Original Article

C3dg and MAC complement depositions in the renal histopathology of patients with lupus nephropathy

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Background: Childhood-onset systemic lupus erythematosus (SLE) refers to SLE with an onset before 18 years old. The key to the pathogenesis of SLE tissue inflammation and injury is complement activation. The presence of complement split C3dg and membrane attack complex (MAC) may indicate a worse prognosis for lupus nephritis (LN). This study investigated whether complement split C3dg and MAC depositions in the pathogenesis of LN are potential biomarkers of disease severity and tissue injury.

Methods: The data on patients with LN were retrospectively analyzed in our center between April 2018 and December 2020. The depositions of C3dg and MAC were detected by immunofluorescence staining.

Results: C3dg and MAC were both detected in specimens from 61.5% of patients. Patients with MAC depositions had a greater proportion of neurological disorders than those without MAC depositions (22.9% *vs.* 3.3%; P=0.044). We found significant differences in serum creatinine, urinary protein, and estimated glomerular filtration rate (eGFR) in all four groups of patients with differing degrees of C3dg and MAC depositions.

Conclusions: This study suggests that C3dg and MAC depositions may be potential biomarkers for disease severity and tissue injury in LN. MAC and C3dg staining may be useful in routine studies of lupus biopsies to identify patients who need more aggressive treatment.

Keywords: Lupus nephritis (LN); complement split; C3dg; membrane attack complex (MAC)

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Introduction

The pathogenesis of systemic lupus erythematosus (SLE) is related to clinical and immunologic manifestations, among which lupus nephritis (LN) is the most common cause of death (1). Childhood-onset SLE refers to SLE with an onset before the age of 18 years (2). The etiology of patients with early-onset SLE often has a larger genetic component, multiple system involvement, and a more severe course. The 5- and 10-year mortality rate is lower than adult SLE (3). LN can occur in up to 50% of cases (4).

The key to the pathogenesis of SLE tissue inflammation and injury is complement activation (5,6). In the past 70 years, many researchers have described the role of the complement system in the pathogenesis of SLE. Complement protein levels (C3, C4) may be used as diagnostic markers for SLE and to monitor disease activity (7). However, the limitation of using them to measure disease level in SLE has been well documented (8). The central component in complement activation is C3.

When activated, C3 is cleaved into two fragments: C3a and C3b. C3b is further cleaved into iC3b and finally to C3dg and C3c. Both C3a and C3c have a shorter half-life than C3dg. Some studies have suggested that C3dg may be a valuable diagnostic biomarker in SLE (9). Since these fragments are formed when the complement cascade is activated, the products of complement split can more accurately reflect complement activation than the level of a single intact protein (9,10). Some studies have also suggested that membrane attack complex (MAC) deposition may be a biomarker for more severe LN disease and poor response to treatment (11-13). Therefore, MAC and C3dg staining of lupus biopsies is essential to investigate whether their presence indicates a worse prognosis.

This study was conducted to observe the changes in complement split C3dg and MAC depositions in children with LN and investigated the value of using complement split C3dg and MAC depositions to predict kidney injury in children with LN. We present the following article in accordance with the STARD reporting checklist (available at https://tp.amegroups.com/article/view/10.21037/tp-22-310/rc).

Methods

Patients

This study was a retrospective case-control study of all active LN patients without treatment or serious contraindications that had undergone renal biopsy in our center. The medical records of 78 patients with renal biopsy-confirmed LN admitted to our hospital (The Children's Hospital of Fudan University) between April 2018 and December 2020 were retrospectively reviewed. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study protocol was approved by the Ethics Committee of The Children's Hospital of Fudan University (No. [2021]240), and individual consent for this retrospective analysis was waived.

The inclusion criteria required a diagnosis of LN as defined by clinical and laboratory manifestations meeting the American College of Rheumatology (ACR) criteria as follows: (I) persistent proteinuria greater than 0.5 gm per day or greater than 3+ by dipstick; (II) and/or cellular casts including red blood cells (RBCs), hemoglobin, granular, tubular, or mixed. A review of the ACR criteria has recommended that a spot urine protein/creatinine ratio >0.5 can be substituted for the 24-hour protein measurement, and "active urinary sediment" [>5 RBCs/high-power field (HPF), >5 white blood cells (WBCs)/HPF in the absence of infection, or cellular casts limited to RBC or WBC casts] can be substituted for cellular casts. An additional, perhaps optimal, criterion is a renal biopsy sample demonstrating immune complex-mediated glomerulonephritis compatible with LN (14).

Patients were excluded if a diagnosis of LN was ruled out during follow-up or if family members refused to sign the consent for their child.

