49.6%)	0.841
Laboratory assessment						
Hemoglobin (mean ± S.D.) (g/l)	100.84 ± 26.05	102.10 ± 25.80	0.720	100.9 ± 26.5	99.6 ± 25.7	0.859
Urine protein (median; IQR) (g/24h)	5.60 ± 3.09	4.97 ± 3.61	0.127	5.7 ± 3.1	4.6 ± 3.3	0.026
Scr (mean ± S.D.) (μmol/l)	147.1 ± 140.1	128.2 ± 145.0	0.252	150.1 ± 121.0	124.7 ± 140.6	0.015
Antinuclear antibody (+), n (%)	31(100.0%)	183(98.4%)	1.000	30 (100.0%)	134(99.3%)	1.000
Anti-dsDNA antibodies (+) no. (%)	23(74.2%)	129(69.4%)	0.676	22 (73.3%)	99(73.3%)	1.000
Anti-Smith antibodies (+), n (%)	9(29.0%)	42(22.6%)	0.493	8 (26.7%)	34 (25.2%)	0.821
Anti-SSA antibodies (+), n (%)	16(51.6%)	85(45.7%)	0.565	15(50.0%)	65(48.1%)	1.000

Abbreviations: SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; dsDNA: double—stranded DNA; SSA: Sjogren's syndrome A antigen; SSB: Sjogren's syndrome B antigen; IQR: interquartile range; AI: Activity indices; CI: Chronicity indices.

	Total patients			Patients with low plasma CFH levels		
Anti-SSB antibodies (+), n (%)	2(6.5%)	22(11.8%)	0.541	2 (6.7%)	15(11.1%)	0.741
Anti-C1q antibodies (+), n (%)	14(45.2%)	44(42.3%)	0.682	11(44.0%)	34(47.9%)	0.818
Plasma C1q (mean ± S.D.) (μg/ml)	29.3 ± 22.3	34.0 ± 19.7	0.134	33.2 ± 18.8	33.2 ± 18.8	0.191
Plasma C3 levels (mean ± S.D.) (g/l)	0.35 ± 0.2	0.48 ± 0.24	0.004	0.3 ± 0.2	0.44 ± 0.22	0.023
Plasma C4 levels (mean ± S.D.) (g/l)	0.21 ± 0.1	0.22 ± 0.14	0.833	0.23 ± 0.17	0.21 ± 0.18	0.756
Plasma CFH levels (mean ± S.D.) (μg/ml)	300.3 ± 155.8	438.2 ± 193.7	< 0.001	294.6 ± 131.1	338.7 ± 124.3	0.052
Soluble C5b-9 (mean ± S.D.) (ng/ml)	1278.0 ± 574.9	1296.3 ± 699.4	0.823	1225.9 ± 571.2	1271.4 ± 657.8	0.785
Pathological parameters						
AI (median; IQR)	8(6,10)	8 (3,12)	0.611	8 (6,10)	8 (4,11)	0.695
Endocapillary hypercellularity	3(2,3)	3 (1,3)	0.404	3 (2,3)	3 (1,3)	0.620
Cellular crescents	0(0,2)	0 (0,2)	0.550	0 (0,2)	0 (0,2)	0.591
Karyorrhexis/fibrinoid necrosis	1(1,3)	0 (0,2)	0.841	0 (0,2)	0 (0,2)	0.640
Subendothelial hyaline deposits	1(1,3)	1(1,2)	0.189	1 (1,3)	1 (0,2)	0.118
Interstitial inflammatory cell infiltration	1(1,1)	1(1,1)	0.722	1 (1,1)	1 (1,2)	0.856
Glomerular leukocyte infiltration	1(1,1)	1(0,1)	0.274	1 (1,1)	1 (0,1)	0.454
CI (median; IQR)	2(2,4)	2 (2,4)	0.458	2.5 (2,4)	2 (2,4)	0.368
Glomerular sclerosis	0(0,1)	0 (0,1)	0.789	0 (0,1)	0 (0,1)	0.970
Fibrous crescents	0(0,1)	0 (0,0)	0.161	0 (0,1)	0 (0,0)	0.228
Tubular atrophy	1(1,1)	1 (1,1)	0.594	1 (1,1)	1 (1,1)	0.481

Abbreviations: SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; dsDNA: double—stranded DNA; SSA: Sjogren's syndrome A antigen; SSB: Sjogren's syndrome B antigen; IQR: interquartile range; AI: Activity indices; CI: Chronicity indices.

