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Research Article

Co-Deposition of IgM and C3 May Indicate Unfavorable Renal Outcomes in Adult Patients with Primary Focal Segmental Glomerulosclerosis

Safak Mirioglu[a] Yasar Caliskan[b] Yasemin Ozluk[c] Ahmet Burak Dirim[a] Zulal Istemihan[a] Arif Akyildiz[a] Halil Yazici[b] Aydin Turkmen[b] Isin Kilicaslan[c] Mehmet Sukru Sever[b]

[a]Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; [b]Division of Nephrology, Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; [c]Department of Pathology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey

Keywords
Focal segmental glomerulosclerosis · IgM · C3 · Complement system

Abstract
Background/Aims: We aimed to investigate the effects of glomerular IgM and C3 deposition on outcomes of adult patients with primary focal segmental glomerulosclerosis (FSGS). ***Methods:*** In this retrospective analysis, 86 consecutive adult patients with biopsy-proven primary FSGS were stratified into 3 groups according to their histopathological features: IgM– C3–, IgM+ C3–, and IgM+ C3+. Primary outcome was defined as at least a 50% reduction in baseline estimated glomerular filtration rate (eGFR) or development of kidney failure, while complete or partial remission rates were secondary outcomes. ***Results:*** Glomerular IgM deposits were found in 44 (51.1%) patients, 22 (25.5%) of which presented with accompanying C3 deposition. Patients in IgM+ C3+ group had higher level of proteinuria (5.6 g/24 h [3.77–8.5], p = 0.073), higher percentage of segmental glomerulosclerosis (20% [12.3–27.2], p = 0.001), and lower levels of eGFR (69 ± 37.2 mL/min/1.73 m^2, p = 0.029) and serum albumin (2.71 ± 0.85 g/dL, p = 0.045) at the time of diagnosis. Despite 86.3% of patients in IgM+ C3+ group (19/22) received immunosuppressive treatment, the primary outcome was more common in patients in the IgM+ C3+ group compared with patients in IgM+ C3– and IgM– C3– groups (11 [50%] vs. 2 [9%] and 11 [26.1%] respectively [p = 0.010]). Complete or partial remission rates were lower in patients in the IgM+ C3+ group (5/22, 22.7%), as well (p = 0.043). Multivariate Cox regression analysis revealed that IgM and C3 co-deposition was an independent risk factor associated with primary outcome (hazard ratio 3.355, 95% CI 1.349–8.344, p = 0.009). ***Conclusions:*** Glomerular IgM and C3 co-deposition is a predictor of unfavorable renal outcomes in adult patients with primary FSGS.

Safak Mirioglu, MD
Istanbul University, Istanbul Faculty of Medicine
Department of Internal Medicine
Capa, Fatih, TR–34093 Istanbul (Turkey)
E-Mail smirioglu@gmail.com

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Introduction

Focal segmental glomerulosclerosis (FSGS), an important cause of end-stage renal disease in adults, is considered "a group of clinical-pathologic syndromes sharing a common glomerular lesion," whose pathology is based on an injury of podocytes caused by diverse insults to glomeruli [1]. Although several factors such as decreased nephron mass, mutations in podocyte proteins and circulating permeability factors, which alter the podocyte cytoskeleton and stability of foot processes, have been identified throughout the years; nearly 80% of the cases are considered to be idiopathic [1–5]. Treatment with immunosuppressive agents can be successful only in 50% of affected individuals with primary FSGS, and approximately 50% of patients who fail to achieve remission of their proteinuria will inexorably lead to end-stage renal disease over a 5–10 year period [1, 5]. Furthermore, disease recurrence still remains as an important risk factor of graft loss in kidney transplant recipients [6, 7]. Nearly 10% of patients are expected to experience disease recurrence in 10 years after renal transplantation, which leads to graft loss in 43% of patients with recurrent FSGS [7].

Diagnosis is based on histopathological findings of focal and segmental sclerosis in light microscopic evaluation with diffuse effacement of podocyte foot processes by electron microscopy, and usually negative immunofluorescence studies [1, 5, 8]. However, segmental deposition of immunoglobulin M (IgM) and complement component 3 (C3) in the sclerotic segments, even in mesangial portions and unaffected areas of glomeruli has been shown in previous studies [5, 9–11]. Until recently, significance of these pathological findings has been unclear, yet Strassheim et al. [12] demonstrated that IgM deposits activated the classical and alternative pathways (APs) of the complement system within the glomerulus and contributed to disease progression in adriamycin nephropathy, an animal model of FSGS. The importance of these depositions on the disease course of patients with primary FSGS is still obscure [8, 13]; we therefore aimed to investigate the significance of glomerular IgM and C3 deposition on clinical and pathological features and outcomes of adult patients with primary FSGS.

Materials and Methods

Patients

A total of 86 consecutive adult patients who were diagnosed with biopsy-proven primary FSGS from 1997 to 2016 and had a follow-up period of at least 6 months or showed progression to primary study outcomes regardless the duration of follow-up were evaluated in this retrospective cohort study. Individuals with familial or genetic FSGS, and secondary FSGS which could be attributable to obesity, diabetes, viral diseases, use of medications or illicit drugs, and other glomerular diseases were excluded. Moreover, patients with a category G5 chronic kidney disease (CKD; estimated glomerular filtration rate [eGFR] <15 mL/min/1.73 m^2) at the time of diagnosis were not included in the analysis [14].

