Clinical Significance of Immune Deposits and Complement System Activation in FSGS

Findings from the Cure Glomerulonephropathy Network Study

Yasar Caliskan[1], *Virginie Royal*[2], *Stéphan Troyanov*[3], *Arnaud Bonnefoy*[4], *Clémence Merlen*[4], *Mark Schnitzler*[1], *John C. Edwards*[1], *Krista L. Lentine*[1], *and Louis-Philippe Laurin*[5], *on behalf of the CureGN Consortium**

Key Points

- Complement activation plays a critical role in FSGS pathogenesis and progression.
- The urine soluble C5b9 protein ratio is emerging as a significant biomarker for FSGS progression.
- Targeting complement pathways and monitoring urinary soluble C5b9 levels may improve risk stratification and therapeutic approaches in FSGS.

Abstract

Background The interplay between complement activation and the immune response in FSGS warrants further investigation. We investigated the association of glomerular C3 and IgM immunostaining with FSGS disease activity, complement system activation, chronicity on kidney biopsy, and initial and follow-up clinical data in the Cure Glomerulonephropathy Network FSGS cohort.

Methods Data for patients with FSGS with available pathology assessment from the Cure Glomerulonephropathy Network cohort were reviewed. We tested associations between glomerular immunoglobulins and C3 staining intensity by immunofluorescence with the Columbia classification, the urinary membrane attack complex (soluble C5b9 [sC5b9]) level, proteinuria, and time to a composite outcome, defined by ESKD or a 40% decline in eGFR. Urinary sC5b9 levels, expressed as ratios to creatinine (sC5b9-to-creatinine ratio) and to protein (urine sC5b9-to-creatinine ratio-to-protein ratio [C5b9uPR]), were also examined.

Results The study cohort comprised 175 patients with FSGS, including 63 (36%) incident subjects enrolled within 6 months of pathology review. Glomerular IgM, C3, and IgG deposits were found in 88 (50%), 48 (27.4%), and 27 (15.4%) patients, respectively. C3 deposition was correlated with global sclerosis (r=0.27, $P < 0.001$), tubular microcystic changes (r=0.19, $P < 0.01$), interstitial fibrosis (IF) tubular atrophy (r=0.17, $P = 0.03$), interstitial inflammation (r=0.17, $P = 0.03$), and tip lesion (r=-0.16, $P = 0.04$). In incident patients, C5b9uPR correlated with total segmental sclerosis (r=0.35, $P < 0.01$), IF (r=0.33, $P = 0.01$), IF tubular atrophy (r=0.35, $P < 0.01$), and interstitial inflammation (r=0.29, $P = 0.03$). Only C5b9uPR (hazard ratio, 1.64 [95% confidence interval, 1.03 to 2.60; $P = 0.03$]) and age at enrollment (hazard ratio, 1.01 [95% confidence interval, 1.00 to 1.03; $P = 0.02$]) were significantly associated with the composite outcome in the adjusted Cox survival models.

Conclusions C5b9uPR is emerging as a significant biomarker for FSGS progression, reflecting the complex interplay between complement activation, inflammation, and kidney injury. The evidence suggests that elevated C5b9uPR levels are associated with poor kidney outcomes and may serve as a valuable tool in the noninvasive assessment of kidney fibrosis and disease progression.

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Due to the number of contributing authors, the affiliations are listed at the end of this article.

Correspondence: Dr. Yasar Caliskan, email: yasar.caliskan@health.slu.edu

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*The list of nonauthor contributors is extensive and has been provided in Supplemental Summary 1.

See related editorial, "Complement and IgM in FSGS: Minor Characters or Leading Actors?," on pages 1265–1267.

Introduction

FSGS is defined as "a group of clinical-pathologic syndromes sharing a common glomerular lesion," whose pathology is based on an injury of podocytes caused by diverse insults.[1,2] Recent studies have highlighted the role of complement activation and deposition in the pathogenesis of FSGS, suggesting that the complement system may be a critical player in the disease's progression and severity.[3]

Complement activation in FSGS has been documented through various mechanisms. The presence of IgM and C3 deposits in the glomeruli is frequently observed in patients with primary FSGS, both in the sclerotic lesions and in the mesangium of unaffected glomeruli.[3–6] It has been hypothesized that these deposits are indicative of complement activation, particularly through the classical pathway, where IgM may act as an activator.[4,5,7] Studies have shown that glomerular IgM and C3 deposition correlate with adverse kidney outcomes, suggesting a potential role in disease progression.[3,6] Furthermore, the colocalization of complement fragments in the glomeruli indicates that these proteins are in their activated forms, which may contribute to glomerular injury.[3,5,7] Moreover, the pathophysiologic mechanisms underlying complement activation in FSGS may involve the generation of neoepitopes within the glomeruli, which can trigger an immune response leading to further complement deposition.[8,9]

The complement system's involvement in FSGS is not merely associative; it seems to have functional implications. Huang *et al.* demonstrated that complement activation profiles in patients with primary FSGS were associated with therapeutic responses and kidney outcomes.[4] In addition, the presence of complement activation products in urine and plasma has been linked to the disease severity, indicating that complement activation may serve as a surrogate biomarker for disease progression.[5,10]

Based on current evidence, we hypothesize that complement immunodeposit, particularly C3, is related to complement system activation in FSGS, contributing to its pathogenesis and potentially serving as a biomarker for disease severity and treatment response.[3,5,7] The interplay between complement activation and the immune response in FSGS warrants further investigation. Therefore, we investigated the association of glomerular C3 and IgM immunostaining with disease activity, chronicity on kidney biopsy, complement system activation, and initial and follow-up clinical data in Cure Glomerulonephropathy Network (CureGN) FSGS cohort.