	Total patients			Patients with low plasma CFH levels		
Interstitial fibrosis	1(1,1)	1 (1,1)	0.581	1 (1,1)	1 (1,1)	0.604

Abbreviations: SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; dsDNA: double—stranded DNA; SSA: Sjogren's syndrome A antigen; SSB: Sjogren's syndrome B antigen; IQR: interquartile range; AI: Activity indices; CI: Chronicity indices.

Association of glomerular IgM deposition and renal outcome in patients with LN

In our cohort study, patients with LN were followed up for a mean duration of 57.3 ± 58.1 months (8–78 months).

We evaluated the association between glomerular IgM deposition and renal survival by Kaplan-Meier survival analysis. It was found that glomerular IgM deposition was not a risk factor for renal outcome in our patients with LN (P = 0.581, HR = 0.854, 95% CI: 0.488–1.495).

Patients were classified into two groups according to the intensity of glomerular IgM deposition. Patients in group 1 had no or less intensity of IgM deposition (0–2+) and those in group 2 had greater intensity IgM deposition (3+-4+). However, there was still no significant difference in renal survival between the two groups (P = 0.33) (Fig. 2).

Discussion

Glomerular IgM staining is commonly shown on the mesangial and capillary wall of glomeruli in patients with lupus nephritis, but its significance has not been elucidated yet. In this large cohort of patients with lupus nephritis, the prevalence of IgM glomerular deposition in patients with lupus nephritis was 84.8% and IgM deposition was associated with glomerular C3 deposition. The high prevalence of IgM staining on glomeruli and its association with C3 indicated that IgM glomerular deposits might involve in the kidney injury of lupus nephritis, especially in the complement activation, rather than a nonspecific trap from circulation. In our study, we found that glomerular IgM deposition was an independent risk factor for glomerular C3 deposition in patients with lupus nephritis, which suggested that the deposit of glomerular IgM participated the complement activation in local tissue.

Interestingly, we found that plasma CFH, which was of importance for inhibiting the complement activation of alternative pathway (AP), hindered the glomerular IgM deposition. However, C1q and C3 deposition was opposite to the plasma CFH, both of which were favorable for IgM deposition. In addition, we also found that the lower plasma CFH levels, the stronger glomerular IgM deposition. As well known, IgM is generally regarded as an activator of the classical pathway (CP), and previous researches have suggested activation of both the CP and the AP in patients with lupus nephritis [14–18]. AP amplification can be triggered by activation of the CP, however, our results suggested it was possible that activation of both pathways could be initiated by deposited IgM. Especially, in a model of $CFH^{-/-}$ mice induced glomerulopathy, IgM was identified as binding to glomerular epitopes and contributing to glomerular damage by complement activation[5]. Perhaps, glomerular IgM deposition and complement activation

represent a common final pathway of injury to the glomerulus after immunologic insults. It is possible that glomerular IgM, which binds to injury-associated epitopes, activates the complement system through the classic pathway and further amplifies it through the alternative pathway. To our knowledge, the association between IgM deposition and plasma CFH in patients with lupus nephritis was the first investigated. Whether the deficiency of CFH facilitated and amplified the complement activation by IgM deposition, which is extremely interesting and worthy of further investigation.

In lupus nephritis, it is mostly suggested to be initiated by the glomerular deposition of nephritogenic IgG type autoantibodies, which caused the complement activation [3, 19–21]. IgM and complement-mediated injury may be secondary phenomena after the IgG type autoantibodies insult [10, 22–25]. Consequently, we further compared the plasma CFH levels among different intensity of glomerular IgG deposition. The finding was that the less intensity of glomerular IgG deposition, the higher plasma CFH levels, which was completely contrary to those of the IgM deposition, which further implied that deposit of IgM in glomeruli might independently take part in the complement activation of AP.

To further investigate the clinical significance of IgM deposition and CFH levels in lupus nephritis, we found that the stronger IgM deposition, the heavier proteinuria, the higher serum creatinine and the lower plasma C3 levels. These findings indicated that the deposit of IgM in glomeruli execrated the renal injury and also affected the complement over-activation.

On the basis of the above relevance of glomerular IgM deposition in patients with lupus nephritis, it is important to evaluate the predictive value of glomerular IgM deposition for long-term renal outcome in lupus nephritis. Recently, several studies showed that IgM deposition predicted renal outcome in patients with IgAN, FSGS and diabetic nephropathy [7, 9, 22, 23].

