

Urinary sC5b-9 is associated with proteinuria and intrarenal complement activation in pediatric kidney transplant recipients

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Abstract

Background

Tubulointerstitial fibrosis is the principal determinant of long-term kidney graft outcome, but its assessment still relies on allograft biopsy, an invasive procedure subject to sampling variability. Proteinuria is associated with fibrosis but remains non-specific. Complement activation is increasingly implicated in tubular injury, and urinary soluble C5b-9 (sC5b-9) has been proposed as a marker of intrarenal complement activation. Whether it reflects genuine local complement activity or simply tracks proteinuria is unresolved, and data in pediatric transplant recipients are scarce.

Methods

We conducted a retrospective single-centre study of 31 pediatric kidney transplant recipients undergoing allograft biopsy; C4d-positive biopsies were excluded. Urinary and plasma sC5b-9 were measured by ELISA on first morning voids collected on the day of biopsy and related to proteinuria, estimated glomerular filtration rate (eGFR), interstitial fibrosis and tubular atrophy (IFTA), and intratubular C5b-9 deposition scored blinded to clinical data. Spearman rank correlation was the primary method; discrimination was assessed by ROC analysis and independence from proteinuria by multivariable logistic regression.

Results

Urinary sC5b-9 was detectable in 61% of patients and correlated with proteinuria ($p = 0.48$, $p = 0.007$) and with the proportion of tubules bearing C5b-9 deposits ($p = 0.56$, $p = 0.001$), supporting intratubular complement activation. Urinary sC5b-9 discriminated above-median intratubular C5b-9 deposition better than proteinuria (AUC 0.77 vs 0.54) and remained independently associated with it after adjustment for proteinuria ($p = 0.023$), improving model fit over proteinuria alone ($p = 0.014$). Urinary sC5b-9 was unrelated to eGFR and did not differ according to the presence of haematuria. Plasma sC5b-9 remained within normal ranges in all patients. No significant association was observed between urinary sC5b-9 and IFTA.

Conclusion

Urinary sC5b-9 is associated with proteinuria and intratubular complement activation in pediatric kidney transplant recipients, and provides information beyond proteinuria. With consistently normal plasma levels, these findings support a locally generated, complement-specific signal rather than passive leak of a urinary protein. No prognostic claim can be made from this cross-sectional cohort, in which no

association with IFTA was found; longitudinal studies are needed to determine whether urinary sC5b-9 precedes and predicts fibrosis progression.

Introduction

In kidney transplantation, allografts are exposed to multiple injuries, including ischemia-reperfusion injury, calcineurin inhibitor toxicity, pyelonephritis and rejection episodes. These insults contribute to tubulointerstitial fibrosis, which is strongly associated with graft survival, making early detection and monitoring essential (1, 2).

Although surveillance biopsies remain the reference standard, assessment of fibrosis using Interstitial Fibrosis and Tubular Atrophy score (IFTA) is limited by sampling variability due to the patchy distribution of lesions (3).

Consequently, there is a need for reliable and minimally invasive biomarkers. Albuminuria is a hallmark of glomerular injury and a risk factor for chronic kidney disease progression (4, 5). Experimental data suggest that glomerular injury may induce secondary tubulointerstitial damage through sustained tubular stress (6, 7). Tubulointerstitial damage occurs with consequent nephron loss and is the final common pathway leading to chronic kidney disease.

The complement system plays a central role in innate immunity and renal injury. Activation of complement leads to formation of C5b-9 and release of pro-inflammatory mediators (C3a, C5a) (8, 9). Renal tissue is particularly susceptible to complement-mediated injury due to limited expression of complement regulatory proteins and local complement components production (10).

Recent studies suggest that intratubular complement activation, secondary to filtration of complement components, may contribute to fibrosis (10–12).

Clinical relevance of urinary sC5b-9 in a range of kidney conditions, highlighting its diagnostic and prognostic values, has been recently reported (13–15).

C5b-9 deposition in fibrotic lesions has been strongly associated with fibrosis severity across various kidney diseases (16–18). Data in pediatric transplant recipients are scarce.

The aim of this study was to evaluate the relationship between complement activation biomarkers (plasma and urinary sC5b-9), proteinuria, tubular C5b-9 deposition and IFTA in pediatric kidney transplant recipients.

Materials and Methods

We conducted a retrospective study of pediatric renal transplant recipients who underwent graft biopsy and provided consent to the DOREGLO biobank between 2018 and 2024 at Sainte-Justine Hospital, Montreal, Canada. Biopsies were performed as protocol biopsies in 21 patients (68%) and for clinical

suspicion of rejection in 10 (32%). Biopsies with C4d-positive peritubular capillaries were excluded to avoid confounding of urinary sC5b-9 levels by rejection-driven complement activation. Immunohistochemical assessment of C5b-9 deposits was performed blinded to clinical data and to urinary and plasma sC5b-9 measurements.