Methods

Study Design and Population

CureGN (https://curegn.org/) is a multicenter prospective observation cohort study of children and adults with four biopsy-proven glomerular diseases (IgA nephropathy, membranous nephropathy, minimal change disease, and FSGS).[11] The first diagnostic biopsies were performed within 5 years before study enrollment. Institutionalized patients or those with ESKD at screening, diabetes, a prior organ/hematopoietic transplant, hepatitis B or C, HIV, malignancy, or SLE at time of biopsy were excluded.

All participants with FSGS enrolled in CureGN from December 2014 to June 2022 with available central pathology assessment by CureGN pathologists were included. We tested associations between the intensity and localization of immunostaining (glomerular C3 and Ig deposition by immunofluorescence) with light microscopy findings. In incident patients, who were enrolled within 6 months of their diagnostic kidney biopsy, we examined the relationship between pathologic findings and clinical as well as urinary markers, specifically urinary membrane attack complex (soluble C5b9) and proteinuria, at the time of enrollment. In all study participants, we assessed the associations between clinical and urinary features, pathologic findings, and a composite outcome, which was defined as the development of ESKD or a 40% decline in eGFR.

Within CureGN, each participating site obtained Institutional Review Board approval, and informed consent, along with assent when applicable, was secured from participants and their legal guardians. The Saint Louis University Institutional Review Board provided a statement of nonhuman subjects' determination for this ancillary study of deidentified data in agreement with the Declaration of Helsinki.

Demographic, Clinical, and Laboratory Measures

Demographic, clinical, laboratory, and medication data of patients with FSGS are extracted from the CureGN database. Demographic data included sex, race, ethnicity, age at the time of enrollment, time since biopsy (years), history of hypertension, solitary kidney, sickle cell anemia, malignancy, tobacco and illicit drug use, and family history of kidney disease. Baseline eGFR, calculated using the CKD in children under 25 formula if age younger than 25 years[12] or the CKD Epidemiology Collaboration 2021 formula for adults age 25 and older.[13] Remission status on the basis of the random urine protein creatinine ratio (uPCR) at the time of enrollment was also obtained from the CureGN data.

Disease Activity at Enrollment

Disease activity was based on CureGN's Disease Activity Working Group definitions for FSGS activity at enrollment, which defines active disease based on ≥1 of the following criteria[11]: 24-hour urine protein >1 g (adults) or 24-hour urine protein >1 g normalized to 1.73 m^2 body surface area (children <1.73 m^2) or uPCR >1 g/g.

Histopathologic Evaluation

Pathology core scoring data, including light microscopy features, immunofluorescence, and electron microscopy data, were reviewed. The CureGN Core Scoring Working Group, composed of 11 nephropathologists, established a standardized approach for scoring kidney pathology features based on routine clinical diagnostic practices. According to the CureGN core scoring protocol,[11,14] cases underwent central review by CureGN pathologists for scoring of glomerular features, including the percentage of globally sclerotic glomeruli (described as quartiles), the percentage of glomeruli showing segmental sclerosis (described as quartiles), and the variant of individual FSGS lesion, as well as the overall FSGS variant defined

according to the Columbia classification.[1,14] Tubulointerstitial and vascular features were also scored. The immunofluorescence study results were based on the review of the enrollment pathology reports using a semiquantitative intensity scale from 0 to 3+. If the pathologist reported a score between two points on the scale, these values were averaged; for example, an Ig stain graded as 1–2+ was reported as 1.5. Trace staining was reported as 0.5. The localization of the deposits, defined as mesangial and/or along the capillary wall, was recorded.

Urinary Biomarkers

Urinary complement components were measured using human enzyme immunoassay Kits: soluble C5b9 [sC5b9] (MicroVue; Quidel Corp., San Diego, CA) in all patients, as previously performed by our group.[15,16] Urine samples were diluted 1:3. The lower threshold sensitivity of the assays is 15 ng/ml for sC5b9. Two hundred and fifty microliters of centrifuged and stored supernatant urine at the time of kidney biopsy and/or at the time of enrollment and urine creatinine levels from the same sample were requested from the CureGN biorepository. Urinary levels of sC5b9 were defined as the sC5b9-to-creatinine ratio (sC5b9CR) and urine sC5b9CR-to-protein ratio (C5b9uPR), which is the ratio of sC5b9CR levels to total protein in the urine.

Definitions and Follow-Up Data

Patients with a follow-up period of more than 4 months or showing progression to ESKD regardless of the duration of follow-up were evaluated. Progression to ESKD was defined as two consecutive eGFR <15 ml/min per 1.73 m^2 more than 1 month apart during follow-up. The number of eGFR measurements, eGFR slope of least-squares regression line using first and last serum creatinine levels ≥8 months apart in ml/min per year, structural equation modeling of the eGFR slope estimate and data of eGFR reduced by 40% from enrollment were obtained from the main CureGN data. Patients were followed until death, study withdrawal, or end of study.