However, owing to the relatively good treatment response of lupus nephritis and our relatively small follow-up population, we failed to draw any convincing conclusions regarding the predictive value of glomerular IgM deposition in our study.

Our study had several limitations. First, it was a retrospectively observational study; thus, a cause-effect relationship could not be established. Second, this is the first report revealing clinical significance of IgM deposition in patients with lupus nephritis; findings from this single-center study require validation from multicenter studies with larger cohorts. Third, a “full-house” pattern of immune deposits in lupus nephritis caused complex pathophysiological process in complement activation, IgM deposition involved in kidney injury progression was not fully interpreted.

In conclusion, this study shows that glomerular IgM deposition of patients with lupus nephritis was associated with glomerular C3 deposition, plasma CFH levels, worse proteinuria and serum creatinine.

This finding indicates that deposit of IgM in glomeruli participated the process of complement activation not only by CP but also AP.

These associations suggested IgM might bind to injury-associated epitopes and be involved in disease progression by activating complement system and provided a possible relevance of CFH and IgM in the process of AP activation.

Methods

Informed consent was obtained for blood sampling and renal biopsy from each patient.
For participants under 16 years old, written informed consent was provided by a parent or guardian.
In addition, all clinical test results were also obtained after patients gave their consent to use them for research purposes.
The research was in compliance with the Declaration of Helsinki and approved by IEC for Clinical Research of Southeast University affiliated Zhongda Hospital (No. 2013-075).

Patients

A total of 217 consecutive patients with renal biopsy–proven lupus nephritis diagnosed at Zhongda Hospital affiliated to Southeast university from 6 September 2013 to 2 March 2019 were enrolled in this study. The patients all fulfilled the 1997 ACR revised criteria for SLE [26].

Clinical evaluations

The following clinical data were collected and analysed: gender, age, fever, anaemia, leucocytopenia, thrombocytopenia, haematuria, leukocyturia, 24-hour proteinuria, serum creatinine and hemoglobulin. Clinical disease activity was assessed using the SLEDAI [27]. eGFR was calculated using a Scr-based equation adjusted for coefficients for age and gender by modified abbreviated MDRD equation based on data from Chinese CKD patients: eGFR (ml/min per $1.73m^2$)=$175\times[\text{Scr (mg/dl)}]^{-1.234}\times age^{-0.179}\times$ (0.79 if female) [28].

Laboratory assessment

Serum ANAs, anti-dsDNA antibodies, anti-extractable nuclear antigen antibodies, including anti-Sm, anti-SSA, anti-SSB antibodies, were detected using immunodotting assays (EUROIMMUN, Lübeck, Germany; normal range). Plasma C3 was determined using a rate nephelometry assay (BeckmanCoulter, IMMAGE, USA; normal range>0.85 g/L).

Anti-C1q IgG autoantibodies were detected using a previously published ELISA method [29]. The results were recorded as the net optical absorbance (average value of antigen wells minus average value of antigen-free wells) at 490 nm in an ELISA reader (BioRad 550, Japan) and expressed as percentage of the known positive sample. The cutoff value was set as the mean+2 SD of healthy blood donors.

Quantification of plasma levels of complement components

Plasma concentrations of human complement components were determined by enzyme-linked immunosorbent assay, including plasma C1q, plasma CFH and soluble C5b-9 (Quidel Corporation, San Diego, CA). The detection of plasma soluble C5b-9 was assayed in accordance with the manufacturer's instructions.

The method to detect plasma C1q was modified as previously described [29]. Rabbit anti-human C1q polyclonal antibodies (Dako, Denmark) were coated on to the microtiter plates (Nunc Immunoplate, Roskilde, Denmark) overnight at 4°C. Then the wells were washed 3 times with 0.01M phosphate-buffered saline (PBS) containing 0.1% Tween20 (PBST) and blocked with PBSTcontaining1% bovine serum albumin (BSA). After standards and serum samples added, horseradish peroxidase–conjugated goat anti-human C1q monoclonal antibody (Abcam, US) was added and incubated. The reaction was developed with tetramethylbenzi-dine (TMB) liquid substrate system and was stopped with 1MH2SO4. The results were recorded as the net optical absorbance at 450 and 570nm in an ELISA reader (Bio-Rad550, Japan).

