Focal Segmental Glomerulosclerosis, Risk Factors for End Stage Kidney Disease, and Response to Immunosuppression

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Abstract

Background FSGS is a heterogeneic glomerular disease. Risk factors for kidney disease ESKD and the effect of immunosuppression treatment (IST) has varied in previously published cohorts. These cohorts were limited by relatively small case numbers, short follow-up, lack of racial/ethnic diversity, a mix of adult and pediatric patients, lack of renin-angiotensin-aldosterone system (RAAS) inhibition, or lack of subgroup analysis of IST.

Methods We compared demographics, clinical characteristics, histopathology, and IST to long-term renal survival in a large, ethnically diverse, adult cohort of 338 patients with biopsy-proven FSGS with long-term follow-up in the era of RAAS inhibition using data from the US Department of Defense health care network.

Results Multivariate analysis showed that nephrotic-range proteinuria (NRP), eGFR <60 ml/min per 1.73 m^2, hypoalbuminemia, interstitial fibrosis and tubular atrophy, and interstitial inflammation at diagnosis and the absence of remission were all associated with worse long-term renal survival. IgM, C3, and a combination of IgM/C3 immunofluorescence staining were not associated with reduced renal survival. IST was not associated with improved renal survival in the whole cohort, or in a subgroup with NRP. However, IST was associated with better renal survival in a subgroup of patients with FSGS with both NRP and hypoalbuminemia and hypoalbuminemia alone.

Conclusions Our study suggests that IST should be reserved for patients with FSGS and nephrotic syndrome. It also introduces interstitial inflammation as a potential risk factor for ESKD and does not support the proposed pathogenicity of IgM and complement activation.

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Introduction

FSGS is a glomerular disease defined by histopathologic lesions. There is significant disease heterogeneity likely due to multiple mechanisms of disease, clinical, and demographic risk factors, and comorbidities (1–3). The incidence of FSGS has risen over the past decades for unclear reasons (1). Previous retrospective cohort studies have significant variability in their findings. Some reported that sex, proteinuria, serum creatinine level, and body mass index (BMI) at diagnosis were associated with long-term survival, whereas others did not (3–15). Immunosuppression was associated with improved renal survival in some cohorts but not others (4,6,7,9–11,13). Results likely differed due to study population and follow-up. Previous studies included both children and adults (4,6,8,11,13,14). A portion of studies included few Black people (6,7,11). Most studies included a large portion of patients diagnosed

before 2000, many of which did not receive renin-angiotensin-aldosterone system (RAAS) blockade (5–7,9–13,15). Many studies included 100 patients or fewer (8–13,15). The follow-up period for the majority of cohorts was <6 years (4–10,12,13,15). Most used serum creatinine to describe renal function and not eGFR (5–13,15). Therefore, in this study we sought to assess the associations between demographics, clinical characteristics, histopathology, immunosuppression treatment (IST), and long-term kidney survival in a large, racially and ethnically diverse, adult FSGS cohort with long-term follow-up.

Materials and Methods

We queried the previously described US Department of Defense health care network for current and previous active-duty military members with the

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International Classification of Diseases, 9th Revision (ICD-9) and 10th Revision (ICD-10) codes 581.1, 582.1, and N04 between 2005 and 2019 (16). The health care network is comprised of 9.6 million people with 65 hospitals and 412 clinics worldwide that have shared a universal electronic medical record (EMR) since 2005. We next reviewed the comprehensive EMR for each potential case to compile the cohort of 338 patients with FSGS. FSGS diagnosis was confirmed by histopathology report or multiple notes from a nephrologist confirming biopsy-proven primary FSGS. Patients were excluded when there was evidence for a secondary cause of FSGS to include HIV, hepatitis B, hepatitis C, parvovirus B19, single kidney, reflux nephropathy, heroin use, and sickle cell disease.

Demographics, presenting clinical characteristics, laboratory, histopathologic, treatment, and outcome data were collected for each FSGS patient in the cohort from the time of biopsy-proven diagnosis to ESKD or last follow-up. Hypertension was defined by a prescription for antihypertensive medications or ≥ 2 systolic or diastolic BP readings of ≥ 140 mm Hg or ≥ 90 mm Hg, respectively, more than 2 weeks apart. Proteinuria was quantified by either 24-hour urine collection or spot urine protein-to-creatinine ratio (UPCR). eGFR was determined using the CKD Epidemiology Collaboration equation. ESKD was defined by the initiation of kidney replacement therapy or preemptive kidney transplant. Glucocorticoid use was defined by ≥ 30mg per day with the intent to treat for up to 4 months to achieve remission unless the risk of an adverse outcome outweighed the perceived benefit of therapy. RAAS inhibitions included any use of angiotensin-converting enzyme inhibitor, angiotensin II receptor blocker, or mineralocorticoid receptor antagonist. Interstitial inflammation and interstitial fibrosis and tubular atrophy (IFTA) were defined by either percent or description. "Scant" was assigned equivalence to $<5\%$, "mild" or "patchy" to 5%–15%, "moderate" to 15%–25%, and "significant" or "severe" to $>25\%$. Categorical variables were established for nephrotic-range proteinuria (NRP; ≥ 3.5 gm/d), CKD (eGFR at diagnosis <60ml/min per 1.73 m^2), IST (any duration of glucocorticoids, calcineurin inhibitor, cyclophosphamide, and/or mycophenolate mofetil), IFTA ($\geq 15\%$ or a description of "moderate," "severe," or "significant"), interstitial inflammation ($\geq 15\%$ or a description of "moderate," "severe," or "significant"), serum albumin (<3.0 mg/dl), IgM staining on immunofluorescence (present), C3 staining on immunofluorescence (present), and achievement of remission. Complete remission was defined by a reduction in proteinuria to <0.3 gm/24 hours with $<25\%$ reduction in eGFR from baseline at biopsy diagnosis. Partial remission was defined by a 50% reduction in proteinuria to <3.5 gm/d or a reduction in proteinuria to <2.0 gm/d.