Urine, plasma, and biopsy samples were collected on the day of biopsy according to our local biobank protocol. All urine samples were collected as the first morning void. This study was approved by the institutional ethics committee, and informed consent was obtained from all participants or their legal guardians. Clinical data included sex, age, cold ischemia time, donor age, donor type (living or deceased), time since transplantation, history of rejection, and indication for biopsy. Induction and maintenance immunosuppression and the underlying cause of end-stage renal disease were also recorded. Estimated glomerular filtration rate (eGFR; ml/min/1.73m²) was calculated using the bedside Schwartz formula ($k = 0.413$) (19). Proteinuria was expressed as the urinary protein-to-creatinine ratio (g/g). Urinary and plasma sC5b-9 were quantified by enzyme-linked immunosorbent assay with the MicroVue™ sC5b-9 Plus EIA (Quidel, Cat. #A029), used according to the manufacturer's protocol, and urinary levels were normalised to urinary creatinine (µg/mmol). Histological analysis included evaluation of IFTA and immunohistochemical detection of C5b-9 deposits in non-atrophic tubules. Deposition was expressed as the percentage of non-atrophic tubules bearing deposits relative to all non-atrophic tubules evaluated in each biopsy section (Fig. 1). Atrophic tubules were excluded from the analysis.

Figure 1. Immunohistochemical detection of C5b-9 deposits in non-atrophic tubules of a pediatric kidney allograft biopsy (original magnification ×10). Brown staining outlines C5b-9 deposition along the basolateral aspect of non-atrophic tubular epithelial cells. The extent of deposition was quantified as the percentage of non-atrophic tubules showing C5b-9 deposits relative to the total number of non-atrophic tubules assessed in each biopsy section. Atrophic tubules were excluded from the analysis, as basement membrane remodelling and non-specific protein entrapment make the signal difficult to interpret in this compartment. Scale bar, 100 µm.

Continuous variables were expressed as median (interquartile range [IQR], 25%-75%), and categorical variables with n (%). Variables were compared using the Mann-Whitney U test for continuous variables and the Fisher exact test for categorical variables; urinary sC5b-9 was also compared according to the presence of haematuria. Correlations were assessed with Spearman's rank correlation coefficient as the primary method: all variables were non-normally distributed (Shapiro–Wilk, all $p < 0.001$). Pearson coefficients are reported alongside as a sensitivity analysis. Undetectable urinary sC5b-9 values ($n = 12$) were assigned the assay limit of detection (0.1 ng/mL) before normalisation to urinary creatinine. Height was unavailable in two patients, so eGFR-based analyses are based on 29 patients. Whether urinary sC5b-9 provided information beyond proteinuria was tested by multivariable logistic regression predicting above-median tubular C5b-9 deposition, with the incremental contribution assessed by the likelihood ratio test; urinary sC5b-9 and proteinuria were log₁₀-transformed for this analysis. Discriminative performance was compared by receiver operating characteristic (ROC) curves, with 95% confidence intervals from 2000 bootstrap resamples. Results were verified by repeating the correlations

after exclusion of the highest urinary sC5b-9 value and of each patient in turn. Analysis was performed using the R version 4.5.0.

Results

The study included 31 patients (21 males, 10 females) with a median age of 13.5 years (IQR 11.6–15.4). Clinical and biological characteristics, including the underlying cause of end-stage renal disease, are summarized in Table 1.

Table 1

Table 1
Clinical and biological characteristics of the study population.

Parameters	n = 31
Gender, male, n (%)	21 (68)
Age (years), median (IQR)	13.5 (11.6, 15.4)
Cold ischemia time (minutes), median (IQR)	330 (167, 540)
Donor age (years), median (IQR)	34.5 (29.3, 43.3)
Living donor, n (%)	15 (50)
Biopsy time from transplantation (months), median (IQR)	32.9 (16.0, 99.9)
Rejection at biopsy (Banff)	
None, n (%)	29 (94)
Cellular (1 acute, 1 chronic), n (%)	2 (6)
Indication for biopsy	
Protocol, n (%)	21 (68)
Suspicion of rejection, n (%)	10 (32)
Induction and maintenance immunosuppression	
Basiliximab induction, n (%)	31 (100)
Prednisone + tacrolimus + MMF, n (%)	31 (100)
Target tacrolimus trough beyond 3 months (ng/mL)	6–8
Cause of end-stage renal disease	
Congenital or genetic nephrotic syndrome, n (%)	9 (29)
Renal hypo-dysplasia, n (%)	6 (19)
Focal segmental glomerulosclerosis, n (%)	4 (13)
Cardio-renal syndrome, n (%)	3 (10)
Lupus nephritis, n (%)	2 (6)
ANCA-associated vasculitis, n (%)	2 (6)
Cystinosis nephropathy, n (%)	2 (6)
Autosomal dominant polycystic kidney disease, n (%)	1 (3)
Alport syndrome, n (%)	1 (3)
Chronic tubulointerstitial nephritis, n (%)	1 (3)