The method of detecting plasma CFH was the same as previously described [30]. The CFH level of each sample was calculated using Curve Expert 1.3 (Hyams Development, http://www.curveexpert.net/). All assays were run in duplicate, and when standard errors were >10%, samples were routinely re-analyzed. Serial concentrations of commercially available highly purified human factor H were used to develop a standard curve. The linear portion of the curve was subsequently used for the measurement of plasma factor H.

Renal histopathology

The renal biopsy specimens were examined by light microscopy, direct immunofluorescence and electron microscopy. LN was re-classified according to the International Society of Nephrology/Renal Pathology Society (ISN/RPS) 2003 classification system [31]. All renal histopathological data of 217 patients with renal biopsy-proven lupus nephritis, were reviewed by two pathologists with 20-year experience. The pathologists classified and scored the biopsies separately, blinded to patients' data and the scores of the other observer. Differences in scoring between the pathologists were resolved by rereviewing the biopsies and reaching a consensus.

Intra- and inter-reader reliability

The two pathologists who undertook the analyses of the pathological data, were unaware of the patients' details. Each evaluation was performed in triplicate and the mean of the values were reported. In addition, in each patient's pathological data evaluation, two expert pathologists reported their results. The inter-rater reliability for immunofluorescence IgG and IgM scores was good, Cohen's kappa=0.953 (P<0.001), Cohen's kappa=0.951(P<0.001), respectively. The intra-rater reliability for immunofluorescence IgG and IgM scores from one pathologist was excellent, ICC=0.962 (95%CI 0.952-0.971), ICC=0.980 (95%CI 0.974-0.985), respectively. And the intra-rater reliability for immunofluorescence IgG and IgM scores from the

other pathologist was also good, ICC=0.946 (95%CI 0.931-0.958), ICC=0.965 (95%CI 0.955-0.973), respectively.

Light microscopy examination

Renal biopsy specimens were fixed in 4.5% buffered formaldehyde for light microscopy. Consecutive serial 3 μm sections were used for histological staining. Stains employed included hematoxylins and eosin (H&E), periodic acid-Schiff, silver methenamine (Meth) and Masson's trichrome.

Immunofluorescence examination

The intensity of fluorescence of immunofluorescence for IgG, IgA, IgM, C3, C1q, fibrin, kappa and lambda deposits were semi-quantitatively graded from 0 to 4, respectively.

Electron microscopy

Renal biopsy specimens were fixed in 2.5% paraformaldehyde for electron microscopy. After embedded in epon, ultrathin sections were mounted on metal grids and stained with uranyl acetate before viewed in a transmission electron microscope (JEM-1230; JEOL,Tokyo, Japan).

Statistical analysis

Statistical software SPSS 25.0 (SPSS, Chicago, IL, USA) was employed for all the statistical analysis. Quantitative data were expressed as mean ± s.d., median with range (IQR) or number (%). One-way analysis of variance was used for the same continuous data in different groups. Differences of quantitative variables between groups were assessed using the t test (for normally distributed data) or Mann–Whitney U test (for non-normally distributed data). Logistic regression analysis was carried out to predict glomerular C3 deposition. Survival analysis was performed using the log-rank test. Statistical significance was considered as $P<0.05$.

Abbreviations

LN: Lupus nephritis; SLE: systemic lupus erythematosus; CFH: complement factor H; FSGS: focal segmental glomerulosclerosis; IgAN: IgA nephropathy; DN: diabetic nephropathy; AP: alternative pathway; CP: classical pathway; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; dsDNA: double—stranded DNA; SSA: Sjogren's syndrome A antigen; SSB: Sjogren's syndrome B antigen; AI: Activity indices; CI: Chronicity indices; PBS: phosphate-buffered saline; BSA: bovine serum albumin; ISN/RPS: International Society of Nephrology/Renal Pathology Society; IQR: interquartile range; OR: odd ratio; CI, confidence interval; AI: Activity indices; CI: Chronicity indices.

Declarations

Ethics approval and consent to participate

Informed consent was obtained for blood sampling and renal biopsy from each patient. For participants under 16 years old, written informed consent was provided by a parent or guardian. In addition, all clinical test results were also obtained after patients gave their consent to use them for research purposes. The research was in compliance with the Declaration of Helsinki and approved by the local ethics committees (No. 2013-075).

Consent for publication

Not applicable.