STATA MP/16.1 (College Station, Tx) was used for all statistical analysis. Univariate analyses were performed with chi-squared testing for categorical variables. The t-test was used for continuous variables (Mann–Whitney test used for non-normally distributed variables). $P<0.05$ was considered statistically significant for all comparisons.

The probability of ESKD by a certain time point was estimated using the Kaplan-Meier method and log-rank test to assess the significant difference between the groups. We conducted both nonstratified and stratified Kaplan-Meier analyses by demographic and clinical variables. We also conducted Cox proportional hazards regression analyses to assess for association between each potential risk factor and renal survival. The categorical variables in the Cox models were age (<60 years old versus >60 years old), race (Black versus non-Black), serum albumin (≤ 3 gm/dl versus >3 gm/dl, proteinuria (>3.5 gm/d versus ≤ 3.5 gm/d), eGFR (<60 ml/min/1.73^2 versus ≥ 60 ml/min/1.73^2), remission state (complete versus partial versus no remission), IST (yes versus no), interstitial inflammation ($\geq 15\%$ versus $<15\%$), and IFTA ($\geq 15\%$ versus $<15\%$). Multivariate analyses were performed for the entire study cohort and were further stratified by race, eGFR, NRP, serum albumin, and by both NRP and hypoalbuminemia. We tested the proportional-hazards assumption on the basis of the graphical method of plotting estimates of $-\ln[-\ln$ (survival probability)] versus $\ln$ (analysis time) and on the basis of testing of Schoenfeld residuals. We found no evidence that our specifications violated the proportional-hazards assumption. The follow-up period for the survival analyses were censored at the last patient follow-up or end of study in January 2020. This study was approved by the Walter Reed National Military Medical Center Institutional Review Board.

Results

The FSGS cohort was predominately Black, male, and <40 years old (Table 1). Nearly all patients with FSGS had hypertension at diagnosis (97%). NRP, a serum albumin ≤ 3.0 mg/dl, and an eGFR <60 ml/min/1.73^2 were present at biopsy-proven FSGS diagnosis in 46%, 32%, and 43% of patients, respectively (Table 1). Median follow-up after biopsy diagnosis was 9.5 years. At 5, 10, and 15 years after biopsy diagnosis, 67%, 42%, and 19% of initial patients with FSGS had clinical follow-up data available (Supplemental Figure 1). The complete renal biopsy report was available for review in 64% of patients with FSGS. Documentation by a nephrologist confirmed the other patients. The majority of biopsy reports did not list a specific variant (86%). RAAS inhibition was almost universal (97%), but only 42% of patients with FSGS received IST. Corticosteroids and calcineurin inhibitors were most common. In total, 27% of patients progressed to ESKD. Obesity (BMI >30) was not associated with progression to ESKD (22% [22/98] versus 24% [31/130], $P=0.8$).

Overall, 5-, 10-, and 15-year renal survival were 86%, 75%, and 55%, respectively (Supplemental Figure 1). On Kaplan-Meier analysis, renal survival at 5, 10, 15 years was lower for patients with FSGS and NRP versus non-NRP, serum albumin ≤ 3.0 mg/dl versus >3.0 mg/dl, inflammation $\geq 15\%$ versus $<15\%$, eGFR <60 ml/min/1.73^2 versus ≥ 60 ml/min/1.73^2, and IFTA $\geq 15\%$ versus $<15\%$ (Figures 1 and 2, Table 2). Black race, Hispanic ethnicity, sex, age, and BMI were not associated with worse long-term renal survival (Supplemental Figure 2).

IgM and C3 staining on immunofluorescence were not individually associated with worse renal survival. The combination of both IgM and C3 staining was also not associated with reduced long-term renal survival to include analysis of both IgM+/C3+ ($n=100$) versus C3$-$/IgM$-$ ($n=44$), and IgM+/C3+ ($n=100$) versus IgM$-$/C3$-$ ($n=44$), IgM+/