Parameters	n = 31
eGFR at biopsy (mL/min/1.73m ²), median (IQR)	67.7 (55.3, 81.9)
Proteinuria (g/g urinary creatinine), median (IQR)	0.18 (0.13, 0.29)
Calcineurin inhibitor toxicity on biopsy, n (%)	5 (16)
Post-transplant urinary tract infection, n (%)	11 (35)
eGFR at last follow-up (mL/min/1.73m ²), median (IQR)	83.3 (58.6, 96.5)

IQR: interquartile range; eGFR: estimated glomerular filtration rate; MMF: mycophenolate mofetil. eGFR was calculated with the bedside Schwartz formula ($k = 0.413$); height was unavailable in 2 patients, so eGFR at biopsy is based on $n = 29$. Donor type was unavailable in 1 patient, so the proportion of living donors is based on $n = 30$. Biopsies with C4d-positive peritubular capillaries were excluded from the study; no antibody-mediated rejection was present in the analysed cohort.

All patients received basiliximab induction and maintenance immunosuppression with prednisone, tacrolimus and mycophenolate mofetil, with a target tacrolimus trough level of 6–8 ng/mL beyond 3 months post-transplantation. Notably, 91% of patients had no history of rejection prior to biopsy. Median baseline eGFR was 67.7 mL/min/1.73m² (IQR 55.3–81.9), median urinary protein-to-creatinine ratio was 0.18 g/g (normal < 0.15), and median time since transplantation was 33 months. Calcineurin inhibitor (CNI) toxicity lesions were reported on biopsy in 5 patients, and 11 patients had a history of urinary tract infection following transplantation.

Urinary sC5b-9 was detectable in 19 patients (61%) with a median (IQR) urinary sC5b-9 -to-creatinine ratio of 0.46 (0.02–2.78) µg/mmol (Table 2a).

Plasma sC5b-9 levels remained within the normal range in all patients, with a median (IQR) of 162 (124–228) ng/mL (normal < 300 ng/mL; $n = 30$, plasma sC5b-9 being unavailable in one patient) (Table 2a).

The median (IQR) percentage of IFTA was 10% (0–20) (Table 2a). The median (IQR) percentage of non-atrophic tubules with C5b-9 deposits was 5.0% (1.5–10.0) (Table 2a).

Table 2b presents these parameters according to detectable ($n = 19$) or undetectable ($n = 12$) urinary sC5b-9. Correlation coefficients are reported in Table 3.

Table 2

a

Parameter	Median (IQR)
Plasma sC5b-9 (ng/mL), n = 30	162 (124, 228)
Urinary sC5b-9 (µg/mmol urinary creatinine), n = 31	0.46 (0.02, 2.78)
IFTA (%), n = 31	10.0 (0.0, 20.0)
Tubular C5b-9 deposits (%), n = 31	5.00 (1.50, 10.00)

Table 2a. Plasma and urinary sC5b-9, IFTA and tubular C5b-9 deposits in the whole cohort.

IQR: interquartile range; IFTA: interstitial fibrosis and tubular atrophy. Urinary sC5b-9 is reported for the whole cohort; undetectable values were assigned the assay limit of detection (0.1 ng/mL) before normalisation to urinary creatinine. Plasma sC5b-9 was unavailable in one patient (n = 30).

Table 2

b

Parameter	Detectable urinary sC5b-9 (n = 19)	Undetectable urinary sC5b-9 (n = 12)	p
Plasma sC5b-9 (ng/mL)	165 (119, 217)	158 (148, 249)	0.34
Urinary sC5b-9 (µg/mmol urinary creatinine)	2.33 (0.58, 4.69)	0.02 (0.01, 0.02)	< 0.001
IFTA (%)	5.0 (0.0, 21.0)	10.0 (0.0, 20.0)	0.82
Tubular C5b-9 deposits (%)	6.00 (3.00, 17.50)	2.00 (0.00, 7.00)	0.063

Table 2b. Plasma and urinary sC5b-9, IFTA and tubular C5b-9 deposits according to detectability of urinary sC5b-9.