Demographic, clinical, and laboratory characteristics of the patients were obtained using their medical records. Patients with systolic blood pressure (BP) ≥140 mm Hg or diastolic BP ≥90 mm Hg or those using antihypertensive treatment were considered hypertensive. Proteinuria levels were determined by using urinary protein-to-creatinine ratio in the first morning specimens. Nephrotic-range proteinuria was defined as proteinuria level of ≥3 g/24 h in the absence of nephrotic syndrome. eGFRs of patients were calculated using CKD Epidemiology Collaboration formula [15].

Our study complied with good medical and laboratory practices and the recommendations of the World Medical Association Declaration of Helsinki: Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects [16].

Histopathological Evaluation

Adequate renal biopsy specimens having at least 8 glomeruli were evaluated using light microscopy, immunofluorescence, and in some cases, electron microscopy. All histochemical and immunofluorescence stains were prepared using 3–4 micrometer sections. 0.4–0.6 cm unfixed tissue was frozen with liquid nitrogen for immunofluorescence staining (IgG, IgM, IgA, C1q, C3 and fibrinogen). Immunofluorescence staining was graded with a semiquantitative scale from 0 to 3 (0, negative; 1, weak; 2, moderate and 3, strong staining). Intensities of IgG and IgA were trace to 1 in patients with these deposits. Segmental deposition of IgM and C3 in the sclerotic glomeruli and segmental or global deposition of IgM and C3 in the mesangium of unaffected areas were taken into consideration. Remaining tissues were fixed in Hollande's fixative, embedded in paraffin, and processed routinely for light microscopic evaluation (hematoxylin and eosin, periodic acid-Schiff, methenamine silver-periodic acid, Masson trichrome and Congo red). Interstitial fibrosis and tubular atrophy were also graded using a semiquantitative scale from 0 to 3: zero, normal; one (mild), <25% of interstitium; 2 (moderate), 25–50% and 3 (severe), >50%. Specimens were fixed in glutaraldehyde and embedded in Epon for electron microscopy. A nephropathologist (Y.O.) who was blinded to all clinical data confirmed the diagnoses by reviewing the biopsy samples.

Treatment

All patients were started on angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs) unless having a category G4 CKD and maintained with these agents as long as they were tolerable [14]. Patients with nephrotic syndrome or nephrotic-range proteinuria were initially treated using corticosteroids on a daily basis. Calcineurin inhibitors, mycophenolate mofetil (MMF), or azathioprine (AZA) were initiated in patients with corticosteroid-resistant disease, or intolerance or contraindications to use of corticosteroids. Oral prednisolone was begun at 1 mg/kg/day and used for up to 16 weeks with tapering. Cyclosporine was started at 3 mg/kg/day with a target trough level of 100–150 ng/mL, and tacrolimus at 0.1 mg/kg/day with a target trough level of 5–10 ng/mL and used for 6–12 months. MMF was initiated at 1 g/day, and then increased to 2 g/day over 2 weeks by monitoring complete blood count. If used, the dosage of AZA was 2 mg/kg/day. Rituximab was not used in the present series. Patients with subnephrotic proteinuria were managed with ACEi/ARBs and dietary sodium restriction at first, and in the face of a worsening kidney function and/or proteinuria, immunosuppressive agents were also taken into consideration for these patients. After 2012, patients were treated according to the Kidney Disease Improving Global Outcomes Clinical Practice Guideline for Glomerulonephritis [17].

Study Outcomes

Primary outcomes of the study were defined as at least a 50% reduction in baseline eGFR or development of kidney failure, which was described as a category G5 CKD (eGFR <15 mL/min/1.73 m^2) [14]. Secondary outcomes were defined as complete or partial remission. Complete remission was determined as proteinuria <0.5 g/24 h and an eGFR of ≥60 mL/min/1.73 m^2 (or a return of ±15% of baseline values in those with eGFR <60 mL/min/1.73 m^2). Partial remission was defined as a proteinuria reduction of >50% (and a proteinuria value of <3 g/24 h in patients with nephrotic-range proteinuria at baseline) and stabilization (±25%) or improvement in renal function. Follow-up period was calculated as the time interval between diagnostic renal biopsy and last outpatient visit, primary outcome, or death.

Associations of clinical features (age, gender, BP, eGFR, hemoglobin, albumin, and proteinuria) and other histopathological lesions (percentage of global and segmental glomerulosclerosis, interstitial fibrosis and tubular atrophy, presence of tip and collapsing lesions) with primary outcomes were also evaluated.