Table 1. Clinical background data for patients with FSGS at diagnosis

Variable	All	No ESKD (%)	ESKD (%)	*P* value
Total, *N*	338	247	91	
Male, *n* (%)	290 (86)	210/247 (85)	80/91 (88)	0.50
Race, *n* (%)				
Black	216 (64)	154/247 (62)	62/91 (68)	0.39
White	109 (32)	84/247 (34)	25/91 (28)	0.31
Asian	11 (3)	9/247 (4)	2/91 (2)	0.73
American Indian	2 (0.6)	0/247 (0)	2/91 (2)	0.07
Ethnicity				
Hispanic	34 (10)	21/247 (9)	13/91 (14)	0.15
Non-Hispanic	98 (29)	72/247 (29)	26/91 (29)	1
Missing	206 (61)	154/247 (62)	52/91 (57)	0.45
Age (yr)	35.7±12.6	35.8±12.4	35.5±13.0	0.88
UPCR at diagnosis (gm)	4.6±4.5	4.4±4.6	5.2±4.2	0.32
Diagnosis proteinuria, *n/N* (%)				
<3.5 gm	150/278 (54)	120/210 (57)	30/68 (44)	0.07
≥3.5 gm	128/278 (46)	90/210 (43)	38/68 (56)	0.07
Diagnosis creatinine (mg/dl)	1.8±1.2	1.5±0.9	2.7±1.6	<0.001
Diagnosis eGFR (ml/min/1.73^2)	65±31	73±30	43±23	<0.001
Diagnosis eGFR <60 m/min/1.73^2	121/281 (43)	71/212 (34)	50/69 (73)	<0.001
Diagnosis albumin (g/dl)		3.5±0.9	3.2±1.0	0.02
Diagnosis albumin, *n/N* (%)				
≤3.0 g/dl	87/271 (32)	60/204 (29)	27/67 (40)	0.01
≤2.5 g/dl	53/281 (19)	34/204 (17)	19/67 (28)	0.04
Immunosuppression, *n/N* (%)	137/329 (42)	104/246 (42)	33/83 (40)	0.69
Prednisone	133/329 (40)	102/246 (42)	31/83 (37)	0.51
CNI	60/329 (18)	45/246 (18)	15/83 (18)	0.96
MMF	28/329 (9)	19/246 (8)	9/83 (11)	0.38
Cyclophosphamide	4/329 (1)	3/246 (1)	1/83 (1)	1
Rituximab	3/329 (0.9)	2/246 (0.8)	1/83 (1)	1
Remission, *n/N* (%)				
Complete	62/238 (26)	59/169 (35)	3/69 (5)	<0.001
Partial	60/238 (25)	49/169 (29)	11/69 (16)	0.04
None	116/238 (49)	61/169 (36)	55/69 (80)	<0.001
Follow-up months		128±93.6		
Months to ESKD			83.6±74.4	
Total glomeruli	17.3±11.1	18.7±11.3	12.9±9.2	0.001
Segmental glomeruli	2.8±2.7	2.9±2.6	2.7±2.9	0.61
Obsolescent glomeruli	3.4±3.9	3.1±3.4	4.6±5.3	0.01
IFTA, *n/N* (%)				
<15% or none/scant/mild	133/215 (62)	117/165 (71)	16/50 (32)	<0.001
≥15% or moderate/significant	82/215 (38)	48/165 (29)	34/50 (68)	<0.001
Inflammation, *n/N* (%)				
<15% or none/scant/mild	154/188 (82)	126/145 (87)	28/43 (65)	0.002
≥15% or moderate/significant	34/188 (18)	19/145 (13)	15/43 (35)	0.002
IgM staining	129/187 (69)	103/147 (70)	26/40 (65)	0.54
C3 staining	114/186 (61)	87/147 (59)	27/39 (70)	0.25
Biopsy variants, *n/N* (%)				
NOS	186/215 (87)	146/167 (87)	40/48 (83)	0.47
Collapsing	11/215 (5)	6/167 (4)	5/48 (10)	0.07
Tip	12/215 (6)	10/167 (6)	2/48 (4)	0.74
Cellular	4/215 (2)	4/167 (2)	0/48 (0)	0.57
Perihilar	2/215 (0.9)	1/167 (0.6)	1/48 (2)	0.40
BMI at diagnosis (kg/m^2)	30.1±5.2	30.1±5.1	30.1±5.6	0.99
HTN, prediagnosis, *n/N* (%)	147/264 (56)	107/198 (54)	40/66 (60)	0.21
HTN, postdiagnosis, *n/N* (%)	327/338 (97)	237/247 (96)	90/91 (99)	0.18
DM, *n/N* (%)	63/337 (19)	41/246 (17)	22/91 (24)	0.24
IDDM, *n/N* (%)	30/337 (9)	20/246 (8)	10/91 (11)	0.59
ACEi/ARB, *n/N* (%)	329/338 (97)	242/247 (98)	87/91 (96)	0.23