Values are median (IQR). Groups were compared with the Mann–Whitney U test. IFTA: interstitial fibrosis and tubular atrophy.

Table 3

Table 3
Correlations between urinary sC5b-9 and proteinuria, tubular C5b-9 deposition, IFTA and eGFR.

Parameter	Spearman's ρ	p-value
Proteinuria (g/g), n = 31	0.48	0.007
Tubular C5b-9 deposition (%), n = 31	0.56	0.001
IFTA (%), n = 31	0.07	0.72
eGFR (mL/min/1.73m ²), n = 29	0.08	0.66

Correlations were assessed with Spearman's rank correlation. Undetectable urinary sC5b-9 values (n = 12) were assigned the assay limit of detection (0.1 ng/mL) before normalisation to urinary creatinine. Height was unavailable in two patients, accounting for the smaller sample size of the eGFR analysis. IFTA: interstitial fibrosis and tubular atrophy; eGFR: estimated glomerular filtration rate.

Urinary sC5b-9 levels were positively correlated with proteinuria ($\rho = 0.48$, $p = 0.007$) (Fig. 2).

Figure 2. Relationship between urinary sC5b-9 and proteinuria in 31 pediatric kidney transplant recipients. Urinary sC5b-9 (normalised to urinary creatinine, $\mu\text{g}/\text{mmol}$) is plotted against the urine protein-to-creatinine ratio (g/g); both axes are logarithmic. Filled circles denote patients with detectable urinary sC5b-9 (n = 19) and open circles those in whom urinary sC5b-9 was undetectable (n = 12) and was assigned the assay limit of detection (0.1 ng/mL) before normalisation. The solid line is the least-squares fit with its 95% confidence band. Untransformed data are shown in Supplementary Figure S1.

Patients with detectable urinary sC5b-9 had a higher proportion of tubules with C5b-9 deposits, although this group comparison did not reach statistical significance (6.0% [3.0–17.5] vs 2.0% [0.0–7.0], $p = 0.063$); the association was significant when both variables were analysed as continuous measures (see below). Urinary sC5b-9 levels did not differ according to the presence of haematuria (0.35 [0.02–0.90] vs 0.49 [0.02–3.42] $\mu\text{g}/\text{mmol}$, $p = 0.56$).

No association was observed between urinary sC5b-9 and IFTA ($\rho = 0.07$, $p = 0.72$). Antibody-mediated rejection was an exclusion criterion and no C4d-positive biopsy was included (see Methods). Two patients had cellular rejection at the time of biopsy according to the Banff classification, one acute and one chronic; urinary sC5b-9 was undetectable in the patient with acute cellular rejection and detectable in the patient with chronic cellular rejection. Given these small numbers, no formal association between rejection status and urinary sC5b-9 levels could be tested. No significant correlation was observed between urinary sC5b-9 and eGFR ($\rho = 0.08$, $p = 0.66$).

Urinary sC5b-9 was also positively correlated with the proportion of tubules bearing C5b-9 deposits ($\rho = 0.56$, $p = 0.001$). In ROC analysis, urinary sC5b-9 discriminated above-median tubular C5b-9 deposition markedly better than proteinuria did (AUC = 0.77, 95% CI 0.57–0.91 vs AUC = 0.54, 95% CI 0.33–0.76; Fig. 3). In multivariable logistic regression predicting above-median tubular C5b-9 deposition, urinary

sC5b-9 remained independently associated ($p = 0.023$) whereas proteinuria did not ($p = 0.30$); adding urinary sC5b-9 to a proteinuria-only model significantly improved model fit (likelihood ratio test, $p = 0.014$). Both associations were robust: excluding the patient with the highest urinary sC5b-9 value did not weaken the correlation with proteinuria ($p = 0.43$, $p = 0.018$) or with tubular C5b-9 deposition ($p = 0.54$, $p = 0.002$), and each correlation remained significant when any single patient was removed. Untransformed data are shown in Supplementary Figure S1.

Figure 3. Discriminative performance of urinary sC5b-9 and proteinuria for the identification of above-median intratubular C5b-9 deposition, assessed by receiver operating characteristic (ROC) analysis. Areas under the curve (AUC) are reported with 95% confidence intervals derived from 2000 bootstrap resamples. The dashed diagonal denotes non-informative discrimination (AUC = 0.50). Urinary sC5b-9 was normalised to urinary creatinine ($\mu\text{g}/\text{mmol}$) and undetectable values were assigned the assay limit of detection (0.1 ng/mL). $n = 31$.