Clinical Outcomes

Composite outcome was defined by the patient meeting at least one of the following criteria: (*1*) ESKD was defined as two consecutive eGFR <15 ml/min per 1.73 m^2 more than 1 month apart and (*2*) eGFR decline ≥40% was based on the decline from enrollment using regression method among patients with a completed enrollment visit.[17]

Statistical Analyses

Descriptive statistics were performed using frequencies with percentages for categorical variables and medians with interquartile range (IQR) for continuous variables. The results are expressed as mean±SD when normally distributed or median (IQR) when not normally distributed. Appropriate parametric and nonparametric tests were used accordingly. Comparisons of continuous variables between the groups were evaluated using *t* tests or the Mann–Whitney *U* test, where appropriate. Differences in the proportions of different patient groups were compared using the chi-squared or Fisher exact test. Periods of kidney survival were analyzed using Kaplan-Meier curves, and this period for each patient was computed from enrollment to the last follow-up or primary outcome. Relationships were determined by Pearson correlation coefficient, and Spearman *rho* was used for nonparametric correlations. Variables found to affect kidney survival in univariate analysis (a P value of ≤0.05 for each variable) were included in the adjusted Cox proportional hazards model. The results of Cox regression models were described as hazard ratios (HRs) and 95% confidence intervals (CIs). Statistical analyses were performed using SPSS for Windows (SPSS version 21.0, IBM Corp., Armonk, NY) and R version 4.3.2 (2023-10-31). Kaplan-Meier curves were computed with R version 4.3.2 (2023-10-31; https://www.R-project.org/). All analyses were two-sided, and a P value of 0.05 or less was considered statistically significant.

Results

Study Population

A total of 175 patients with FSGS were included in this study, with 63 (36%) incident participants (Table 1). The median follow-up time after enrollment was 5.2 years (IQR, 3.7–6.5 years). Most patients self-reported as White (n=93, 53%). The mean age at enrollment was 39.6±21.9 years. Seventy-four patients (42.3%) had a family history of kidney disease. Active disease at presentation was found in 102 (58.3%) patients. A total of 130 patients were exposed to immunosuppression. Regarding Columbia classification, patients were classified as follows: 74 (43%) with not otherwise specified FSGS, 35 (20%) with tip lesion, 30 (17%) with collapsing, 20 (12%) perihilar, and 5 (2.9%) cellular.

Glomerular IgM, C3, and IgG deposits were found in 88 (50%), 48 (27%), and 27 (15%) patients, respectively (Figure 1). Forty-four (91%) patients with C3 deposition presented concomitant IgM deposition, and 20 (48%) showed IgG deposition.

BP and current medications provide important context for assessing the effect of our findings. In our cohort, renin-angiotensin-aldosterone system blockers were the most commonly used medication prescribed in 88% of patients. Diuretics were the next most common, used in approximately 60% of patients. Calcium channel blockers were used in 43% of patients, whereas β blockers were used in about a third (34%). Less commonly used antihypertensive medications included vasodilators (16%), α-blockers (9%), and central-acting agents (7%).

Associations of Immune Deposits with Pathology Findings

C3 intensity of staining from zero to three correlated with global sclerosis (r=0.27, P < 0.001), tubular microcystic changes (r=0.19, P < 0.01), interstitial fibrosis tubular atrophy (IFTA; r=0.17, P = 0.03), and interstitial inflammation (r=0.17, P = 0.03) and showed negative correlation with tip lesion (r=−0.16, P = 0.04). IgM intensity of staining only showed a weak correlation with IFTA (r=−0.16, P = 0.04), whereas IgG intensity of staining showed a correlation with collapsing segmental lesion (r=0.24, P < 0.01), interstitial inflammation (r=0.17, P = 0.02), and tip lesion (r=−0.17, P = 0.03). There was no correlation of C3, IgM, and IgG intensity

Table 1. Baseline demographics, clinical, and laboratory characteristics of patients with FSGS

Characteristics	*n*=175
Male/female, *n* (%)	85 (48.6)/90 (51.4)
Age, yr, mean±SD	39.6±21.9
BMI, kg/m^2, median (IQR)	27.7 (23.2–32.8)
Ethnicity, *n* (%)	
Hispanic or Latino	33 (18.9)
Not Hispanic or Latino	140 (80.0)
Unknown	2 (1.1)
Race, *n* (%)	
Asian	14 (8.0)
Black	49 (28.0)
Multiracial	3 (1.7)
Native American	2 (1.1)
Pacific Islander	1 (0.6)
White	93 (53.1)
Unknown	13 (7.4)
Enrollment time, *n* (%)	
Incident[a]	63 (36.0)
Prevalent	112 (64.0)
Hypertension, *n* (%)	110 (62.9)
Family history of kidney disease, *n* (%)	74 (42.8)
Active disease at enrollment	102 (58.3)
Serum creatinine at enrollment, mg/dl, median (IQR)	1.24 (0.86–1.78)
eGFR at enrollment, ml/min per 1.73 m^2, median (IQR)	59.9 (36.4–91.9)
uPCR, g/g, median (IQR)	2.02 (0.5–3.98)
Immunosuppressive treatment ever, *n* (%)	
Yes (≥1 med is for glomerular disease)	119 (68.0)
Yes (no med is for glomerular disease)	11 (6.3)
No	45 (25.7)
Columbia classification, *n* (%)	
NOS	74 (42.8)
Perihilar	20 (11.6)
Tip	35 (20.2)
Cellular	5 (2.9)
Collapsing	30 (17.3)
Nondiagnostic	9 (5.2)
Unknown	2 (1.1)

BMI, body mass index; IQR, interquartile range; med, medication; NOS, not otherwise specified; uPCR, urine protein creatinine ratio.
[a]Kidney biopsy was ≤6 months from time of enrollment.

of staining with mesangial hypercellularity, mesangial matrix expansion, interstitial edema, arteriosclerosis, and arteriolar hyalinosis.