UPCR, urine protein-to-creatinine ratio; CNI, calcineurin inhibitor; MMF, mycophenolate mofetil; IFTA, interstitial fibrosis and tubular atrophy; NOS, not otherwise specified; BMI, body mass index; HTN, hypertension; DM, diabetes mellitus; IDDM, insulin dependent diabetes mellitus; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker. Continuous variables presented with mean ± standard deviation.
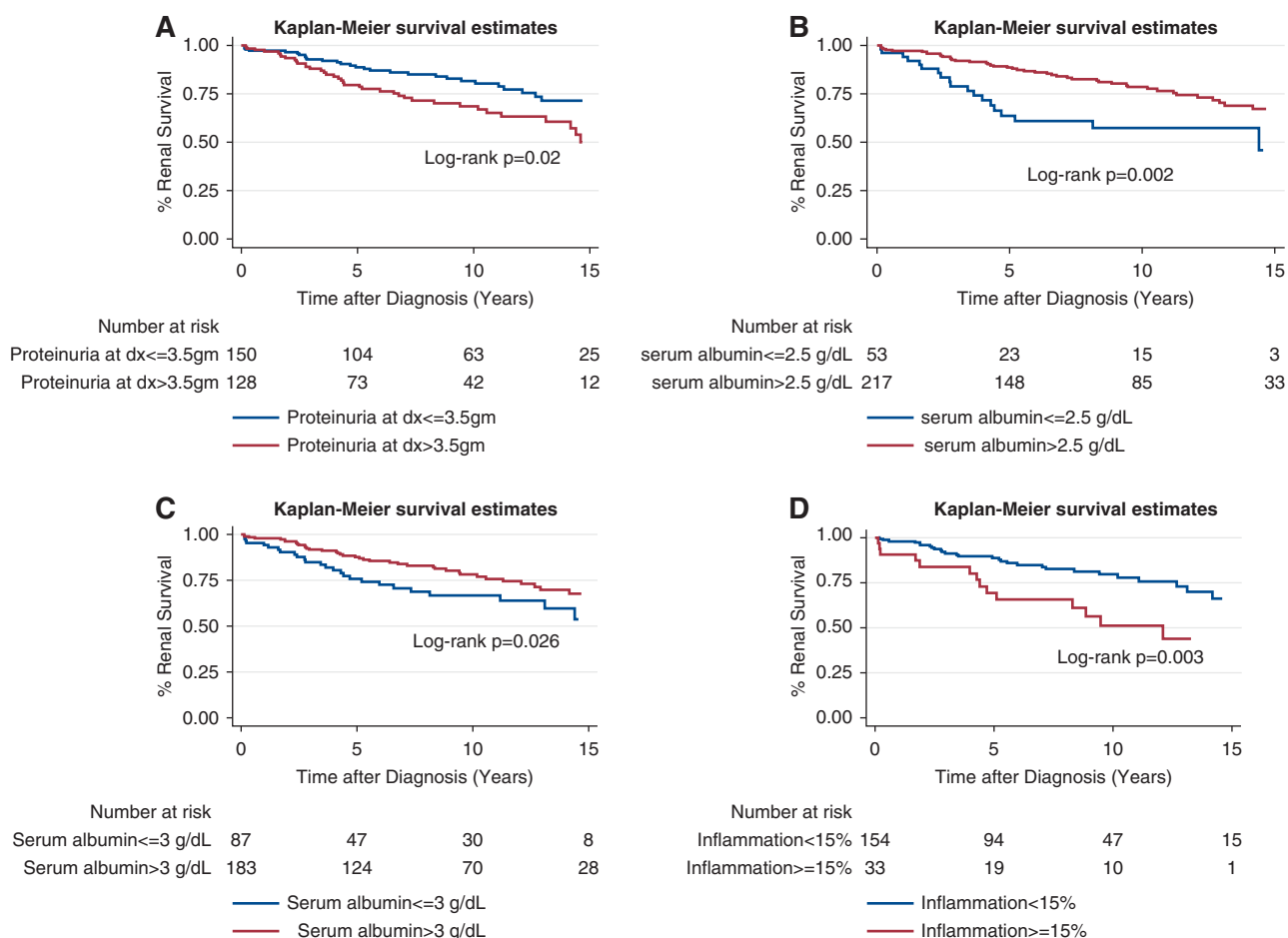
Figure 1. | **FSGS Kaplan-Meier curves for renal survival on the basis of disease acuity variables.** These include (A) nephrotic range proteinuria versus non-nephrotic range proteinuria; (B) serum albumin ≤2.5 g/dl versus >2.5 g/dl; (C) serum albumin ≤3.0 mg/dl versus >3.0 mg/dl; and (D) interstitial inflammation ≥15% versus <15%. Dx, diagnosis.

C3− (n=28), and IgM−/C3+ (n=14) (Supplemental Figure 3).

In total, 42% of patients received immunosuppression. Immunosuppression was not associated with improved renal survival for the whole FSGS cohort (P=0.99; Figure 3) or groups stratified by eGFR ≥60 ml/min per 1.73 m² (P=0.96), eGFR <60 ml/min per 1.73 m2 (P=0.88), NRP (P=0.09), and non-NRP (P=0.78, Figure 3, Supplemental Figure 4). But, when stratified by albumin ≤3.0 mg/dl and ≤2.5 mg/dl, IST was associated with improved renal survival (P=0.01 and P<0.001, respectively; Figure 3). IST was also associated with increased renal survival in a subgroup stratified for both NRP and albumin ≤3.0 mg/dl (P=0.001) and a subgroup stratified for both NRP and albumin ≤2.5 mg/dl (Figure 3, P=0.03). When stratified by Black race (P=0.9), Hispanic ethnicity (P=0.79), age (0.80), sex (P=0.95), BMI (0.54), interstitial inflammation (P=0.34), IFTA (P=0.64), and IgM+/C3+ immunofluorescence (P=0.31), IST was not associated with improved renal survival (Supplemental Figures 5 and 6).

Multivariable Cox regression analysis found that serum albumin, eGFR, percent IFTA, percent inflammation, and NRP at biopsy-confirmed FSGS diagnosis all remained

significantly associated with ESKD (Tables 3 and 4). Black race was not associated with ESKD. Two separate Cox regression analyses were performed, which included either IFTA or interstitial inflammation because of high clinical correlation. RAAS inhibition and hypertension were not included in the model because they were present in over 97% of patients with FSGS.

Complete and partial remission occurred in 26% and 25% of patients with FSGS, respectively. Renal survival was significantly better with complete and partial remission than no remission at 5, 10, and 15 years (Figure 4). Remission remained significantly associated with renal survival after adjustment for confounding variables (Table 3).