Discussion

In this study, we demonstrate that urinary sC5b-9 is associated with proteinuria and intratubular complement activation in pediatric kidney transplant recipients. Our findings support the concept that complement activation occurs locally within the tubular compartment and may represent an early event in graft injury.

Proteinuria is both a marker and a mediator of kidney graft injury and a driver of fibrosis (5). Beyond reflecting glomerular barrier dysfunction, proteinuria promotes tubular damage through filtration of complement components able to interact with locally produced complement factors (20). This process may trigger local complement activation within the tubular lumen, leading to inflammation and subsequent fibrosis (12, 21, 22). In this context, our observation that urinary sC5b-9 correlates with proteinuria and intratubular C5b-9 deposition provides *in vivo* clinical support for this mechanistic link in pediatric transplant recipients.

Importantly, plasma sC5b-9 levels remained within the normal range in all patients. This finding argues against systemic complement activation and strongly supports a compartmentalized intrarenal process. Given the molecular size of the sC5b-9 complex, its presence in urine is unlikely to result from passive filtration, even in the setting of proteinuria (15, 23). Rather, it is more plausibly generated locally within the tubular lumen, secondary to complement activation triggered by filtered proteins.

A key finding of our study is the absence of association between urinary sC5b-9 and IFTA. This result should be interpreted with caution and does not contradict a role for complement activation in fibrogenesis. Several factors may explain this observation. First, our cohort is characterized by a low burden of fibrosis (median IFTA 10%) and relatively preserved graft function (median eGFR 67.7 mL/min/1.73m²). The low fibrosis burden and the very low rate of rejection, together with the relatively young donor age, are consistent with a cohort sampled early in the course of chronic allograft injury,

before fibrosis has accumulated. Second, fibrosis is a cumulative and relatively late process, whereas complement activation is likely to occur at earlier stages of injury. Therefore, urinary sC5b-9 may reflect ongoing active injury rather than established structural damage.

This distinction is clinically relevant. Current monitoring strategies rely heavily on biopsy-based assessment of fibrosis, which reflects irreversible damage. In contrast, biomarkers such as urinary sC5b-9 may provide insight into dynamic and potentially reversible processes. Identifying patients with early complement-mediated tubular injury could allow for closer monitoring and therapeutic optimisation before the development of irreversible fibrosis.

Our findings are consistent with previous studies in adult transplant populations showing an association between urinary sC5b-9 and proteinuria (14). However, pediatric transplant recipients represent a unique population with fewer comorbidities making them particularly well suited to investigate early pathogenic processes.

Importantly, urinary sC5b-9 provided information that was not captured by proteinuria alone. In multivariable logistic regression predicting above-median tubular C5b-9 deposition, log-transformed urinary sC5b-9 remained an independent predictor, whereas log-transformed proteinuria did not, and adding urinary sC5b-9 to a proteinuria-based model significantly improved model fit (LRT $p = 0.014$). Discrimination of intra-tubular complement activation by urinary sC5b-9 was substantially superior to that of proteinuria (AUC 0.77 vs 0.54). Together with the persistently normal plasma sC5b-9 levels, these findings support the interpretation that urinary sC5b-9 reflects the biological output of local, intra-tubular complement activation rather than being merely a passive readout of proteinuric protein leak. This distinction is directly relevant to the question of whether urinary sC5b-9 could serve as a clinically informative complement-specific biomarker in this population. Nevertheless, as recently highlighted by a systematic review of prognostic biomarkers in kidney transplantation (24), candidate biomarkers evaluated in retrospective single-centre cohorts without external validation should be regarded as hypothesis-generating until confirmed in larger prospective cohorts with pre-specified handling of nondetectable values, adjustment for standard-of-care monitoring parameters and, ideally, longitudinal follow-up.

Several observations further support the specificity of the urinary sC5b-9 signal in this cohort. First, urinary sC5b-9 did not track alloimmune activity: it was undetectable in the patient with acute cellular rejection, and the very small number of rejection episodes in this cohort precluded any formal association, arguing against urinary sC5b-9 behaving as a marker of alloimmune activation in this setting. Second, urinary sC5b-9 was not associated with eGFR ($p = 0.08$, $p = 0.66$), which is consistent with a marker of ongoing tubular injury that operates upstream of measurable functional decline rather than a surrogate of graft function. Third, the concern that the observed association with proteinuria might be driven by a single extreme observation is not supported by our sensitivity analyses: the association persisted after exclusion of the most influential data point and remained significant in every leave-one-out analysis, as did the association between urinary sC5b-9 and tubular C5b-9 deposition.

Rank-based methods, which are insensitive to the magnitude of extreme values, were used throughout for this reason.