Table 1. Baseline demographic, clinical, and laboratory characteristics of patients with regard to IgM and C3 deposition

Characteristic	IgM– C3– (*n* = 42)	IgM+ C3– (*n* = 22)	IgM+ C3+ (*n* = 22)	*p* value
Male/female, *n*	26/16	8/14	10/12	0.125
Age, years, mean ± SD	39.2±11.7	33.8±12.4	33.2±13	0.107
Hypertension, *n* (%)	23 (54.7)	12 (54.5)	15 (68.1)	0.542
Clinical presentation, n (%)				
Nephrotic syndrome or nephrotic-range proteinuria	26 (61.9)	15 (68.1)	21 (95.4)	0.016
Subnephrotic proteinuria	16 (38)	7 (31.8)	1 (4.5)	
Laboratory data				
Proteinuria, g/24 h, median (IQR)	3.5 (2.15–7.92)	4.05 (2.3–7.2)	5.6 (3.77–8.5)	0.073
Serum creatinine, mg/dL, median (IQR)	1 (0.8–1.42)	0.85 (0.7–1)	1.25 (0.8–1.8)	0.021
eGFR, mL/min/1.73 m^2, mean ± SD	81.2±34	96.8±30.1	69±37.2	0.029
Serum albumin, g/dL, mean ± SD	3.28±0.84	3.1±0.86	2.71±0.85	0.045
Hemoglobin, g/dL, mean ± SD	13.2±2.3	12.6±2.6	12.8±3.4	0.617
Stages of CKD, mL/min/1.73 m^2, n (%)				
1 (≥90)	18 (42.8)	15 (68.1)	5 (22.7)	0.088
2 (60–89)	12 (28.5)	4 (18.1)	7 (31.8)	
3 (30–59)	8 (19)	3 (13.6)	6 (27.2)	
4 (15–29)	4 (9.5)	0	4 (18.1)	

IQR, interquartile range; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease.

Statistical Analyses

Results are expressed as mean ± SD when normally distributed or as median (interquartile range [IQR]) when not normally distributed. Appropriate parametric and nonparametric tests were used accordingly. Comparisons of continuous variables between the groups were evaluated by using *t* tests or the Mann-Whitney U test, where appropriate. Differences in the proportions of different patient groups were compared using the chi-square or Fisher's exact test. Periods of renal survival were analyzed using Kaplan-Meier curves and this period for each patient was computed from baseline evaluation to the last follow-up or primary outcome. Relationships were determined by Pearson's correlation coefficient and Spearman *rho* was used for nonparametric correlations. Variables found to affect the renal survival in univariate analysis (a *p* value of ≤0.05 for each variable) were included in the multivariate Cox proportional hazards model. Results of Cox regression models were described as hazard ratios (HRs) and 95% CIs. Receiver operating characteristic (ROC) analysis was used and area under the curve was calculated to determine the predictive power of clinical, laboratory and histopathological markers for the primary outcome. Statistical analyses were performed by using SPSS for Windows (SPSS version 21.0, IBM Corp., Armonk, NY, USA). Kaplan-Meier and ROC curves were computed with MedCalc for Windows (MedCalc version 18.0, MedCalc Software, Ostend, Belgium). All analyses were 2 sided and a *p* value of 0.05 or less was considered statistically significant.

This study was approved by the Istanbul Faculty of Medicine Ethical Committee, and registered with ClinicalTrials.gov, number NCT03126201.

Results

Demographic and Clinical Features

In total, 86 patients (44 male, 42 female) who were followed up for a median of 51 (IQR 17.75–93.25) months were included in the study. The mean age was 36.3 ± 12.4 years. All of

Table 2. Histopathological characteristics of patients with regard to IgM and C3 deposition

Characteristic	IgM– C3– (*n* = 42)	IgM+ C3– (*n* = 22)	IgM+ C3+ (*n* = 22)	*p* value
Global glomerulosclerosis, median (IQR)	7.1 (0–23.5)	7.9 (0–21.8)	12.4 (0–30.8)	0.671
Segmental glomerulosclerosis, median (IQR)	8 (0–14.8)	12.6 (8.9–19.8)	20 (12.3–27.2)	0.001
IgG deposition, *n* (%)	1 (2.3)	0	0	0.589
IgA deposition, *n* (%)	0	3 (13.6)	2 (9)	0.064
C1q deposition, *n* (%)	0	1 (4.5)	3 (13.6)	0.048
Collapsing lesion, *n* (%)	3 (7.1)	0	0	0.196
Tip lesion, *n* (%)	8 (19)	3 (13.6)	2 (9)	0.558
Interstitial fibrosis, n (%)				
None	20 (47.6)	11 (50)	8 (36.3)	0.592
Mild	16 (38)	10 (45.4)	12 (54.5)	
Moderate	6 (14.2)	1 (4.5)	2 (9)	
Severe	0	0	0	
Tubular atrophy, n (%)				
None	15 (35.7)	5 (22.7)	6 (27.2)	0.484
Mild	21 (50)	16 (72.7)	13 (59)	
Moderate	6 (14.2)	1 (4.5)	3 (13.6)	
Severe	0	0	0	

IQR, interquartile range.

the patients presented with proteinuria. Median serum creatinine, serum albumin and level of proteinuria were 0.8 (IQR 1–1.42) mg/dL, 3.1 (IQR 2.27–3.9) g/dL and 4 (IQR 2.49–7.82) g/24 h, respectively, at the time of diagnosis. Of these 86 individuals, 44 (51.1%) had IgM deposition in glomeruli. Patients with IgM deposition were further classified into 2 groups according to presence of glomerular C3 deposition: IgM+ C3– (*n* = 22, 25.5%) and IgM+ C3+ (*n* = 22, 25.5%). Baseline demographic, clinical, and laboratory features of these groups are summarized in Table 1.