Associations between Immune Deposits and Clinical Features at Enrollment

There was no statistically significant difference in mean IgM, C3, and IgG intensity scores between the disease activity groups (P = 0.20, P = 0.55, and P = 0.21). The number of patients with concomitant IgM/C3 deposition is similar in patients with and without active disease (26.5% and 26.2%, P = 0.48). There was no correlation between C3, IgM, and IgG deposition and age, sex, active disease, race, ethnicity, or family history of kidney disease.

Associations of Urinary Markers with Pathology and Clinical Findings

At enrollment, the median urinary sC5b9CR was 0.82 μg/mmol (IQR, 0–5.5 μg/mmol), and the median uPCR was 2.02 (IQR, 0.50–3.98) g/g. The median urinary sC5b9CR was 2.05 μg/mmol (IQR, 0–8.0 μg/mmol) in incident patients and 0.72 μg/mmol (IQR, 0–3.7 μg/mmol) in prevalent patients.

In incident patients, uPCR levels correlated with urinary sC5b9CR levels (r=0.51, $P < 0.001$). Regarding pathology findings, uPCR correlated with mesangial dense deposits (r=0.31, P = 0.01) and degree of foot process effacement (r=0.27, P = 0.04). Urinary sC5b9CR levels correlated with total segmental sclerosis (r=0.38, $P < 0.01$), interstitial fibrosis (IF; r=0.34, $P < 0.01$), IFTA (r=0.33, P = 0.01), interstitial inflammation (r=0.34, $P < 0.01$), capillary wrinkling (r=0.34, $P < 0.01$), IgG deposition intensity (r=0.29, P = 0.02), and collapsing segmental lesion (r=0.26, P = 0.04). After adjusting urinary sC5b9CR levels for the degree of proteinuria, C5b9uPR correlated with total segmental sclerosis (r=0.35, $P < 0.01$), IF (r=0.33, P = 0.01), IFTA (r=0.35, $P < 0.01$), interstitial inflammation (r=0.29, P = 0.03), but no association was observed between IgG and C3 deposition, collapsing segmental lesion and C5b9uPR. Figure 2 presents the mean C5b9uPR levels for histologic findings that show significant associations. There was no correlation of urinary sC5b9CR and C5b9uPR levels with age, sex, active disease, race, family history of kidney disease, mesangial hypercellularity, mesangial matrix expansion, tubular microcystic changes, interstitial edema, arteriosclerosis, arteriolar hyalinosis, the intensity of C3, and IgM deposition.

Factors Associated with Kidney Disease Progression

Sixty-five (37.1%) of 175 patients reached a composite outcome after a median follow-up of 1.74 (IQR, 0.91–3.41) years after enrollment. Progression to ESKD was noted in 46 patients (26.2%) after a median of 1.63 (IQR, 0.97–3.37) years, and 19 patients (10.9%) had an at least 40% reduction in baseline eGFR at enrollment after a median of 4.23 (IQR, 2.85–4.78) years of follow-up.

Cox proportional hazard regression models were used to analyze time-to-event data, with clinical and pathologic prognostic factors examined separately for the composite outcome of ESKD or ≥40% eGFR reduction.

Clinical and Urinary Factors

In unadjusted Cox survival models, several clinical characteristics including age (HR, 1.01 [95% CI, 1.00 to 1.02], P = 0.024), active disease at enrollment (HR, 1.51 [95% CI, 1.05 to 2.19], P = 0.03), eGFR at enrollment (HR, 0.97 [95% CI, 0.96 to 0.98], $P < 0.001$), uPCR (HR, 1.01 [95% CI, 1.00 to 1.01] $P < 0.001$), urinary sC5b9CR (HR, 1.00 [95% CI, 1.00 to 1.01], $P < 0.001$), and C5b9uPR (HR, 2.12 [95% CI, 1.50 to 3.01], $P < 0.001$) were associated with composite ESKD and >40% eGFR decline (Table 2). Among those, only C5b9uPR (HR, 1.87 [95% CI, 1.12 to 3.13; P = 0.02]), eGFR at enrollment (HR, 0.95 [95% CI, 0.94 to 0.97], $P < 0.001$), uPCR (HR, 1.01 [95% CI, 1.00 to 1.01], P = 0.02), and age

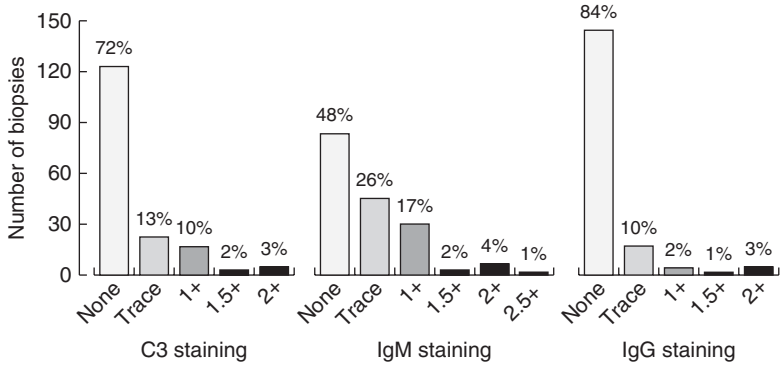

Figure 1. Histogram illustrating the intensity of glomerular C3, IgM, and IgG deposits in CureGN FSGS cohort. CureGN, Cure Glomerulopathy Network.

at enrollment (HR, 0.98 [95% CI, 0.97 to 1.00; $P = 0.01$]) remain associated with an increased risk of ESKD and >40% decline in eGFR in the adjusted Cox survival models (Figure 3). Active disease at enrollment did not show statistically significant association with the composite outcome.