Two methodological points deserve comment. C5b-9 staining was deliberately restricted to non-atrophic tubules. Atrophic tubules display basement membrane remodelling and non-specific protein entrapment, which makes immunohistochemical signal difficult to interpret and may reflect residua of past injury rather than ongoing complement turnover. Restricting the analysis to viable tubules was therefore intended to capture active, ongoing intra-tubular complement activation; the trade-off is that our pathological readout is, by design, a marker of active injury rather than of established fibrosis, which is consistent with the absence of association with IFTA. In addition, the tubular compartment in this cohort was exposed to several concurrent insults beyond proteinuria: calcineurin inhibitor toxicity lesions were present in 8 patients and 9 patients had experienced post-transplant urinary tract infection. Urinary sC5b-9 may therefore integrate complement activation arising from multiple sources of tubular stress, which would be consistent with its independence from proteinuria in multivariable analysis and warrants dedicated evaluation in larger cohorts.

This study has several limitations. Its retrospective design and relatively small sample size limit statistical power and preclude causal inference. Given the low burden of fibrosis and rejection in this cohort, the absence of correlation between urinary sC5b-9 and IFTA should be interpreted as reflecting limited statistical power in this cross-sectional analysis rather than definitive evidence against a role for complement activation in fibrogenesis; our findings should therefore be considered hypothesis-generating. In addition, the lack of longitudinal follow-up prevents evaluation of whether elevated urinary sC5b-9 precedes fibrosis progression. Albuminuria was not measured in this cohort; as urinary sC5b-9 has previously been linked to albuminuria (15), this limits our ability to fully characterize the state of the glomerular filtration barrier in relation to urinary sC5b-9.

Despite these limitations, our study provides a strong rationale for further investigation. Prospective longitudinal studies are needed to determine whether urinary sC5b-9 can predict fibrosis progression and graft outcomes, and whether it may serve as a tool to guide therapeutic interventions, particularly in the context of emerging complement-targeted therapies.

Conclusion

Urinary sC5b-9 is associated with proteinuria and intratubular complement activation in pediatric kidney transplant recipients. It may represent an early, non-invasive biomarker of complement mediated tubular injury. Longitudinal studies are required to determine its role in predicting fibrosis and graft outcomes.

Abbreviations

ANCA

anti-neutrophil cytoplasmic antibody

AUC

area under the curve
C5b-9
terminal complement complex (membrane attack complex)
CI
confidence interval
CNI
calcineurin inhibitor
EIA
enzyme immunoassay
eGFR
estimated glomerular filtration rate
ELISA
enzyme-linked immunosorbent assay
IFTA
interstitial fibrosis and tubular atrophy
IQR
interquartile range
LRT
likelihood ratio test
MMF
mycophenolate mofetil
ROC
receiver operating characteristic
sC5b-9
soluble C5b-9 (soluble terminal complement complex)

Declarations

Ethics approval and consent to participate

The DOREGLO biobank was approved in 2016 by the Research Ethics Board of CHU Sainte-Justine (Comité d'éthique de la recherche du CHU Sainte-Justine), Montreal, Canada [approval number: 2017 – 1274]. The present study was conducted under this protocol. Written informed consent was obtained from all participants and/or their parents or legal guardians, with assent from minors where appropriate. The study was performed in accordance with the Declaration of Helsinki and Good Clinical Practices regulations.

Consent for publication:

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Supplementary Figure S1.

Relationship between urinary sC5b-9 and proteinuria, shown as untransformed values. (A) Whole cohort (n = 31). (B) The same relationship after exclusion of the patient with the highest urinary sC5b-9 value (n = 30); the dashed line indicates the least-squares fit. Pearson and Spearman coefficients with corresponding p values are shown in each panel. Undetectable urinary sC5b-9 values were assigned the assay limit of detection (0.1 ng/mL). Excluding this patient did not weaken the association.

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Author Contribution

LB and ALL wrote the main manuscript text and prepared all tables and figures. AF performed the statistical analyses. SS and AB performed the sC5b-9 measurements. KC supervised the evaluation of C5b-9 deposits. All authors read and approved the final manuscript.

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Not applicable

Data Availability

The datasets generated and analysed during the current study are not publicly available because they consist of individual-level clinical, biological and histological data from a small cohort of pediatric kidney transplant recipients, including rare underlying diagnoses, which carries a risk of participant re-identification. The data were collected under the DOREGLO biobank protocol and their use is governed by the research ethics board of CHU Sainte-Justine, Montreal, Canada. De-identified data are available from the corresponding author on reasonable request, subject to approval by the research ethics board of CHU Sainte-Justine and to a data transfer agreement.