Availability of data and materials

Further clinical data and images of this case are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

FMW drafted the manuscript and did the whole revision process. JRY, LZ and YZ participated in collecting the related clinical and pathological data. JZ conducted partly statistical analysis. XWY acted as the corresponding author did the whole study design and the manuscript revision. BCL acted as the co-corresponding author and did the partly study design and the manuscript revision. All authors read and approved the final manuscript.

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Figures

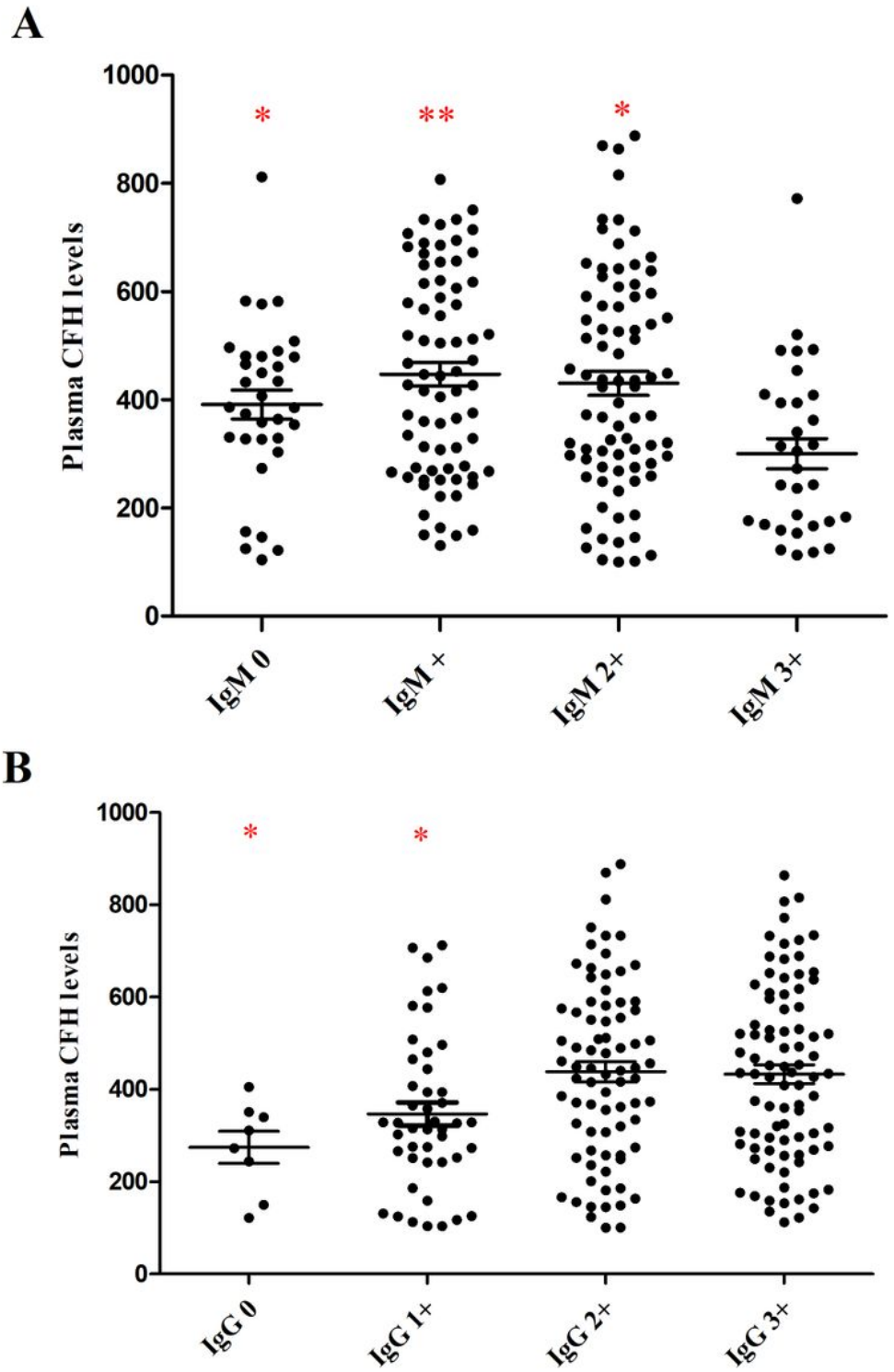

Figure 1

Plasma CFH levels in different groups. A) Plasma CFH levels in different intensity of glomerular IgM deposition; B) Plasma CFH levels in different intensity of glomerular IgG deposition. Note: P value*: compared with the plasma CFH levels in lupus nephritis patients with glomerular IgM3+ deposition. *:P<0.05; ** P<0.001.