Pathology Features

IFTA (HR, 2.76 [95% CI, 2.07 to 3.67], $P = 0.001$), interstitial inflammation (2.47 [95% CI, 1.74 to 3.49], $P < 0.001$), tubular microcystic change (HR, 2.40 [95% CI, 1.62 to 3.57], $P < 0.001$), IF (HR, 2.31 [95% CI, 1.70 to 3.12], $P < 0.001$), C3 ≥1 (HR, 2.23 [95% CI, 1.26 to 3.95], $P = 0.006$), mesangial hypercellularity (0.53 [95% CI, 0.32 to 0.88], $P = 0.01$), arteriosclerosis (HR, 1.20 [95% CI, 1.04 to 1.39], $P = 0.01$), and global sclerosis (HR, 2.17 [95% CI, 1.12 to 4.19], $P = 0.02$) were significantly associated with ESKD and >40% eGFR decline in unadjusted Cox survival models (Table 3). Among these, C3 ≥1 was the most critical immunofluorescence staining factor in predicting composite outcome, highlighting its central role in risk stratification.

In our adjusted Cox survival model, IFTA was associated with a higher likelihood of reaching our composite end point (HR, 2.30 [95% CI, 1.53 to 3.43; $P < 0.001$]), whereas mesangial hypercellularity was associated with

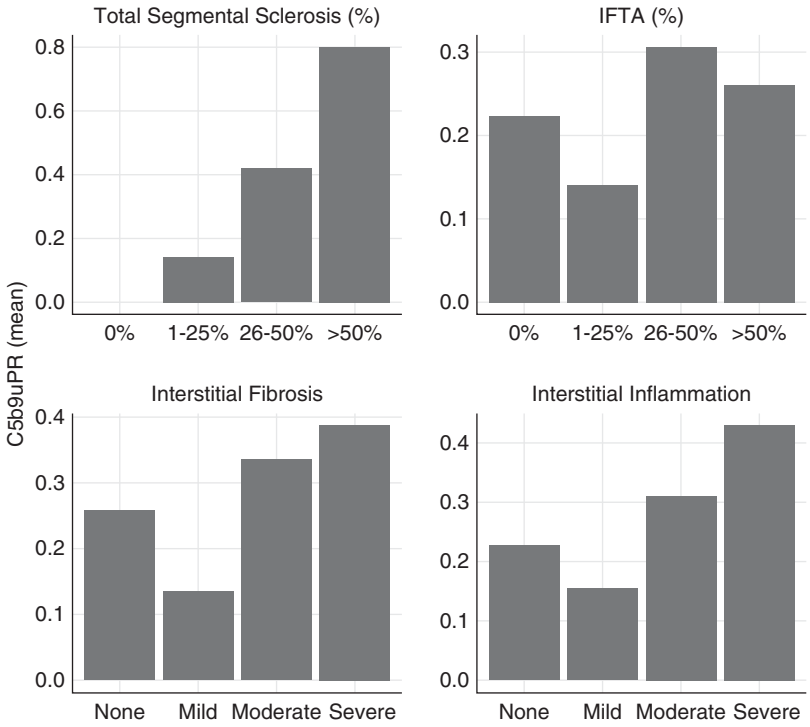

Figure 2. Bar charts illustrate the association between histologic findings and C5b9uPR levels. The *x* axis represents different histologic findings (categorical variables), while the *y* axis represents C5b9uPR levels. Data are presented as (mean). C5b9uPR, urine sC5b9-to-creatinine ratio-to-protein ratio; IFTA, interstitial fibrosis tubular atrophy; sC5b9, soluble C5b9.

Table 2. Univariate Cox proportional hazards ratios for various clinical characteristics associated with the composite outcome of ESKD or ≥40% eGFR reduction

Variable	HR (95% CI)	*P* Value
C5b9uPR	2.12 (1.50 to 3.01)	<0.001
Urinary sC5b9 per creatinine	1.00 (1.00 to 1.01)	<0.001
eGFR at enrollment	0.97 (0.97 to 0.98)	<0.001
uPCR	1.00 (1.00 to 1.01)	<0.001
Age at consent	1.01 (1.00 to 1.02)	0.02
Active disease	1.51 (1.05 to 2.19)	0.03
Race	0.90 (0.78 to 1.03)	0.12
Sex	0.89 (0.55 to 1.45)	0.64
Ethnicity	1.08 (0.58 to 2.03)	0.80
Family history of ESKD	1.03 (0.63 to 1.70)	0.89
Immunosuppressive treatment ever, *n* (%)		
No	Reference	
Yes (≥1 med is for glomerular disease)	0.68 (0.38 to 1.21)	0.19
Yes (no med is for glomerular disease)	0.88 (0.26 to 3.03)	0.84

C5b9uPR, urine sC5b9-to-creatinine ratio-to-protein ratio; CI, confidence interval; HR, hazard ratio; med: medication; sC5b9, soluble C5b9; uPCR, urine protein creatinine ratio.

lower hazards of our composite event (HR, 0.57 [95% CI, 0.33 to 0.99; $P = 0.04$]; Figure 4). Tubular microcystic change, interstitial inflammation, IF, C3≥1 deposition, and global sclerosis did not significantly correlate with the composite outcome.

The binary variables for C5b9uPR and uPCR were created based on their median values. The Cox proportional hazards models were fitted for each variable and showed that high C5b9uPR (HR, 2.37 [95% CI, 1.42 to 3.96], $P < 0.001$) and high uPCR (HR, 2.93 [95% CI, 1.74 to 4.95], $P < 0.001$) were associated with composite end point. Kaplan-Meier plots for the binary variables, C5b9uPR, and uPCR are shown in Figure 5, A and B.