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25. Supplementary Figure S. 1.

Figures

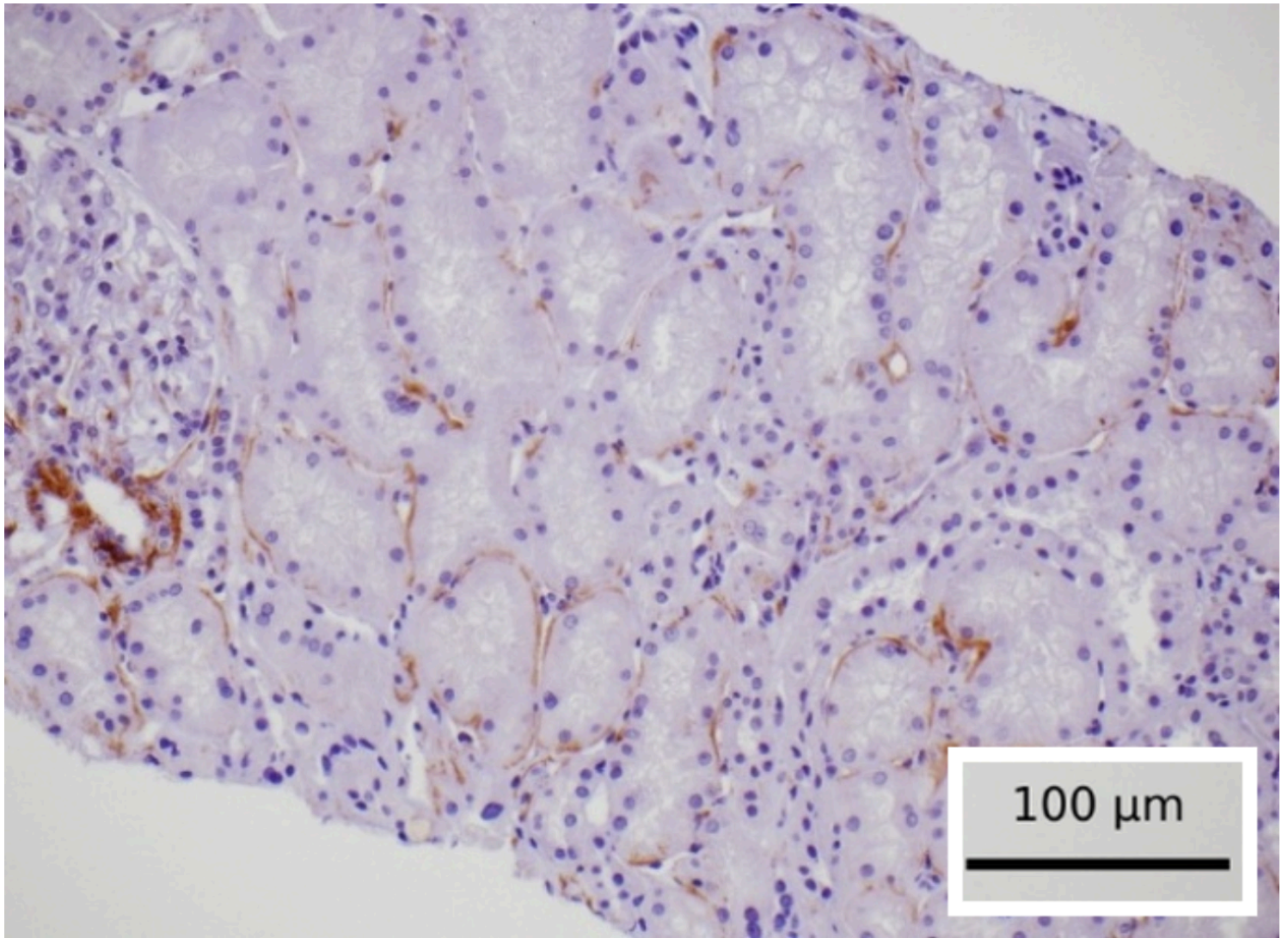


Figure 1

Immunohistochemical detection of C5b-9 deposits in non-atrophic tubules of a pediatric kidney allograft biopsy (original magnification $\times 10$). Brown staining outlines C5b-9 deposition along the basolateral aspect of non-atrophic tubular epithelial cells. The extent of deposition was quantified as the percentage of non-atrophic tubules showing C5b-9 deposits relative to the total number of non-atrophic tubules assessed in each biopsy section. Atrophic tubules were excluded from the analysis, as basement membrane remodelling and non-specific protein entrapment make the signal difficult to interpret in this compartment. Scale bar, 100 μm .