Discussion

This large racially and ethnically diverse adult FSGS cohort with a long clinical follow-up period provides valuable prognostic and IST treatment outcome data to instruct patient counseling and care, and guide future research. Because of our large cohort size, we were able to demonstrate that NRP, eGFR <60 ml/min per 1.73 m², hypoalbuminemia, IFTA, interstitial inflammation, and absence of
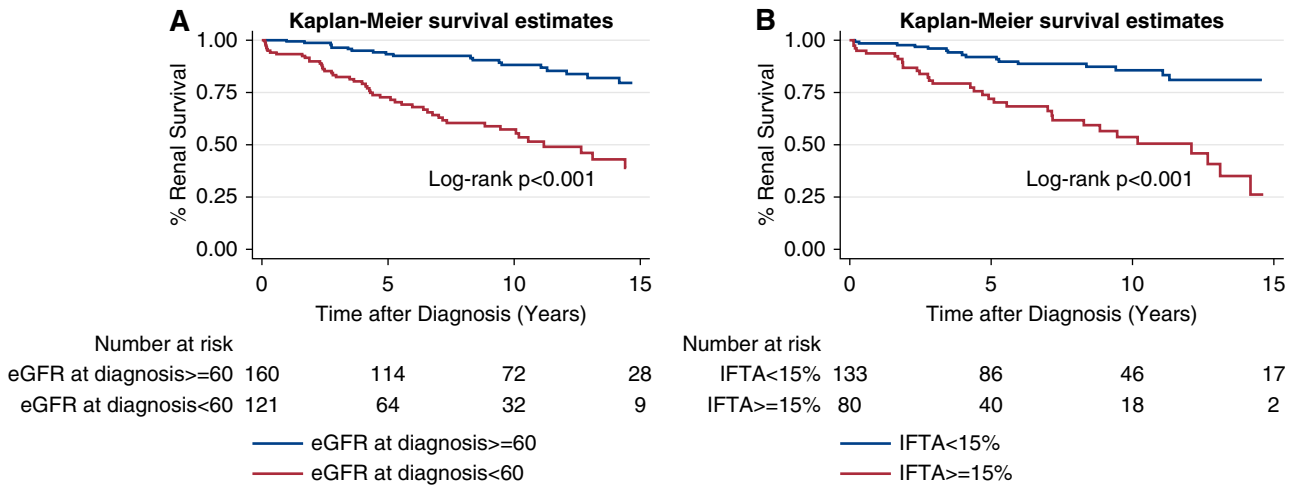
Figure 2. | **FSGS Kaplan-Meier curves for renal survival on the basis of disease chronicity variables.** These include (A) eGFR $\geq$60 ml/min/1.73^2 versus <60 ml/min/1.73^2 at diagnosis; and (B) interstitial fibrosis and tubular atrophy (IFTA) $\geq$15% versus <15%.

remission were all associated with reduced renal survival even after adjusting for potential confounding variables (Tables 3 and 4). Previous studies reported mixed and sometimes conflicting results on these potential risk factors. Many did not have a large enough cohort for multivariate analysis (3–15). Similar to previous studies, Black and White race had similar long-term renal survival. We also report for the first time that Hispanic ethnicity had similar long-term renal survival to non-Hispanic patients with FSGS.

The overall 5-year renal survival in our cohort was similar to the cohort of 250 patients reported by Bohle *et al.* (86% versus 90%), but significantly better at 10 years (75% versus 50%) and 15 years (65% versus 35%) (Supplemental Table 1) (6). A similar pattern of improved renal survival was noted in between NRP subgroups at 10-year follow-up (69% versus 50%) and 15-year follow-up (50% versus 30%) and between CKD subgroups at 15-year follow-up (39% versus 25%) (Figure 1). In addition, patients in our cohort that did

not achieve remission had a higher percent renal survival than the 281-patient cohort published by Troyanov *et al.* at 10-year (56% vs 40%) and 15-year (37% versus 20%) follow-up (4,5). The same trends were seen when comparing our cohort to other smaller cohorts (7,9–11,13). The most likely explanation for the higher observed long-term renal survival in these high-risk FSGS subgroups is the ubiquitous use of RAAS inhibition in our cohort (97%). However, we were limited in our ability to compare renal outcomes on the basis of RAAS inhibition because only nine patients were not treated. Previous retrospective FSGS studies found that RAAS inhibition reduced proteinuria, but did not affect renal survival (17–20). However, these studies were limited by their small cohorts and short follow-up periods. Three larger cohort studies documented 50%–90% RAAS inhibition but they neither specifically analyzed the association of RAAS inhibition and renal survival nor had overall renal survival data at 10 and 15 years (3,4,14). The studies that did