Definition of LN

The diagnosis of LN was established based on the criteria of the American Rheumatism Association and verified by its characteristic changes, including glomerular hypertrophy, thickened capillary basement membranes, and nodular mesangial sclerosis on renal histology. The biopsies were assessed as per the International Society of Nephrology/Renal Pathology Society (ISN/RPS) classification for LN (15).

Clinical evaluation

The disease activity was assessed by the SLE disease activity index (SLEDAI) (16). Also, the following data from the study subjects were collected through the Electronic Health Information System: gender, fever, malar rash, photosensitivity, oral ulcer, alopecia, arthritis, serositis, neurological disorder, anemia, leukocytopenia, thrombocytopenia, hematuria, and leukocyturia.

Highlight box

Key findings
- C3dg and MAC depositions may be potential biomarkers for disease severity and tissue injury in lupus nephritis.

What is known, and what is new?
- The key to the pathogenesis of SLE tissue inflammation and injury is complement activation.
- C3dg and MAC depositions may be potential biomarkers for disease severity and tissue injury in lupus nephritis.

What is the implication, and what should change now?
- C3dg and MAC should be included in the routine staining of lupus nephritis biopsies.

Laboratory assessment

For the laboratory parameters, the study subjects' serum and plasma were collected on the day of their renal biopsy to measure renal function and complement values.

Renal histopathology

LN was classified according to the ISN/RPS 2018 LN classification system by two experienced renal pathologists (HL and GL) who also scored the indicators independently based on the average score according to the activity index of renal tissue in lupus nephropathy (17). The intensity of immunostaining was reported by GL and blindly assessed by HL. Based on our previous research on the treatment of complement inhibitors in lupus mice, our center has carried out C3dg and MAC immunostaining in renal tissue since April 2018 (18).

Statistical analysis

The statistical analysis was performed with SPSS version 19.0 software (IBM, Armonk, NY, USA). Continuous variables are presented as means ± standard deviations. Differences between groups were determined using a one-way analysis of variance (ANOVA), and a P value less than 0.05 was considered statistically significant.

Results

General data

Of our 78 patients with LN, 15 were male, and 63 were female, with a mean age of 12.12±2.91 years at the time of renal biopsy.

Comparison of clinical manifestations

On direct immunofluorescence microscopy, C3dg and MAC were both detected in specimens from 48/78 (61.5%) patients. Of the 48 patients with C3dg deposition, 40 (83.3%) also had MAC deposition. Specimens with C3dg deposition were evaluated as grades. 1+, 2+, and 3+ after pathological staining in 16/48 (33.3%), 23/48 (47.9%), and 9/48 (18.8%), respectively. The degree of MAC deposition in the 48 patients was also evaluated in three grades: 1+, 2+, and 3+ in 19/48 (39.6%), 21/48 (43.8%), and 8/48 (16.7%), respectively.

There was a significantly greater proportion of neurological disorders in patients with MAC depositions *vs.* those without such deposits (22.9% *vs.* 3.3%, P=0.044). Similarly, a significantly greater proportion of patients with nephrotic syndrome (77.1% *vs.* 46.7%, P=0.006, respectively) and acute renal failure had MAC and C3dg depositions *vs.* those without such deposits (27.1% *vs.* 3.3%, P=0.018, respectively). In addition, patients with *vs.* without MAC and C3dg depositions on renal histopathology had significantly higher levels of serum creatinine (65.5±20.6 *vs.* 47.9±11.8 μmol/L, P=0.001; 63.9±21.5 *vs.* 50.5±12.4 μmol/L, P=0.001, respectively), urinary protein (2.9±2.2 g/24 h *vs.* 0.8±1.9 g/24 h, P=0.003; 3.1±2.4 g/24 h *vs.* 0.5±0.6 g/24 h, P<0.001, respectively), urinary RBC (67.2±75.6/HP *vs.* 20.9±40.1/HP, P<0.001; 70.0±74.3/HP *vs.* 16.5±38.1/HP, P<0.001, respectively), and urinary WBC (16.6±24.5/HP *vs.* 4.8±12.4/HP, P=0.001; 18.2±25.5/HP *vs.* 2.2±1.6/HP, P<0.001, respectively), but significantly lower proportions of serum C3 decreased (79.2% *vs.* 36.7%, P<0.001; 79.2% *vs.* 36.7%, P<0.001, respectively) and C4 decreased (68.7% *vs.* 33.3%, P=0.002; 66.7% *vs.* 36.7%, P=0.010, respectively). Moreover, circulating hemoglobin levels were significantly lower in patients with MAC depositions than those without such deposits (107.3±16.4 *vs.* 117.7±24.3 g/L, P=0.003) (*Table 1*).