Patients with IgM and C3 deposition were characterized by a higher level of serum creatinine (1.25 [IQR 0.8–1.8] vs. 0.85 [IQR 0.7–1] and 1 [IQR 0.8–1.42] mg/dL, respectively [*p* = 0.021]) and a lower level of serum albumin (2.71 ± 0.85 vs. 3.1 ± 0.86 and 3.28 ± 0.84 g/dL, respectively [*p* = 0.045]) as compared to patients in IgM+ C3– and IgM– C3– (*n* = 42, 48.8%) groups at the time of diagnosis. Also, patients in IgM+ C3+ group had higher levels of proteinuria when compared with patients in IgM+ C3– and IgM– C3– groups (5.6 [IQR 3.77–8.5] vs. 4.05 [IQR 2.3–7.2] and 3.5 [IQR 2.15–7.92] g/24 h respectively). Although this difference was not statistically significant (*p* = 0.073), more patients with IgM and C3 deposition presented with nephrotic syndrome or nephrotic-range proteinuria compared to patients in IgM+ C3– and IgM– C3– groups (21 [95.4%] vs. 15 [68.1%] and 26 [61.9%], respectively [*p* = 0.016]).

Histopathological Features

The percentage of global glomerulosclerotic lesions was not different among study groups (7.1% [IQR 0–23.5], 7.9% [IQR 0–21.8], and 12.4% [IQR 0–30.8] for IgM– C3–, IgM+ C3–, and IgM+ C3+ groups respectively [*p* = 0.671]); however, segmental glomerulosclerosis was more common in patients in IgM+C3+ group as compared to patients in IgM+ C3– and IgM– C3– groups (20% [IQR 12.3–27.2] vs. 12.6% [IQR 8.9–19.8] and 8% [IQR 0–14.8], respectively [*p* = 0.001]). Rates of interstitial fibrosis and tubular atrophy were similar among all study groups (*p* = 0.592 and *p* = 0.484, respectively). There were no significant differences among 3 study groups in terms of IgG (*p* = 0.589) and IgA (*p* = 0.064) deposition, yet more

Table 3. Treatment modalities and study outcomes in various groups

	IgM– C3– (*n* = 42)	IgM+ C3– (*n* = 22)	IgM+ C3+ (*n* = 22)	*p* value
Primary outcome, *n* (%)	11 (26.1)	2 (9)	11 (50)	0.010
Kidney failure, *n* (%)	6 (14.2)	2 (9)	8 (36.3)	0.040
Remission, n (%)				
No remission	22 (52.3)	9 (40.9)	17 (77.2)	0.154
Partial remission	8 (19)	4 (18.1)	2 (9)	
Complete remission	12 (28.5)	9 (40.9)	3 (13.6)	
Complete or partial remission (secondary outcome)	20 (47.6)	13 (59)	5 (22.7)	0.043
Follow-up time, months, median (IQR)	52.5 (16.75–88.5)	61.5 (16.75–132)	42 (33.25–89.25)	0.620
ACEi/ARB use, *n* (%)	39 (92.8)	20 (90.9)	17 (77.2)	0.166
Immunosuppressive treatment, *n* (%)*	25 (59.5)	11 (50)	19 (86.3)	0.030
Use of calcineurin inhibitors, *n* (%)	12 (28.5)	7 (31.8)	10 (45.4)	0.389

* Use of immunosuppressive agents was further described in Methods.
IQR, interquartile range; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

patients in IgM+ C3+ group (3/22) showed C1q deposition when compared to IgM+ C3– (1/22) and IgM– C3– (0/42) groups ($p = 0.048$). The intensity of IgM deposition showed a strong correlation with intensity of C3 deposition ($r = 0.699$, $p < 0.001$). Histopathological characteristics of the patients are shown in Table 2.

Study Outcomes

Twenty-four (27.9%) of 86 patients reached primary outcome after a median follow-up of 64.5 (IQR 16.5–120.75) months. Progression to kidney failure was noted in 16 patients (18.6%) after a median of 59 (IQR 13.5–120.75) months, and 8 patients (9.3%) had an at least a 50% reduction in baseline eGFR after a median of 69 (IQR 20.5–130.5) months of follow-up. Primary outcome was more common in patients in IgM+ C3+ group as compared to patients in IgM+ C3– and IgM– C3– groups (11 [50%] vs. 2 [9%] and 11 [26.1%], respectively [$p = 0.010$]). A considerable amount of patients in IgM+ C3+ group progressed to kidney failure when compared to patients in IgM+ C3– and IgM– C3– groups (8 [36.3%] vs. 2 [9%] and 6 [14.2%], respectively [$p = 0.040$]). Kaplan-Meier analysis revealed that 5-year and 10-year renal survival rates were 63.6 and 54.5%, 100 and 95.5%, and 92.9 and 85.7% for IgM+ C3+, IgM+ C3– and IgM– C3– groups respectively ($p = 0.002$; Fig. 1).