Table 2. The 5-, 10-, and 15-yr renal survival rates for various risk factors			
Kidney Survival (%)	5 Yr	10 Yr	15 Yr
Overall	86	75	55
NRP	80	69	50
NNRP	89	82	71
Albumin <3.0 g/dl	76	67	54
Albumin >3.0 g/dl	88	78	68
eGFR <60 m/min/1.73^2	73	57	39
eGFR >60 m/min/1.73^2	93	88	80
IFTA $\geq$15%	72	54	26
IFTA <15%	92	86	81
Interstitial inflammation $\geq$15%	79	62	49
Interstitial inflammation <15%	90	83	71
Complete remission	98	96	93
Partial remission	88	78	68
No remission	72	56	37
NRP, nephrotic-range proteinuria; NNRP, non-NRP; IFTA, interstitial fibrosis and tubular atrophy.			
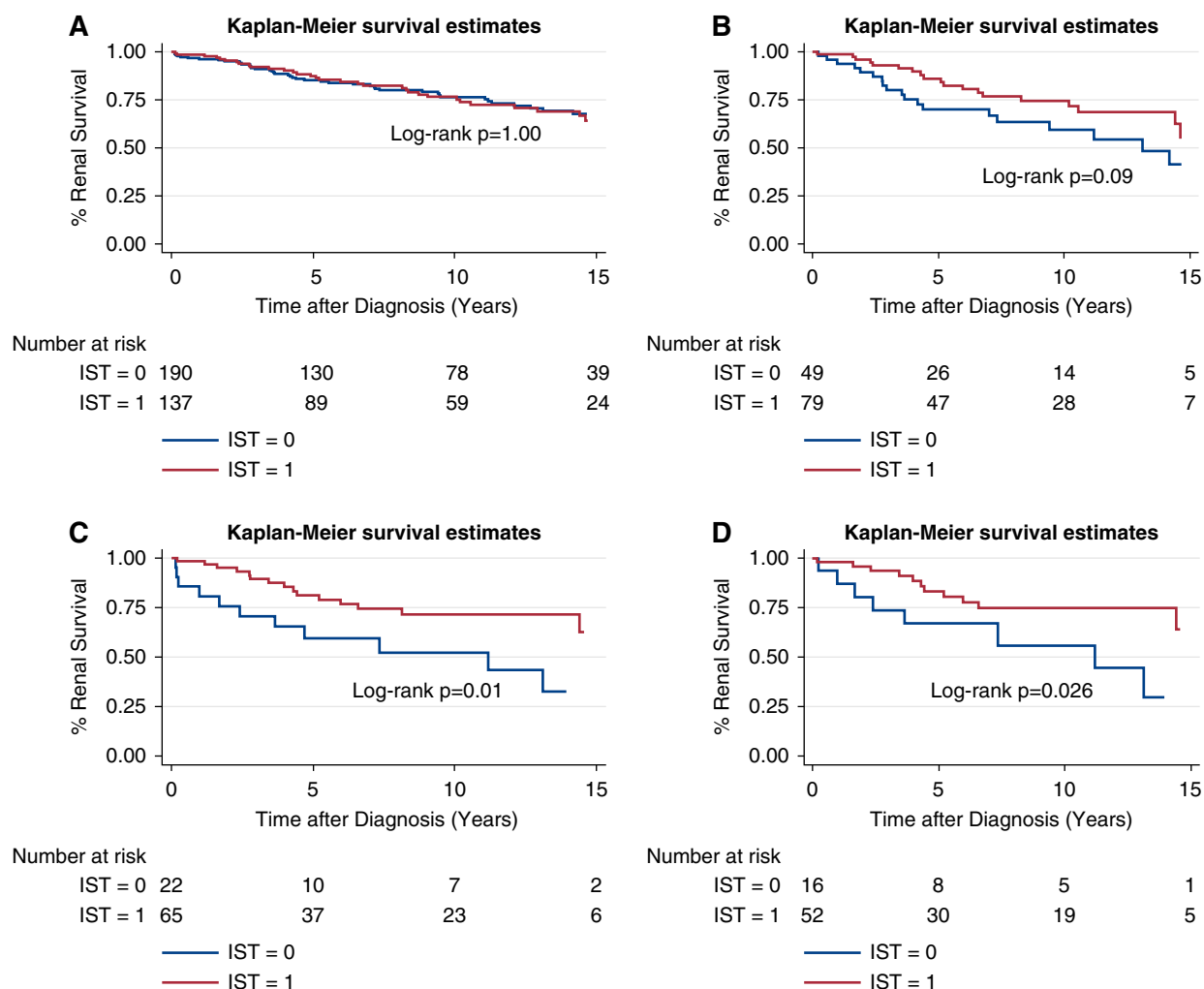
Figure 3. | FSGS Kaplan-Meier curves for renal survival curves. These are for (A) immunosuppression treatment (IST) versus no IST; (B) IST versus no IST stratified by nephrotic-range proteinuria; (C) IST versus no IST stratified by albumin ≤3.0 mg/dl; and (D) IST versus no IST stratified by both nephrotic-range proteinuria (NRP) and albumin ≤3.0 mg/dl.

have long-term renal survival data included patients predominantly from time periods before significant RAAS inhibition use (5–7,9–11,13). Although significantly limited by historical comparison, our study suggests RAAS blockade may provide some long-term renal survival benefit even in high-risk patients with FSGS, which supports current Kidney Disease: Improving Global Outcomes guidelines. Alternatively, our study had a larger number of patients still tracked at 10 and 15 years, which decreases bias in our estimates. In addition, we provide the first renal survival

Table 3. Multivariate analysis including IFTA

Variable	Hazard Ratio (95% Confidence Interval)	P Value
Age	1.03 (1.00 to 1.06)	0.008
Black race	0.9 (0.5 to 1.8)	0.77
Albumin ≤3.0 mg/dl	4.2 (1.7 to 11.1)	0.003
Proteinuria >3.5 gm	3.1 (1.3 to 7.3)	0.009
eGFR >60 ml/min/1.73^2	0.23 (0.09 to 0.53)	0.001
IFTA ≥15%	1.5 (1.0 to 2.2)	0.05
Complete remission	0.06 (0.02 to 0.24)	<0.001
Partial remission	0.18 (0.07 to 0.48)	0.001
IST	0.62 (0.26 to 1.5)	0.28

Each variable is adjusted for the others listed in the table. IFTA, interstitial fibrosis and tubular atrophy; IST, immunosuppression treatment.