In general, there were significant differences in serum creatinine, urinary protein, and the estimated glomerular filtration rate (eGFR) in the four groups of patients with differing degrees of C3dg and MAC depositions. Further analyses showed that compared with patients with neither depositions, patients with ++ C3dg or MAC staining depositions had significantly higher serum creatinine, urinary protein, and SLEDAI levels and significantly lower eGFRs and serum C3 and C4 levels (all P<0.05) (*Table 2*).

In a further investigation of patients with two types of deposition and patients with only one or no complement deposition, significant differences were found for serum creatinine, urinary protein, serum C3 and C4, SLEDAI, and eGFR in the four study groups (*Table 3*). In particular, when comparing patients with only C3d deposition and patients with two types of complement protein deposition, the latter's urinary protein, RBC, WBC, and SLEDAI levels were significantly increased, but serum C3 and C4 levels were lower (all P<0.05).

Comparison of renal histopathology

Patients with C3dg depositions had a significantly greater proportion of type II (P=0.005), type III (P=0.029), and

Table 1 Baseline data in patients with and without C3dg or MAC depositions on renal histology

Variables	C3dg			MAC		
	With deposition (n=48)	Without deposition (n=30)	P	With deposition (n=48)	Without deposition (n=30)	P
Clinical evaluation						
Age (years)	12.3±2.7	11.9±3.2	0.678	12.0±2.9	12.4±3.0	0.838
Gender (male/female), (% for male)	10/38 (20.8)	5/25 (16.7)	0.650	8/40 (16.7)	7/21 (23.3)	0.379
Duration of LN (years)	13.0±19.4	12.0±20.8	0.940	12.0±18.6	13.6±22.0	0.464
Fever (non-infectious)	13 (27.1)	11 (36.7)	0.372	12 (25.0)	12 (40.0)	0.163
Malar rash	28 (58.3)	14 (46.7)	0.315	16 (33.3)	16 (53.3)	0.081
Photosensitivity	30 (62.5)	15 (50.0)	0.277	18 (37.5)	16 (53.3)	0.170
Oral ulcer	21 (43.8)	16 (53.3)	0.410	20 (41.7)	17 (56.7)	0.197
Alopecia	16 (33.3)	12 (40.0)	0.550	15 (31.3)	13 (43.3)	0.279
Arthralgia	6 (12.5)	5 (16.7)	0.607	7 (14.6)	4 (13.3)	1.000
Serositis	10 (20.8)	8 (26.7)	0.552	9 (18.8)	9 (30.0)	0.251
Neurologic disorder	10 (20.8)	1 (3.3)	0.068	11 (22.9)	1 (3.3)	0.044
Anemia	24 (50.0)	15 (50.0)	1.000	25 (52.1)	14 (46.7)	0.642
Acute renal failure	13 (27.1)	1 (3.3)	0.018	13 (27.1)	1 (3.3)	0.018
Nephrotic syndrome	37 (77.1)	14 (46.7)	0.006	36 (75.0)	15 (50.0)	0.024
SLEDAI	18.2±6.5	11.4±7.4	0.382	18.5±6.2	10.9±7.3	0.346
Laboratory assessment						
Leukocytopenia	15 (31.3)	6 (20.0)	0.276	17 (35.4)	4 (13.3)	0.061
Thrombocytopenia	5 (10.4)	1 (3.3)	0.481	2 (4.2)	4 (13.3)	0.298
Hematuria	26 (54.2)	12 (40.0)	0.223	25 (52.1)	13 (43.3)	0.452
Hemoglobin (g/L)	109.0±19.1	114.9±21.9	0.337	107.3±16.4	117.7±24.3	0.003
CRP (mg/L)	8.7±2.9	9.9±5.4	0.026	9.5±4.9	8.5±1.9	0.029
ESR (mm/h)	36.7±26.4	38.3±29.9	0.514	40.6±26.3	32.1±29.3	0.727
Serum creatinine (μmol/L)	65.5±20.6	47.9±11.8	0.001	63.9±21.5	50.5±12.4	0.001
BUN (mmol/L)	8.67±8.5	6.7±4.3	0.210	8.1±5.5	7.5±9.4	0.966
Urinary protein (g/24 h)	2.9±2.2	0.8±1.9	0.003	3.1±2.4	0.5±0.6	<0.001
Decrease of serum C3 level (%)	38 (79.2)	11 (36.7)	<0.001	38 (79.2)	11 (36.7)	<0.001
Decrease of serum C4 level (%)	33 (68.8)	10 (33.3)	0.002	32 (66.7)	11 (36.7)	0.010
eGFR (mL/min/1.73 m^2)	122.0±35.9	157.1±41.71	0.810	125.1±41.2	152.1±37.4	0.137
Urinary RBC (unit/HP)	67.2±75.6	20.9±40.1	<0.001	70.0±74.3	16.5±38.1	<0.001
Urinary WBC (unit/HP)	16.6±24.5	4.8±12.4	0.001	18.2±25.5	2.2±1.6	<0.001
ANA (+)	48 (100.0)	30 (100.0)		48 (100.0)	30 (100.0)	
Anti-dsDNA antibody (+)	39 (81.3)	19 (63.3)	0.078	38 (79.2)	20 (66.7)	0.219
Anti-SSA antibody (+)	17 (35.4)	12 (40.0)	0.684	14 (29.2)	15 (50.0)	0.064
Anti-SSB antibody (+)	1 (2.1)	5 (16.7)	0.056	1 (2.1)	5 (16.7)	0.056
Anti-Smith antibody (+)	12 (25.0)	5 (16.7)	0.386	11 (22.9)	6 (20.0)	0.761