Considering the secondary outcome, complete or partial remission was less common in patients with IgM and C3 deposition compared to patients in IgM+ C3– and IgM– C3– groups (5 [22.7%] vs. 13 [59%] and 20 [47.6%] respectively [$p = 0.043$]). ACEi/ARB use did not differ among 3 study groups ($p = 0.166$); however, more patients in IgM+ C3+ group had been treated by using immunosuppressive agents compared with IgM+ C3– and IgM– C3– groups (19 [83.6%] vs. 11 [50%] and 25 [59.5%] respectively [$p = 0.030$]). Details of study outcomes are shown in Table 3.

Levels of eGFR Throughout the Follow-Up Period

Patients with IgM and C3 deposition presented with lower levels of eGFR at the time of diagnosis compared to IgM– C3– and IgM+ C3– groups (69 ± 37.2 vs. 81.2 ± 34 and 96.8 ± 30.1 mL/min/1.73 m^2 respectively [$p = 0.029$]). Moreover, eGFRs of patients in the IgM+ C3+ group significantly decreased when compared with patients in IgM– C3– and IgM+ C3+ groups

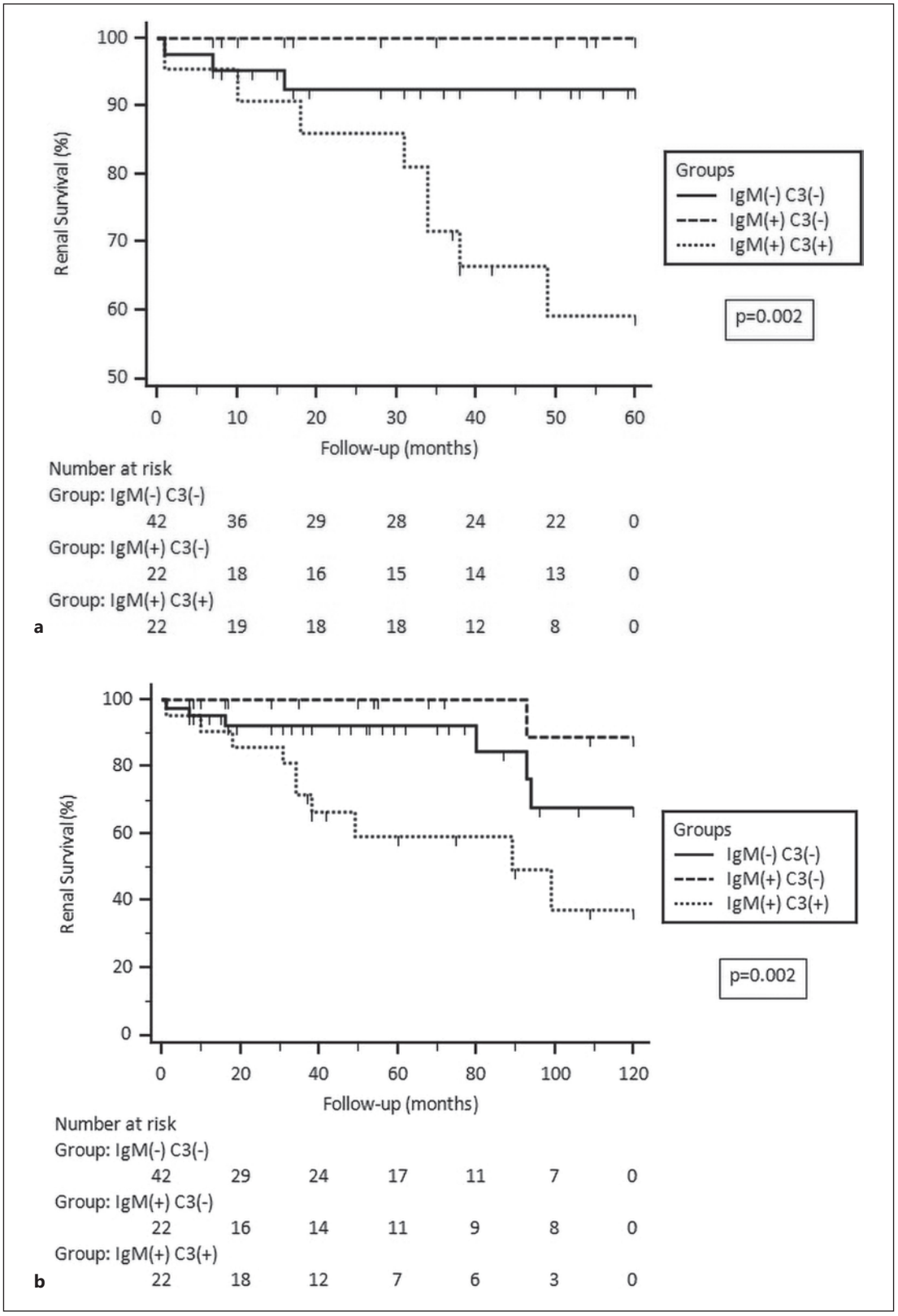

Fig. 1. a. Kaplan-Meier analysis of 5-year renal survival in study groups. Patients with IgM and C3 deposition tended to have worse outcomes (p = 0.002). **b** Kaplan-Meier analysis of 10-year renal survival in study groups. Patients with IgM and C3 deposition continued to have worse outcomes (p = 0.002).