Table 4.   Multivariate analysis including interstitial inflammation

Variable	Hazard Ratio (95% Confidence Interval)	P Value
Age	1.05 (1.02 to 1.08)	0.001
Black race	0.7 (0.3 to 1.6)	0.38
Albumin ≤3.0 mg/dl	2.0 (0.7 to 5.5)	0.19
Proteinuria >3.5 gm	5.7 (2.3 to 14.3)	<0.001
eGFR >60 ml/min/1.73^2	0.17 (0.07 to 0.41)	<0.001
Interstitial inflammation ≥15%	2.6 (1.1 to 6.4)	0.04
Complete remission	0.04 (0.01 to 0.18)	<0.001
Partial remission	0.12 (0.04 to 0.32)	<0.001
IST	0.55 (0.23 to 1.3)	0.18

Each variable is adjusted for the others listed in the table. IST, immunosuppression treatment.

curves using eGFR at diagnosis which will provide more robust prognostic information for reduced renal function present at diagnosis.

Interstitial inflammation was independently associated with worse long-term renal survival (Figure 1, Table 4). The most likely explanation is that interstitial inflammation transitioned to fibrosis. However, IST was not associated with improved renal survival in an FSGS subgroup with interstitial inflammation. Given observed trends, it is possible that IST treatment in patients with FSGS and interstitial inflammation is associated with improved renal survival after 10 years, but statistical significance was not demonstrated due to the low number of patients at these time intervals (Supplemental Figure 5). Interstitial inflammation has not been previously assessed as a risk factor for progression to ESKD in an FSGS cohort. Our data suggest it could help assess kidney prognosis and be a topic for future study.

There has been renewed focus on the potential pathogenic role of IgM antibody and complement activity in FSGS (21,22). Strassheim *et al.* (21) reported compelling evidence from a murine model that IgM binds in the glomerulus and activates complement to cause increased proteinuria. Two FSGS cohort studies found no association between IgM

Kaplan-Meier survival estimates

Log-rank p<0.001

% Renal Survival — Time after Diagnosis (Years)

Number at risk

Complete remission	62	52	33	9
Partial remission	60	40	23	7
No remission	114	63	31	15

Complete remission
Partial remission
No remission

Figure 4. | FSGS Kaplan-Meier curves for renal survival curve for complete remission (blue), partial remission (red), and no remission (green).

staining on immunofluorescence and long-term renal survival in FSGS (23,24). C3 staining was associated with worse renal outcomes in one study but not another (23,25). The combination of IgM and C3 staining was associated with both renal dysfunction and poor renal survival previously (23,24). In our cohort, C3, IgM, or a combination of IgM/C3 staining on immunofluorescence were not associated with reduced renal survival (Supplemental Figure 3). Previous cohorts that investigated IgM/C3 staining included predominantly Asian patients, had significantly fewer patients, and a shorter follow-up than our cohort.

Immunosuppression was not associated with better renal survival in the whole FSGS cohort (Figure 3). Immunosuppression was also not associated with a renal survival benefit in subgroups stratified separately by proteinuria and eGFR at diagnosis. However, it is possible that immunosuppression may have provided benefit in patients with FSGS and NRP (P=0.09) if the stratified cohort had been larger to provide more power. Interestingly, when further stratified by hypoalbuminemia and proteinuria together and hypoalbuminemia alone, immunosuppression was associated with improved renal survival (Figure 3). Specifically, the lower the serum albumin at diagnosis, the more IST was associated with improved long-term renal survival. Analysis of hypoalbuminemia alone is relevant because it is possible that some patients with FSGS with proteinuria ≤3.5 gm on spot urine protein-to-creatinine ratio would have had >3.5 gm if measured by the gold-standard 24-hour urine collection, and therefore met the criteria for nephrotic syndrome if accompanied by a serum albumin ≤3.0 g/dl. Our findings suggest that immunosuppression may only significantly benefit patients with FSGS and nephrotic syndrome and not FSGS of undetermined cause with significant proteinuria but preserved serum albumin. This is the strongest evidence to date to support the 2012 and 2020 draft Kidney Disease: Improving Global Outcomes guidelines for FSGS treatment, which were based in part on two previous publications, but acknowledges that treatment strategy for FSGS of undetermined cause is a "conundrum" (26,27). Laurin *et al.* found that immunosuppression was not associated with improved renal survival in the whole FSGS cohort. However, immunosuppression was beneficial in the subgroup of collapsing patients with FSGS, which had 300% higher mean proteinuria and significantly lower mean serum albumin to suggest a much higher incidence of nephrotic syndrome in that subpopulation (14). Schwartz *et al.*
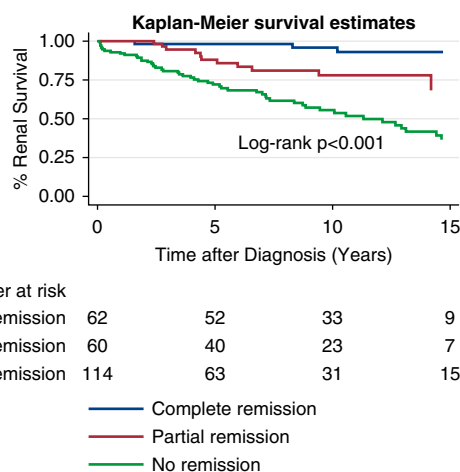
(13) also found that immunosuppression was associated with better renal survival in patients with NRP FSGS but not the whole FSGS cohort. However, serum albumin levels were not reported. The other IST outcome studies did not provide subgroup analysis of hypoalbuminemia or nephrotic syndrome. Most studies demonstrate that IST is associated with increased remission (5,7,8,10,11). Other studies show that remission is associated with improved long-term renal survival (5,7,8,10,11,13). However, this does not guarantee that IST is associated with long-term renal survival. At least three studies reported that IST was not associated with improved renal outcomes, despite it being associated with remission and remission being associated with renal survival (7,10,11). Two other studies found that IST was not associated with long-term renal survival, but did not also have robust analysis of both the IST/remission and the remission/renal survival relationships (6,13). One possible explanation for this pattern is that the renal survival benefit from of IST-induced remission in a nephrotic syndrome subpopulation could be negated by increased IST-related renal risk factors such as hypertension, hyperglycemia, increased BMI, and interstitial fibrosis within the whole cohort. When stratified by race, IST was not associated with improved renal survival in Black patients. Although we did not measure APOL1 genotype, this would suggest IST does not directly treat the specific pathophysiology of APOL1-associated FSGS (8,28,29). Therefore, developing more targeted therapy would likely be beneficial.