Table 1 (*continued*)

Table 1 (*continued*)

Variables	C3dg			MAC		
	With deposition (n=48)	Without deposition (n=30)	P	With deposition (n=48)	Without deposition (n=30)	P
Pathological characteristics						
ISN/RPS classification						
II	4 (8.3)	11 (36.7)	0.005	4 (8.3)	11 (36.7)	0.005
III	4 (8.3)	9 (30.0)	0.029	8 (16.7)	5 (16.7)	1.00
IV	36 (75.0)	7 (23.3)	<0.001	34 (70.8)	9 (30.0)	<0.001
V	4 (8.3)	3 (10.0)	1.00	2 (4.2)	5 (16.7)	0.141
Activity indices score	9.7±3.6	5.0±2.3	0.037	9.8±3.4	4.8±2.1	0.051
Endocapillary hypercellularity	2.6±0.6	1.3±0.5	0.171	2.6±0.6	1.3±0.6	0.466
Cellular crescents	3.0±1.7	0.3±0.9	<0.001	3.0±1.6	0.3±0.9	<0.001
Karyorrhexis/fibrinoid necrosis	0.7±1.0	0.7±1.1	0.939	0.9±1.2	0.5±0.9	0.005
Subendothelial hyaline deposits	0.7±0.8	0.2±0.6	0.031	0.6±0.8	0.3±0.6	0.156
Interstitial inflammatory cell infiltration	1.5±0.7	1.3±0.6	0.072	1.4±0.7	1.3±0.6	0.351
Glomerular leukocyte infiltration	1.3±0.8	1.2±0.6	0.049	1.3±0.8	1.1±0.6	0.028
Chronicity indices score	1.1±1.5	1.1±1.5	0.752	1.0±1.4	1.2±1.6	0.960
Glomerular sclerosis	0.4±0.6	0.3±0.7	0.752	0.3±0.6	0.4±0.7	0.146
Fibrous crescents	0.3±0.6	0.3±0.5	0.262	0.4±0.6	0.2±0.4	0.074
Tubular atrophy	0.1±0.3	0.1±0.6	0.833	0.1±0.3	0.2±0.4	0.118
Interstitial fibrosis	0.2±0.4	0.4±0.6	0.016	0.2±0.4	0.4±0.6	0.002

Data were presented as mean ± SD or n (%). MAC, membrane attack complex; LN, Lupus nephritis; SLEDAI, systemic lupus erythematosus disease activity index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rates; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; ANA, antinuclear antibody; ISN/RPS, International Society of Nephrology/Renal Pathology Society.

type IV LN (P<0.001) than patients without C3dg deposits. Patients with C3dg deposition also had significantly higher cellular crescents, subendothelial hyaline deposits, glomerular leukocyte infiltration, and activity index scores (all P<0.05). Similarly, patients with MAC deposition had a significantly greater proportion of type IV LN (P<0.001). They also had significantly higher cellular crescents, fibrinoid necrosis, glomerular leukocyte infiltration, and interstitial fibrosis (all P<0.05) (*Table 1*).

Overall, there were significant differences in activity index scores and cellular crescents in all four groups of patients with varying degrees of C3dg deposition. On the other hand, there were no significant differences in glomerular category or interstitial inflammation and vascular lesion scores between patients with and without MAC deposition. Those with ++ MAC-stained deposition had a greater proportion of interstitial fibrosis (P=0.002) (*Table 2*).

On further investigation of patients with both C3dg and MAC depositions *vs.* those with only one or no complement deposition, significant differences were found in the four study groups (*Table 3*). In particular, when comparing patients with only C3dg deposition *vs.* those with deposits of both complement proteins, the latter showed significantly higher levels of interstitial inflammatory cell infiltration and glomerular leukocyte infiltration but lower tubular atrophy (all P<0.05). Similarly, when comparing patients with only MAC deposition *vs.* those with deposits of both complement proteins, the latter showed significantly higher levels of interstitial inflammatory cell infiltration (all P<0.05).