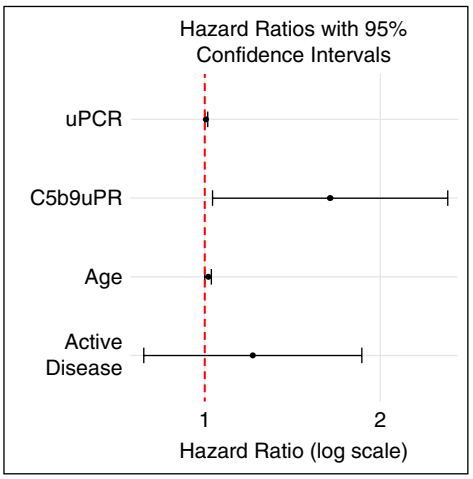

Figure 3. Multivariable Cox regression analysis with least absolute shrinkage and selection operator regularization revealed that urinary C5b9uPR and age were the clinical factors most significantly associated with the composite outcome. uPCR, urine protein creatinine ratio.

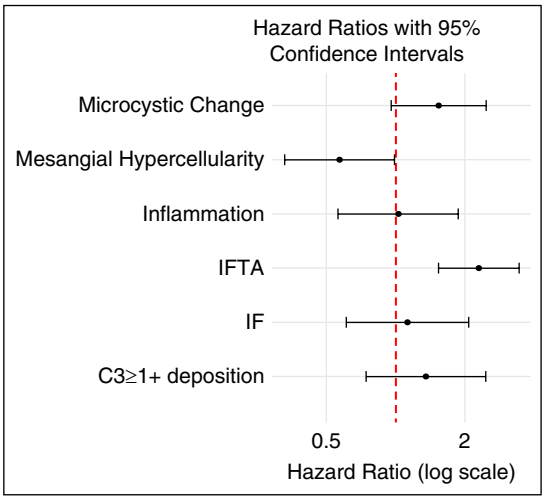

Figure 4. Multivariable Cox regression analysis with least absolute shrinkage and selection operator regularization revealed that IFTA was the pathologic lesion most significantly associated with the composite outcome. IF, interstitial fibrosis.

Table 3. Univariate Cox proportional hazards ratios for various pathologic characteristics associated with the composite outcome of ESKD or ≥40% eGFR reduction

Variable	HR (95% CI)	*P* Value
IFTA	2.76 (2.07 to 3.67)	<0.001
Interstitial inflammation	2.47 (1.74 to 3.49)	<0.001
Tubular microcystic change	2.40 (1.62 to 3.60)	<0.001
IF	2.31 (1.70 to 3.12)	<0.001
C3 ≥1	2.23 (1.26 to 3.95)	0.006
Mesangial hypercellularity	0.53 (0.32 to 0.88)	0.01
Arteriosclerosis	1.20 (1.04 to 1.39)	0.01
Global sclerosis	2.17 (1.12 to 4.19)	0.02
IgM and C3 deposition	1.95 (0.99 to 3.85)	0.05
Tip lesion	0.68 (0.37 to 1.25)	0.21
Arteriolar hyalinosis	1.11 (0.93 to 1.33)	0.25
Foot effacement	1.07 (0.87 to 1.32)	0.53
Interstitial edema	1.17 (0.59 to 2.31)	0.66
IgG staining score	1.04 (0.60 to 1.80)	0.89
Mesangial matrix expansion	0.96 (0.55 to 1.67)	0.89
IgM staining score	1.00 (0.68 to 1.47)	0.98
Columbia classification, *n* (%)		
NOS	Reference	
Perihilar	1.53 (0.76 to 3.12)	0.24
Tip	0.39 (0.15 to 1.00)	0.05
Cellular	0.82 (0.20 to 3.46)	0.79
Collapsing	1.33 (0.69 to 2.54)	0.39

CI, confidence interval; HR, hazard ratio; IF, interstitial fibrosis; IFTA, interstitial fibrosis and tubular atrophy; NOS, not otherwise specified.