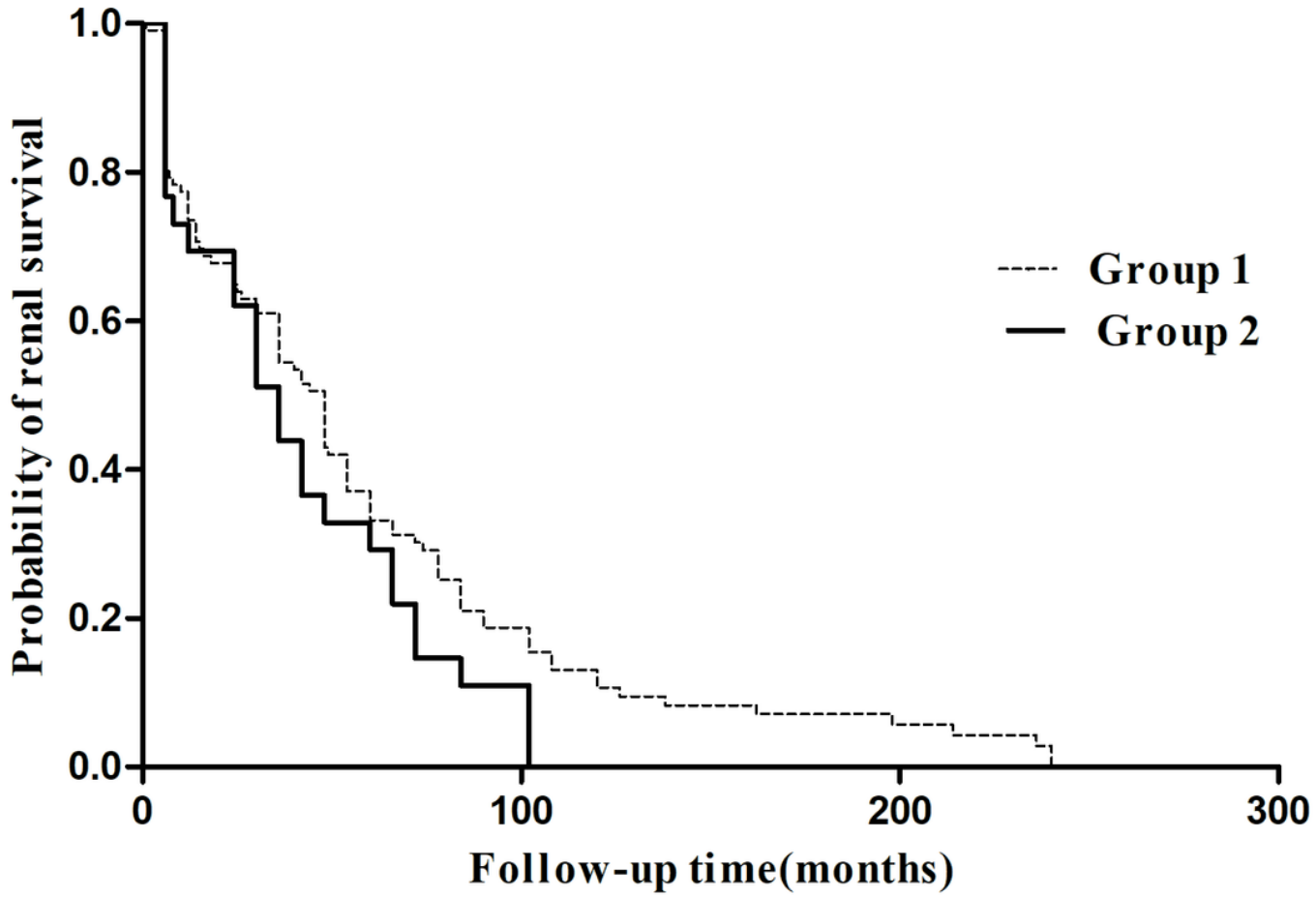

Figure 2

The correlation between intensity of glomerular IgM deposition and renal survival in patients with lupus nephritis.