The strength of this study is that it is the largest racially and ethnically diverse adult FSGS cohort from the era of RAAS inhibition with the longest follow-up period. In addition, patients were predominantly managed within the same health care system. This minimized potential confounding due to differences in access to care, quality of care, and socioeconomic factors. It also reports on the effect of interstitial inflammation and IgM and C3 staining on immunofluorescence on renal survival.

Our study had limitations intrinsic to the retrospective cohort design. Specifically, there was significant variability in treatment strategies, follow-up intervals, and laboratory data. Some patients did not have all of the desired data available in the EMR for collection. A detailed histopathology report was not available for each patient nor was each biopsy reviewed independently by a study pathologist. The majority of detailed pathology reports did not comment on a specific FSGS lesion. Interstitial inflammation and IFTA severity were assigned by either reported percent or description. Both are estimates subject to pathologist interpretation and sample variability. Correlation between percent and descriptive report of interstitial inflammation and IFTA has not been previously investigated. These limitations could explain why we found no renal survival benefit for IST in patients with higher interstitial inflammation. Our cohort was predominantly male likely because it was derived from prior and current military members. Despite our large cohort with long median follow-up, we were not able to adjust for all potential confounding variables in multivariable analyses because it would reduce study power and significance. We therefore focused principally on variables with statistical significance in univariate analysis. Specifically, we did not include BMI in the multivariate analysis, because obesity was not associated with ESKD on univariate

analysis or renal survival on Kaplan Meier analysis. We did not have genetic analysis for the study cohort (2). Uneven distribution of established FSGS risk mutations that are generally more resistant to steroid treatment, could have been a confounding variable in data analysis. However, inadvertent inclusion of steroid-resistant genetic causes of FSGS would have only reduced or negated the significance of our treatment comparisons. Specifically, we were not able to adjust for high-risk APOL1 genotype, but most clinicians do not currently have access to this information, so our data can still be instructive for real-time patient care. We did not record the exact duration of prednisone treatment for each patient due to the challenges of tracking the exact timing of not only cessation of treatment, but also the variable weaning strategies. However, any abridged steroid courses would most likely have only reduced the significance of our treatment analysis. We also cannot fully delineate between proteinuria and a negative acute phase reactant of systemic inflammation as the cause of hypoalbuminemia, although IST would theoretically improve both. Finally, Hispanic ethnicity data were missing from a large number of patients with FSGS, so data analysis in this group may have been underpowered to show existing significant differences. Future study of FSGS in the Hispanic population is warranted.

This large, racially and ethnically diverse adult FSGS cohort with long-term follow-up provides important prognostic information for clinicians and suggests IST should be reserved only for FSGS with nephrotic syndrome, but a prospective trial is required to confirm these findings.

Supplemental Material

This article contains the following supplemental material online at http://kidney360.asnjournals.org/lookup/suppl/doi:10.34067/KID.0006172020/-/DCSupplemental.

Supplemental Figure 1. FSGS Kaplan-Meier curves for renal survival in the whole cohort.

Supplemental Figure 2. FSGS Kaplan-Meier curves for renal survival: A) Black patients vs. White patients and B) Hispanic patients vs. Non-Hispanic patients C) Patients with BMI >30 vs. BMI <30.

Supplemental Figure 3. FSGS Kaplan-Meier curves for renal survival: A) IgM positive immunofluorescence versus IgM negative immunofluorescence; B) C3 positive immunofluorescence versus C3 negative immunofluorescence; C) IgM and C3 positive immuno-fluorescence versus IgM and C3 negative immunofluorescence.

Supplemental Figure 4. FSGS Kaplan-Meier curves for renal survival: A) Immunosuppression treatment (IST) versus no IST stratified by estimate glomerular filtration rate ≥ 60 ml/min; B) Immunosuppression treatment (IST) versus no IST stratified by estimate glomerular filtration rate < 60 ml/min.

Supplemental Figure 5. FSGS Kaplan-Meier curves for renal survival for Immunosuppression treatment (IST) versus no IST stratified by interstitial inflammation ≥ 15%.

Supplemental Figure 6. FSGS Kaplan-Meier curve for renal survival for immunosuppression treatment (IST) versus no IST stratified by Black race.

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