The treatment follow-up of C3dg/MAC positive patients at 6 and 12 months

All patients received steroids as part of their induction

Table 2 Clinical and pathological characteristics of patients with different degrees of C3dg or MAC depositions in kidney tissue

Variables	C3gd				MAC			
	Without deposition (n=30)	With deposition + (n=16)	With deposition ++ (n=23)	With deposition +++ (n=9)	Without deposition (n=31)	With deposition + (n=19)	With deposition ++ (n=21)	With deposition +++ (n=8)
Clinical characteristics								
Serum creatinine (μmol/L)	47.9±11.8	53.4±11.1	68.7±19.9* (P<0.001)# (P=0.005)	78.7±25.5* (P<0.001)# (P<0.001)	50.5±12.4	57.7±22.2	64.5±20.0* (P=0.008)	77.1±19.8* (P<0.001)# (P=0.013)
BUN (mmol/L)	6.7±4.3	10.1±12.9	9.1±5.8	5.2±1.5	7.5±9.4	7.4±4.1	8.1±4.8	10.1±9.4
Urinary protein (g/24 h)	0.76±1.94	1.5±1.6	3.2±1.8* (P<0.001)# (P=0.006)	4.8±2.3* (P<0.001)# (P<0.001)& (P=0.037)	0.5±0.7	1.7±1.7* (P=0.015)	3.7±2.1* (P<0.001)# (P<0.001)	5.1±3.0* (P<0.001)# (P<0.001)
Serum C3 (g/L)	0.8±0.3	0.6±0.3	0.4±0.2* (P<0.001)# (P=0.012)	0.5±0.2* (P=0.008)	0.7±0.3	0.7±0.4	0.4±0.2* (P<0.001)# (P=0.004)	0.4±0.1* (P=0.001)# (P=0.005)
Serum C4 (g/L)	0.2±0.1	0.2±0.2	0.1±0.1* (P=0.012)	0.06±0.07* (P=0.015)	0.2±0.2	0.1±0.1	0.07±0.07* (P=0.001)	0.05±0.07* (P=0.007)
eGFR (mL/min/1.73 m^2)	157.1±41.7	142.8±24.3	116.0±38.9* (P<0.001)# (P=0.028)	100. 6±28.9* (P<0.001)# (P=0.007)	152.1±37.44	135.6±37.5	127.9±43.2* (P=0.03)	93.0±30.8* (P<0.001)# (P=0.011)& (P=0.032)
Urinary RBC (unit/HP)	20.9±40.1	55.1±68.5	68.0±66.1* (P=0.01)	86.8±109.6* (P=0.009)	16.50±38.10	53.1±57.2	77.4±85.8* (P=0.001)	90.8±78.8* (P=0.004)
Urinary WBC (unit/HP)	4.8±12.4	14.6±20.9	17.0±24.5* (P=0.039)	19.0±32.4	2.17±1.62	15.4±19.7* (0.029)	18.9±29.4* (P=0.005)	23.0±29.0* (P=0.012)
SLEDAI	11.4±7.4	17.3±7.6* (P=0.007)	19.0±6.1* (P<0.001)	17.7±5.7* (P=0.019)	10.93±7.25	16.2±6.8* (P=0.007)	20.6±5.3* (P<0.001)# (P=0.038)	18.5±5.3* (P=0.005)
Pathological characteristics								
Activity indices score	5.0±2.2	7.6±2.5* (P=0.004)	10.1±3.7* (P<0.001)# (P=0.01)	12.2±3.0* (P<0.001)# (P<0.001)	7.2±3.8	8.6±5.1	7.3±2.3	9.4±3.4
Endocapillary hypercellularity	1.3±0.5	2.4±0.7* (P<0.001)	2.7±0.5* (P<0.001)	2.9±0.3* (P<0.001)	2.0±0.9	2.2±0.9	2.1±0.9	2.38±0.74
Cellular crescents	0.3±0.9	1.9±1.2* (P<0.001)# (P=0.003)	3.1±1.5* (P<0.001)# (P=0.003)	4.4±1.7* (P<0.001)# (P<0.001)& (P=0.009)	1.5±1.8	2.2±2.0	1.8±1.7	3.0±2.4
Karyorrhexis/fibrinoid necrosis	0.7±1.1	0.4±0.8	0.7±1.2	1.4±0.9# (P=0.016)	0.6±1.1	0.7±1.2	0.7±1.0	1.0±1.1
Subendothelial hyaline deposits	0.2±0.6	0.6±0.6	0.7±0.9* (P=0.039)	0.7±0.7	0.5±0.6	0.7±1.1	0.3±0.6	0.6±0.7
Interstitial inflammatory cell infiltration	1.3±0.6	1.2±0.7	1.7±0.8# (P=0.029)	1.3±0.5	1.3±0.6	1.6±0.9	1.4±0.6	1.1±0.4