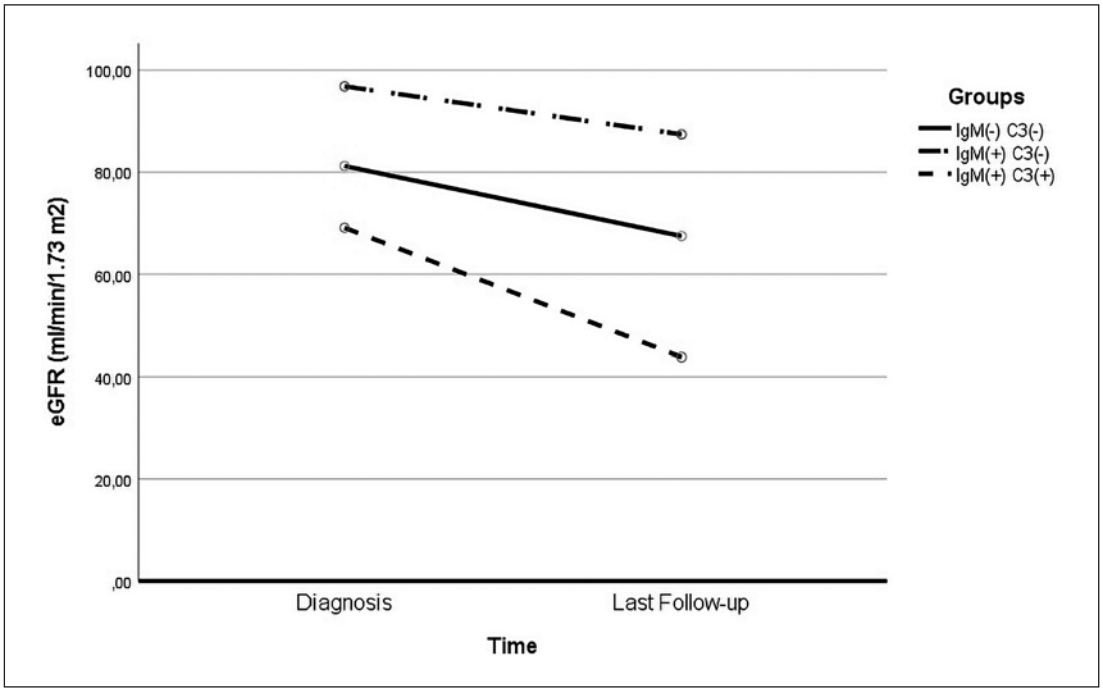

Fig. 2. Slope of eGFRs across study groups throughout the follow-up. eGFR, estimated glomerular filtration rate.

at the end of the follow-up period (43.7 ± 36.2 vs. 71.5 ± 35.8 and 87.4 ± 41.5 mL/min/1.73 m^2 respectively [$p = 0.001$]; Fig. 2).

Decrease of Proteinuria and the Primary Outcome

Over time, 52 patients (60.5%) exhibited at least a 50% decrease in their proteinuria levels, and only 7 of these 52 individuals (13.4%) reached the primary outcome. On the other hand, primary outcome was found in half of the patients who did not demonstrate this decrease of proteinuria (34, 39.5%) throughout the follow-up ($p < 0.001$).

Predictors of Primary Outcome

In univariate analysis of all patients, eGFR (HR 0.976, 95% CI 0.961–0.991, $p = 0.002$), use of ACEi/ARBs (HR 0.316, 95% CI 0.114–0.876, $p = 0.027$), the percentage of global glomerulosclerosis (HR 1.032, 95% CI 1.014–1.050, $p = 0.001$), IgM and C3 co-deposition (HR 2.884, 95% CI 1.283–6.484, $p = 0.010$) and tubular atrophy (HR 1.914, 95% CI 1.104–3.319, $p = 0.021$) were associated with primary outcome. However, IgM deposition (HR 0.932, 95% CI 0.415–2.089, $p = 0.863$), level of proteinuria (HR 0.953, 95% CI 0.833–1.089, $p = 0.479$), presentation with nephrotic syndrome or nephrotic range proteinuria (HR 1.095, 95% CI 0.443–2.705, $p = 0.844$) or use of immunosuppressive agents (HR 0.863, 95% CI 0.364–2.048, $p = 0.739$) were not identified as risk factors for primary outcome.