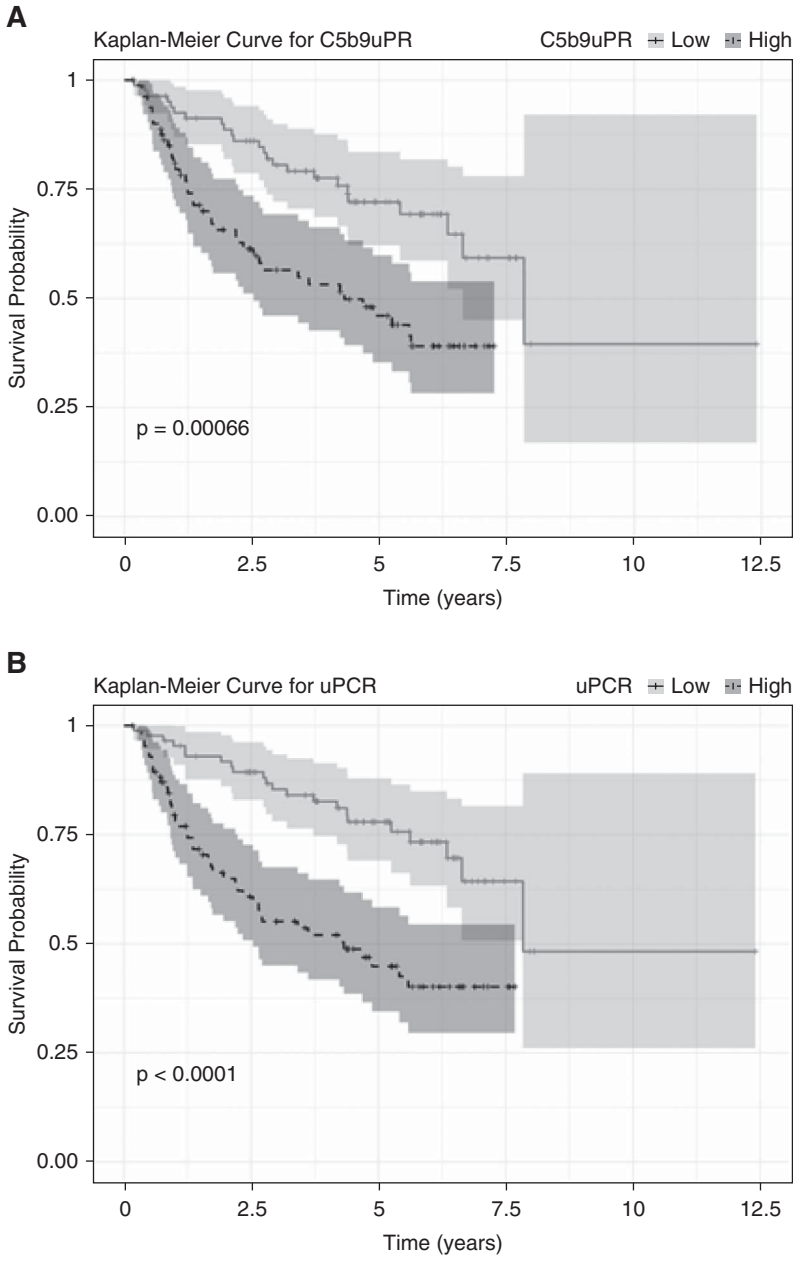

Figure 5. Kaplan-Meier survival curves illustrating time to composite outcome based on significant binary predictors. (A) Kaplan-Meier plot stratified by low vs. high C5b9uPR levels. (B) Kaplan-Meier plot stratified by low vs. high uPCR levels. These plots demonstrate the influence of each variable on time to the composite outcome, providing a clear visual comparison of survival distributions between groups.

Discussion

In this CureGN FSGS cohort, glomerular C3 deposition correlated with global sclerosis, interstitial inflammation, and IFTA. In incident patients, urinary C5b9uPR levels were associated with total segmental sclerosis, IFTA, and interstitial inflammation. Adjusted Cox regression analyses identified urinary C5b9uPR as a risk factor for kidney disease progression, suggesting a potential role of complement activation in FSGS pathogenesis.

C3 deposition in kidney tissues has been associated with the progression of kidney fibrosis through several mechanisms. Studies have shown that C3 promotes renal fibrosis by inducing IL-17A secretion, facilitating M1 macrophage polarization, and activating toll-like receptor 4/NFκB signaling, contributing to podocyte injury and fibrosis. In this study, urine C5b9uPR levels served as a surrogate urine marker, and C3 $\geq$1 emerged as the most critical immunofluorescent staining factor in predicting the composite outcome. These findings underscore the central role of complement system activation in risk stratification. C3 deposition in the glomeruli has been linked to adverse renal outcomes in patients with FSGS. Peng *et al.*[10] found that glomerular capillary C3 deposition is a significant risk factor for unfavorable kidney outcomes in pediatric

patients with primary FSGS, particularly when associated with IgM deposition.[10] Mirioglu *et al.* emphasized that the code position of IgM and C3 serves as a predictor of unfavorable kidney outcomes in adult patients with primary FSGS.[3] The correlation between C3 staining intensity and C5b9uPR levels with interstitial inflammation, total segmental sclerosis, and IFTA in our study highlights ongoing complement activation, which may aggravate glomerular injury and drive fibrosis. This relationship emphasizes the interplay between C3 deposition, urinary sC5b9 levels, and FSGS pathology, offering clinical insight into the mechanisms influencing disease progression and potential therapeutic targets. In our study, this was particularly well demonstrated in collapsing FSGS lesions where an association with higher urinary sC5b9CR levels was observed. However, as a terminal complement activation complex, urinary levels of sC5b9 reflect overall complement activation but do not allow differentiation between the classical, lectin, or alternative complement pathways.

Elevated levels of urinary soluble C5b9 and its association with kidney fibrosis have been explored in the context of various kidney diseases. Our group reported that urinary sC5b9 levels, a surrogate marker complement activation, are substantially higher in patients with FSGS than diabetic nephropathy.[18] The study emphasizes the potential of sC5b9 levels in monitoring kidney disease progression and fibrosis without the need for invasive procedures such as biopsies. In addition to its role in fibrosis, C5b9 has been implicated in the broader context of kidney injury and inflammation. For instance, Burwick *et al.* noted that elevated plasma concentrations of C5b9 in patients with gestational hypertension reflect systemic inflammation and endothelial dysfunction, which are critical factors in the development of kidney fibrosis.[19] This systemic activation of the complement system can exacerbate kidney injury, leading to a cycle of inflammation and fibrosis. Beyond C5b9, C3a–C3aR signaling in podocytes contributes to cytoskeletal rearrangements and nephrin loss. Angeletti *et al.*[20] demonstrated that C3a-induced IL-1β production, facilitated by the absence of decay-accelerating factor, promotes podocyte injury and correlates with proteinuria severity. Genetic variation in complement-related genes can significantly affect the course of glomerular diseases, including FSGS. These insights suggest potential therapeutic targets for glomerulosclerosis. Traditional immunosuppressive therapies, such as corticosteroids and calcineurin inhibitors, do not effectively suppress complement activation. Targeted therapies against B cells or complement components may provide better outcomes for patients with evidence of complement deposition by mitigating complement-driven podocyte injury and glomerular damage.