Table 2 (*continued*)

Table 2 (*continued*)

Variables	C3gd				MAC			
	Without deposition (n=30)	With deposition + (n=16)	With deposition ++ (n=23)	With deposition +++ (n=9)	Without deposition (n=31)	With deposition + (n=19)	With deposition ++ (n=21)	With deposition +++ (n=8)
Glomerular leukocyte infiltration	1.2±0.6	1.1±0.7	1.3±0.9	1.4±0.9	1.3±0.7	1.3±1.0	1.1±0.6	1.3±0.7
Chronicity indices score	1.1±1.5	1.3±1.5	0.7±1.3	1.6±1.7	1.2±1.6	1.3±1.7	0.4±0.9	1.6±1.4
Glomerular sclerosis	0.3±0.7	0.4±0.6	0.2±0.4	0.8±0.8[&] (P=0.014)	0.4±0.7	0.4±0.7	0.2±0.4	0.4±0.5
Fibrous crescents	0.3±0.5	0.4±0.5	0.3±0.7	0.4±0.5	0.2±0.4	0.4±0.5	0.2±0.4	0.6±1.1
Tubular atrophy	0.1±0.4	0.2±0.4	0.1±0.3	0.1±0.3	0.2±0.4	0.2±0.4	0.1±0.2	0
Interstitial fibrosis	0.4±0.6	0.3±0.5	0.2±0.4	0.2±0.4	0.4±0.6	0.3±0.5	0* (P=0.002)	0.6±0.5[&] (P=0.001)

Data were presented as mean ± SD. *, compared to the without deposition group; [#], compared to the with deposition group +; [&], compared to the with deposition group ++. MAC, membrane attack complex; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; RBC, red blood cell; WBC, white blood cell; SLEDAI, systemic lupus erythematosus disease activity index.

therapy (as shown in *Table 4*), defined as between 0 and 6 months. A complete response (CR) was defined as a urinary protein creatinine ratio (UPCR) <500 mg/g, eGFR decrease <10% of pre-treatment level, or eGFR ≥90 mL/min per 1.73 m^2, and was not considered a treatment failure. Treatment aims to preserve and improve kidney function, represented by at least a 50% reduction in proteinuria 6 months after treatment and a UPCR level <500 mg/g at 12 months after treatment. Thirty-one out of 48 patients who were glomerular C3dg positive received cyclophosphamide. Similarly, 30 out of 48 patients who were glomerular MAC positive received cyclophosphamide. At 12 months, there were more non-responders to therapy in the C3dg positive group (22.9% *vs.* 3.3%, P=0.017) and in the MAC positive group (22.9% *vs.* 0%, P<0.001) than in the negative groups.

Receiver operating characteristic (ROC) curves and the predictive value of C3dg and MAC depositions

We used the treatment outcome at 12 months as the primary outcome variable and constructed ROC curves with C3dg deposition, MAC deposition, C3dg deposition with MAC simultaneously, and C3dg or MAC deposition as four independent predictors to compare the area under the ROC curve of the four prediction models. The modeling results showed that the area under the curve was 0.868 when C3dg deposition was an independent predictor, 0.939 when MAC deposition was an independent predictor, 0.958 when C3dg simultaneous deposition with MAC (in series) was an independent predictor, and 0.925 when C3dg or MAC deposition (in parallel) was an independent predictor. When C3dg simultaneous deposition with MAC (in series) was the independent predictor, the model had the highest sensitivity of 100%. When C3dg or MAC deposition (in parallel) was an independent predictor, the model had the highest specificity, at 75% (*Figure 1*).

Discussion

Due to the importance of complement binding fragments in LN, our center began to detect C3dg and MAC in kidney tissues from 2018. In this study, we explored the distribution of complement split C3dg and MAC depositions in the pathogenesis of LN. Our results indicate that C3dg and MAC depositions may be very important indicators of LN.