In multivariate Cox regression analysis, only IgM and C3 co-deposition predicted primary outcome (HR 3.355, 95% CI 1.349–8.344, $p = 0.009$), whereas eGFR (HR 0.989, 0.972–1.007, $p = 0.247$), use of ACEi/ARBs (HR 0.353, 95% CI 0.113–1.105, $p = 0.074$), percentage of global glomerulosclerosis (HR 1.020, 95% CI 0.994–1.046, $p = 0.142$) or tubular atrophy (HR 1.415, 95% CI 0.619–3.236, $p = 0.411$) did not. In ROC analysis, for primary outcome, area under the curve of IgM and C3 co-deposition was calculated as 0.637 ($p = 0.050$; Fig. 3). Results of univariate and multivariate Cox regression analyses are detailed in Table 4.

Table 4. Univariate and multivariate Cox regression analyses regarding primary outcome in all patients (n = 86)

Characteristic	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p value	HR (95% CI)	p value
Gender, male	1.190 (0.529–2.673)	0.674	–	–
Age, years*	0.969 (0.931–1.009)	0.127	–	–
Hypertension	1.242 (0.545–2.829)	0.606	–	–
Proteinuria*, g/24 h	0.953 (0.833–1.089)	0.479	–	–
Presentation with nephrotic syndrome or nephrotic-range proteinuria	1.095 (0.443–2.705)	0.844	–	–
eGFR*, mL/min/1.73 m^2	0.976 (0.961–0.991)	0.002	0.989 (0.972–1.007)	0.247
Serum albumin*	1.098 (0.676–1.785)	0.705	–	–
Hemoglobin*	0.895 (0.760–1.055)	0.185	–	–
ACEi/ARB use	0.316 (0.114–0.876)	0.027	0.353 (0.113–1.105)	0.074
Immunosuppressive treatment	0.863 (0.364–2.048)	0.739	–	–
Histopathological features				
Percentage of global glomerulosclerosis	1.032 (1.014–1.050)	0.001	1.020 (0.994–1.046)	0.142
Percentage of segmental glomerulosclerosis	1.029 (1.000–1.059)	0.054	–	–
IgM deposition	0.932 (0.415–2.089)	0.863	–	–
IgM and C3 co-deposition	2.884 (1.283–6.484)	0.010	3.355 (1.349–8.344)	0.009
Collapsing lesion	0.811 (0.108–6.066)	0.838	–	–
Tip lesion	1.854 (0.624–5.507)	0.266	–	–
Interstitial fibrosis	1.508 (0.854–2.664)	0.157	–	–
Tubular atrophy	1.914 (1.104–3.319)	0.021	1.415 (0.619–3.236)	0.411

* Age, proteinuria, eGFR, serum albumin and hemoglobin values at the time of diagnosis were taken into consideration.

HR, hazard ratio; eGFR, estimated glomerular filtration rate; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Discussion

Glomerular IgM and C3 deposits are commonly demonstrated in patients with primary FSGS. These depositions are assumed to be the result of a passive trapping rather than primary immune mechanisms, hence they do not show a significant effect on disease course [9, 10]. However, it was recently shown that IgM deposits activated complement system in the glomerulus in an animal model of FSGS [12]. Zhang et al. [8] took a step forward and showed the clinical significance of these depositions in patients with primary disease. In this study, we identified glomerular IgM and C3 co-deposition as a predictor of unfavorable renal outcomes in adult patients with primary FSGS confirming the previous findings.

Heterogeneous nature of FSGS is an important obstacle in the path of our understanding of the disease, while injury of podocytes remains as the common denominator in all cases. The disease has different histological subtypes, which can originate from diverse insults to glomeruli, such as systemic disorders, infections and use of various drugs; it also may be idiopathic or caused by a genetic mutation [1, 18]. Therefore, the activation of complement system may be involved in disease pathogenesis in a subgroup of patients. In our cohort, 51.1% (n = 44) had glomerular IgM deposition and 50% (n = 22) of these patients (25.5% of all patients) had accompanying C3 deposits, which indicates that IgM deposition does not have a role in onset of the disease. However, these deposits may be an additional phenomenon. A previous study showed that following the insults to glomeruli causing podocyte injury, IgM antibodies could bind to new epitopes exposed in glomeruli, thereby activating the complement system [12]. IgM is considered an activator of classical pathway of complement cascade; however, our results demonstrate not only IgM but also C3 deposition in glomeruli, showing activation