Urinary sC5b9 levels are gaining recognition as a prognostic biomarker in FSGS. Studies show significantly higher urinary C5b9 levels in patients with FSGS compared with health controls, with strong correlations to kidney damage and tubulointerstitial fibrosis that urinary excretion of C5b9 is significantly higher in patients with FSGS compared with healthy controls, suggesting a strong correlation between complement activation and kidney damage in this condition.[4,21] The mechanism by which C5b9 contributes to FSGS progression may involve its role in promoting tubulointerstitial damage. Studies have also shown that C5b9 can induce inflammatory responses and cellular injury within the renal tubules, leading to fibrosis and further deterioration of kidney function.[22,23] This is particularly relevant as the accumulation of C5b9 in the tubulointerstitium has been observed in patients with FSGS, correlating with the extent of renal injury.[24]

Our findings may support the use of urinary levels of C5b9CR per proteinuria as a noninvasive biomarker associated with disease progression in patients with FSGS.

There are several limitations of this study. The current CureGN FSGS cohort is not powered to detect small differences in rare outcomes such as ESKD. Kidney biopsies in CureGN are processed locally rather than centrally, leading to potential image variability because of differing processing techniques. In addition, the time gap between biopsy and study enrollment, along with exclusion of patients experiencing the composite outcome before enrollment, may introduce bias.

In conclusion, C5b9uPR is emerging as a significant biomarker for FSGS progression, reflecting the complex interplay between complement activation, inflammation, and kidney injury. This study strengthens evidence that elevated urinary C5b9uPR levels are associated with poor kidney outcomes and may serve as a valuable tool in the noninvasive assessment of kidney fibrosis and disease progression. Continued investigations into the role of membrane attack complex in FSGS may further elucidate its potential as a target for therapeutic strategies aimed at mitigating disease progression.

Disclosures

Disclosure forms, as provided by each author, are available with the online version of the article at http://links.lww.com/KN9/A998.

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Author Contributions

Conceptualization: Yasar Caliskan, John C. Edwards, Louis-Philippe Laurin, Krista L. Lentine, Virginie Royal, Stéphan Troyanov.

Data curation: Yasar Caliskan, John C. Edwards, Louis-Philippe Laurin, Krista L. Lentine, Virginie Royal, Stéphan Troyanov.

Formal analysis: Yasar Caliskan, Virginie Royal, Stéphan Troyanov.

Funding acquisition: Yasar Caliskan.

Investigation: Yasar Caliskan, Krista L. Lentine, Virginie Royal, Stéphan Troyanov.

Methodology: Arnaud Bonnefoy, Yasar Caliskan, John C. Edwards, Louis-Philippe Laurin, Krista L. Lentine, Clémence Merlen, Mark Schnitzler, Stéphan Troyanov.

Project administration: Arnaud Bonnefoy, Clémence Merlen, Mark Schnitzler.

Resources: Arnaud Bonnefoy, Virginie Royal.

Supervision: Yasar Caliskan, John C. Edwards, Louis-Philippe Laurin, Krista L. Lentine.

Validation: Clémence Merlen.

Writing – original draft: Arnaud Bonnefoy, Yasar Caliskan, John C. Edwards, Louis-Philippe Laurin, Krista L. Lentine, Clémence Merlen, Virginie Royal, Mark Schnitzler, Stéphan Troyanov.
Writing – review & editing: Arnaud Bonnefoy, Yasar Caliskan, John C. Edwards, Louis-Philippe Laurin, Krista L. Lentine, Clémence Merlen, Virginie Royal, Mark Schnitzler, Stéphan Troyanov.

Data Sharing Statement

Original data created for the study are or will be available in a persistent repository upon publication. Partial restrictions to the data and/or materials apply. Raw Data/Source Data. NIDDK Repository. https://repository.niddk.nih.gov/study/171. Data collected in CureGN studies are made available to qualified researchers to advance understanding of glomerular diseases via a data sharing and analytic platform supported by the i2b2 tranSMART Foundation (i2b2transmart.org). TranSMART is a password-protected open-source web-based software platform containing curated, clinical, laboratory, and pathology data, which is updated at regular intervals. Other datasets (genomic, proteomic, *etc.*) that are generated will be added for data analysis. Researchers can submit a proposal detailing their objectives, study design, and data requirements. The proposal must be approved by CureGN's Data Use Committee. Data are not anonymized to protect participant confidentiality.

Supplemental Material

This article contains the following supplemental material online at http://links.lww.com/KN9/B43.

Supplemental Summary 1. CureGN Consortium.

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AFFILIATIONS

[1]Division of Nephrology, SSM Health Saint Louis University Hospital, Saint Louis, Missouri
[2]Division of Pathology, Hôpital Maisonneuve-Rosemont, University of Montreal, Montreal, Quebec, Canada
[3]Division of Nephrology, Hôpital du Sacré-Coeur-de-Montréal, University of Montreal, Montreal, Quebec, Canada
[4]Division of Hematology, Centre Hospitalier Universitaire Sainte-Justine, University of Montreal, Montreal, Quebec, Canada
[5]Division of Nephrology, Hôpital Maisonneuve-Rosemont, University of Montreal, Montreal, Quebec, Canada