After the complement system activation, the activation of immune complexes can drive type III hypersensitivity, leading to inflammatory responses in target tissues (18-20). Tissue targeting is accomplished through the binding of the fusion protein to complement C3 fragments that contain a surface-exposed C3d domain and which are covalently deposited on tissues where complement is being activated (21). Complement activation on cell surfaces leads to the massive deposition of C3dg, the main complement opsonin. Complement receptor type 3 (CR3) fosters pathogen opsonophagocytosis by macrophages and the stimulation of

Table 3 Characteristics of patients according to C3dg and/or MAC deposition

Variables	Both C3dg and MAC deposition (n=40)	Only C3dg deposition (n=8)	Only MAC deposition (n=8)	No C3dg and MAC deposition (n=22)
Clinical characteristics				
Serum creatinine (μmol/L)	67.3±21.3	56.4±13.9	46.9±13.5* (P=0.004)	48.3±11.4* (P<0.001)
BUN (mmol/L)	7.9±5.2	12.8±17.6	9.5±7.0	5.6±2.2$^{\#}$ (P=0.016)
Urinary protein (g/24 h)	3.4±2.1	0.9±1.1* (P=0.002)	2.0±3.6	0.3±0.2* (P<0.001)$^{\&}$ (P=0.034)
Serum C3 (g/L)	0.5±0.2	0.7±0.4* (P=0.015)	0.9±0.5* (P<0.001)	0.72±0.23* (P<0.001)
Serum C4 (g/L)	0.1±0.1	0.3±0.2* (P<0.001)	0.2±0.2* (P=0.002)	0.2±0.1* (P=0.004)$^{\#}$ (P=0.032)
eGFR (mL/min/1.73 m^2)	119.1±36.3	136.4±32.4	155.1±53.2* (P=0.018)	157.8±38.2* (P<0.001)
Urinary RBC (unit/HP)	75.4±77.7	26.5±49.1 12.86±33.91* (P=0.049)	43.1±49.4	12.9±33.9* (P<0.001)
Urinary WBC (unit/HP)	19.5±26.0	2.3±1.8* (P=0.0031)	12.0±23.5	2.1±1.6* (P=0.002)
SLEDAI	19.1±5.6	13.9±8.8* (P=0.043)	15.6±8.3* (P=0.043)	9.9±6.5* (P<0.001)$^{\&}$ (P=0.036)
Pathological characteristics				
Activity indices score	10.5±3.1	5.5±2.4* (P<0.001)	6.3±2.3* (P<0.001)	4.6±2.0* (P<0.001)
Endocapillary hypercellularity	2.8±0.4	2.00±0.75* (P<0.001)	1.9±0.6* (P<0.001)	1.0±0.2* (P<0.001)$^{\#}$ (P<0.001)$^{\&}$ (P<0.001)
Cellular crescents	3.4±1.4	1.0±1.5* (P<0.001)	1.3±1.5* (P<0.001)	0* (P<0.001)$^{\#}$ (P=0.047)$^{\&}$ (P=0.014)
Karyorrhexis/fibrinoid necrosis	0.8±1.1	0.3±0.71	1.0±1.5	0.5±0.9
Subendothelial hyaline deposits	0.7±0.8	0.5±0.5	0.1±0.4	0.5±0.5* (P=0.038)
Interstitial inflammatory cell infiltration	1.5±0.7	1.0±0.5* (P=0.035)	1.0±0.0* (P=0.035)	1.5±0.6
Glomerular leukocyte infiltration	1.4±0.8	0.8±0.5* (P=0.029)	1.0±0.5	1.2±0.6
Chronicity indices score	0.9±1.4	1.9±1.6	1.4±1.7	1.0±1.5
Glomerular sclerosis	0.3±0.6	0.8±0.7	0.4±0.5	0.3±0.7
Fibrous crescents	0.4±0.6	0.3±0.5	0.4±0.5	0.2±0.4
Tubular atrophy	0.1±0.3	0.4±0.5* (P=0.021)	0.3±0.5	0.1±0.3
Interstitial fibrosis	0.2±0.4	0.5±0.5	0.4±0.5	0.4±0.6

Data were presented as mean ± SD. *, compared to the combined C3dg and MAC deposition group; $^{\#}$, compared to the only C3dg deposition group; $^{\&}$, Compared to the only MAC deposition group. MAC, membrane attack complex; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; RBC, red blood cell; WBC, white blood cell; SLEDAI, systemic lupus erythematosus disease activity index.

Table 4 Comparison of therapies between C3dg/MAC positive versus negative patients at 6 and 12 months