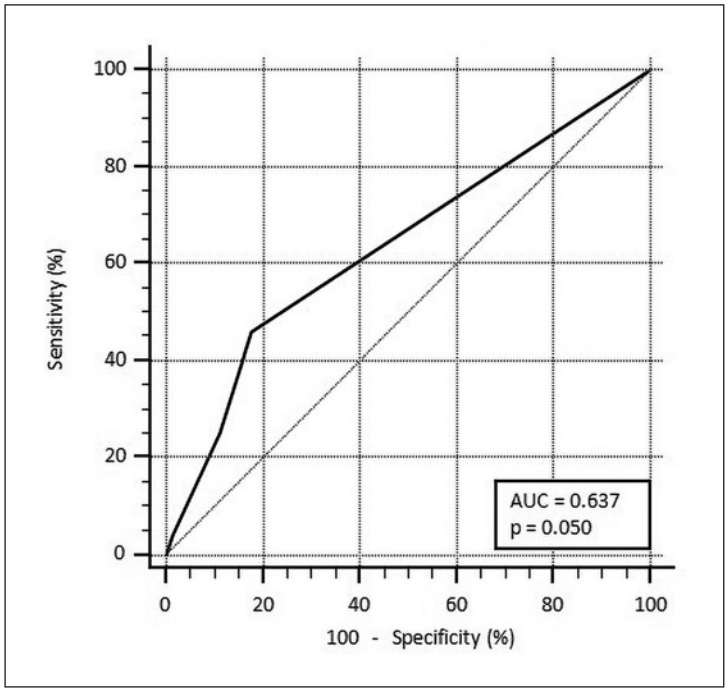

Fig. 3. ROC analysis to determine the predictive power of IgM and C3 co-deposition for primary outcome (AUC 0.637, $p = 0.050$). AUC, area under the curve.

of the AP. Activation of the classical pathway by IgM antibodies may trigger the AP, and glomerular injury may have a negative impact on complement regulatory proteins controlling the amplification of the AP [5, 12]. Moreover, a recent study showed that low serum C3 levels could be an independent risk factor for renal dysfunction in patients with primary FSGS, thus indicating the activation of the AP [19].

In our study, patients with IgM and C3 deposition were characterized by higher levels of proteinuria, higher percentage of glomerular sclerosis, lower levels of eGFR and serum albumin as compared to other patients at the time of diagnosis. Rates of interstitial fibrosis and tubular atrophy were comparable, as well as glomerular IgG and IgA deposition. More patients in IgM+ C3+ group demonstrated C1q deposition (3/22, $p = 0.048$); however, the overall number of patients was quite low to draw a firm conclusion (5/86, 5.8%). Hence, patients in this group presented with more severe disease, and over a comparable duration of follow-up, these patients continued to have worse renal outcomes. It can be speculated that this co-deposition of IgM and C3 may represent a higher glomerular permeability secondary to more severe disease, which results in worse prognosis. Other option is that co-deposition of IgM and C3 may further contribute to glomerular damage. Similar degrees of IgG and IgA depositions make the first possibility unlikely. Indeed, the findings of the multivariate Cox regression analysis (IgM and C3 co-deposition was the sole independent factor associated with renal dysfunction [HR 3.355, 95% CI 1.349–8.344, $p = 0.009$]) confirm that IgM and C3 deposits contribute to glomerular damage.

Considering the heterogeneous nature of the disease, patients in this large spectrum have variable responses to different treatment regimens [20, 21]. Our patients had been mostly treated by using corticosteroids and calcineurin inhibitors based regimens, whereas MMF was used in 13 (15.1%) patients, who showed resistance to aforementioned agents. AZA was used only in 9 (9.3%) cases, before implementation of the 2012 kidney disease improving global outcomes Guideline [17]. Complete or partial remission was seen only in 22.7% of patients with IgM and C3 deposition, although 86.3% of them received immunosuppressive treatment. This finding suggests that standard immunosuppressive treatment options have

limited efficacy in withholding the activity of the disease [22–24]. Agents against B cells, such as rituximab successfully reduced glomerular IgM deposition and thereby albuminuria in animal models [12]; however, its efficacy is still controversial in several patients with primary FSGS [25, 26]. We suggest that, at least on a theoretical basis, treatment options targeting complement system, such as eculizumab may be another choice in this particular subset of patients. Proper selection of individuals with IgM and C3 co-deposition might constitute the first step toward the judicious use of targeted therapies.

Our study had several limitations. First, it was designed as a retrospective cohort study, and thus, drawing conclusions to establish a cause-effect relationship is not feasible. Second, we did not perform the Columbia histopathological classification systematically [18]; however, the presence of tip and collapsing lesions were included in the analyses. Third, serum levels of IgM and C3 could not be obtained in all patients, and therefore these variables were not taken into consideration.

In conclusion, this study demonstrates that glomerular IgM and C3 co-deposition is associated with unfavorable renal outcomes and lower treatment responses to conventional immunosuppressive agents in adult patients with primary FSGS. We speculate that these findings are consistent with the accumulating evidence that shows that after the insults causing injury of podocytes, IgM antibodies can bind to neoepitopes exposed in the glomerulus, thereby activating the complement system in a subset of patients.

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Preliminary results of this study were presented in an abstract form in 54th ERA-EDTA Congress in Madrid, Spain, on June 4, 2017.

Statement of Ethics

This study was approved by our institutional research committee and conducted in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from the patients involved in the study.

Disclosure Statement

The authors declare that they have no conflicts of interest to disclose.

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Author Contributions

Y.C. designed the study. S.M., Y.C., Y.O., A.B.D., Z.I., A.A., H.Y., A.T., I.K., and M.S.S. collected the data. S.M. and Y.C. carried out data analyses. S.M. prepared the manuscript with help of the input from Y.C. and Y.O. M.S.S. critically revised the paper. All authors read and approved of the final version.

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