	C3dg			MAC		
	Positive for glomerular (n=48)	Negative for glomerular (n=30)	P	Positive for glomerular (n=48)	Negative for glomerular (n=30)	P
Therapy from 0–6 months, n (%)						
Cyclophosphamide	31 (64.6)	10 (33.3)	0.007	30 (62.5)	11 (36.7)	0.026
Mycophenolate mofetil	10 (20.8)	13 (43.3)	0.034	8 (16.7)	15 (50.0)	0.002
Multitarget therapy	4 (8.3)	4 (13.3)	0.479	5 (10.4)	3 (10.0)	1.0
Tacrolimus	3 (6.3)	4 (13.3)	0.511	5 (10.4)	1 (3.3)	0.481
Hydroxychloroquine at 6 months	48 (100.0)	30 (100.0)		48 (100.0)	30 (100.0)	
Noncompliance at 6 months	43 (89.6)	30 (100.0)	0.150	42 (87.5)	30 (100.0)	0.077
Response to therapy at 6 months[a], n (%)						
No	0	0		0	0	
Yes	48 (100.0)	30 (100.0)		48 (100.0)	30 (100.0)	
Therapy from 6–12 months, n (%)						
Cyclophosphamide	0	0		0	0	
Mycophenolate mofetil	45 (93.8)	23 (76.7)	0.028	42 (87.5)	28 (93.3)	0.658
Multitarget therapy	2 (4.2)	3 (10.0)	0.584	3 (6.3)	1 (3.3)	0.968
Tacrolimus	3 (6.3)	4 (13.3)	0.511	5 (10.4)	1 (3.3)	0.481
Noncompliance at 12 months	32 (66.7)	28 (93.3)	0.015	35 (72.9)	29 (96.7)	0.018
Response to therapy at 12 months[b], n (%)						
No	11 (22.9)	1 (3.3)	0.017	11 (22.9)	0	<0.001
Yes	37 (77.1)	29 (96.7)	0.044	37 (77.1)	30 (100.0)	<0.001

[a], response to therapy at 6 months is defined as a reduction in proteinuria of at least 50%; [b], response to therapy at 12 months is defined as complete response. MAC, membrane attack complex.

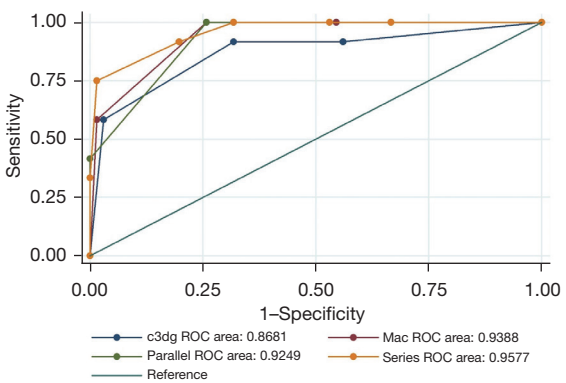

Figure 1 ROC curves and predictive value of C3dg and MAC deposition. ROC, receiver operating characteristic; MAC, membrane attack complex.

adaptive immunity by complement-opsonized antigens that recognize the complement opsonin (22). In the European multinational initial cohort of 200 newly diagnosed (within two years) lupus patients, many had active disease, and only 54% had low C3 and/or C4 levels at baseline (23). In a recent study, although 30% of patients converted to SLE at follow-up, only 36% of patients historically had low C3 and/or C4 levels (24). Low complement level as a marker of disease activity in SLE is not reliable because the level is persistently low or normal, may not be related to disease activity, and is not sensitive to predicting disease onset (25,26). We found that patients with MAC deposition had a greater proportion of neurological disorders. Patients with MAC and C3dg deposition on renal histopathology had significantly

higher levels of serum creatinine, urinary protein, urinary RBC, and urinary WBC. There were significant differences in serum creatinine, urinary protein, and eGFR in all four groups of patients with differing degrees of C3d and MAC deposition. Complement C3dg and MAC fragments are expected to be used as biomarkers for LN.

There were significantly more patients with C3dg and MAC depositions that had type IV LN (P<0.001). They also had significantly higher cellular crescents, fibrinoid necrosis, glomerular leukocyte infiltration, and interstitial fibrosis. These findings indicate that C3dg and MAC depositions can sensitively reflect the severity of disease in children with LN. Lupus children with more C3dg and MAC depositions require more aggressive treatment. The differences between the treatment outcomes at 6 and 12 months reminded us that although lupus children with C3dg and MAC depositions are given active hormone and immunosuppressive therapy at the initial stage of treatment, the effect is still not good. The deposition of C3dg and MAC in the kidney may indicate a poor prognosis in LN.

C3dg and MAC are also good clinical predictors of treatment outcome in LN. When C3d simultaneous deposition with MAC (in series) was the independent predictor, the model had the highest sensitivity at 100%. When C3d or MAC deposition (in parallel) was an independent predictor, the model had the highest specificity, at 75%.

Conclusions

This study suggests that C3dg and MAC depositions may be potential biomarkers for greater disease severity and tissue injury in LN. MAC and C3dg staining may be useful in routine studies of lupus biopsies to identify patients at risk of severe disease who may need more aggressive treatment.

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Footnote

Reporting Checklist: The authors have completed the STARD reporting checklist. Available at https://tp.amegroups.com/article/view/10.21037/tp-22-310/rc

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Ethical Statement: The authors are 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. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study protocol was approved by the Ethics Committee of Children's Hospital of Fudan University (No. [2021]240), and individual consent for this retrospective analysis was waived.

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