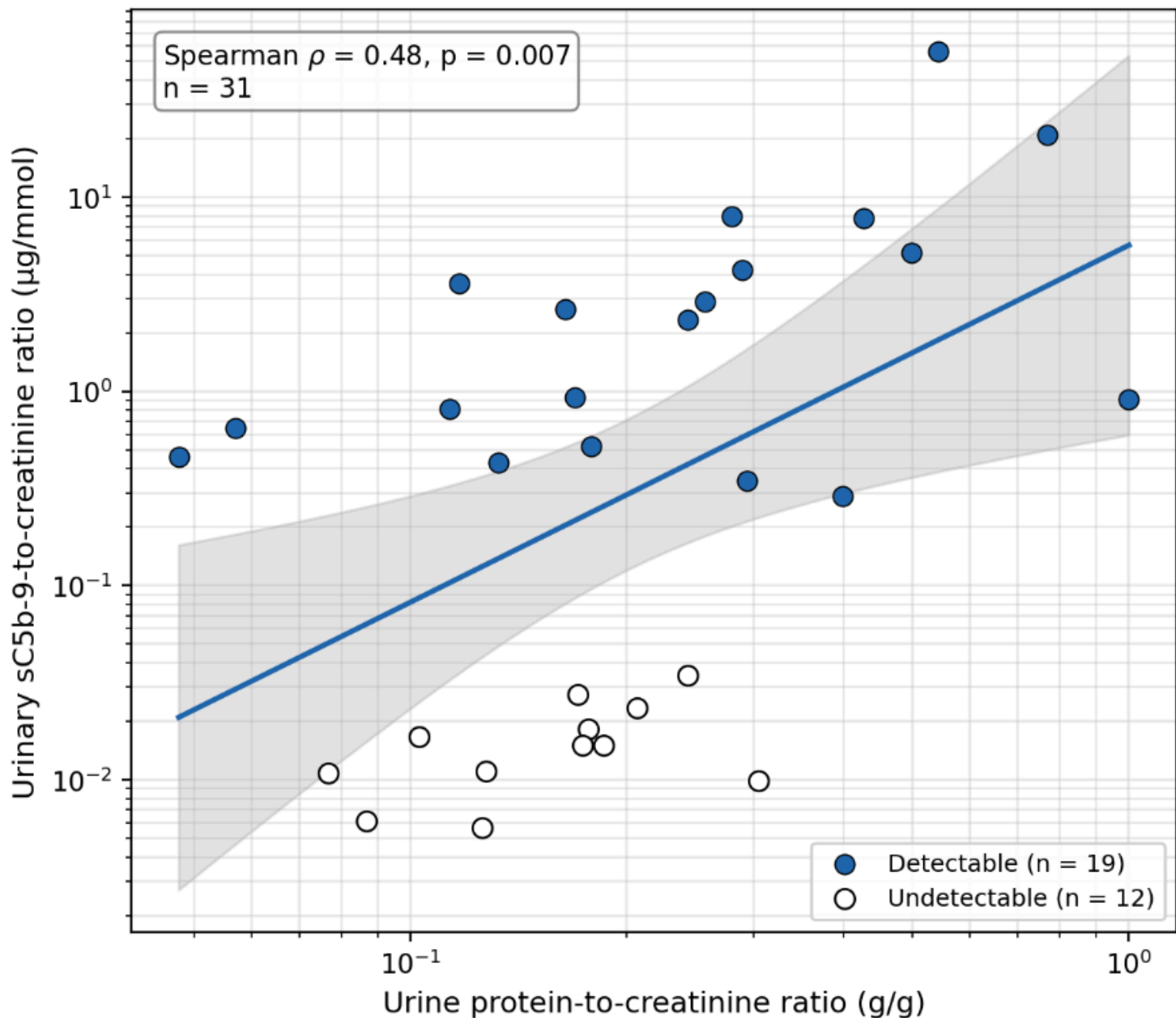


Figure 2

Relationship between urinary sC5b-9 and proteinuria in 31 pediatric kidney transplant recipients. Urinary sC5b-9 (normalised to urinary creatinine, $\mu\text{g}/\text{mmol}$) is plotted against the urine protein-to-creatinine ratio (g/g); both axes are logarithmic. Filled circles denote patients with detectable urinary sC5b-9 ($n = 19$) and open circles those in whom urinary sC5b-9 was undetectable ($n = 12$) and was assigned the assay limit of detection (0.1 ng/mL) before normalisation. The solid line is the least-squares fit with its 95% confidence band. Untransformed data are shown in Supplementary Figure S1.

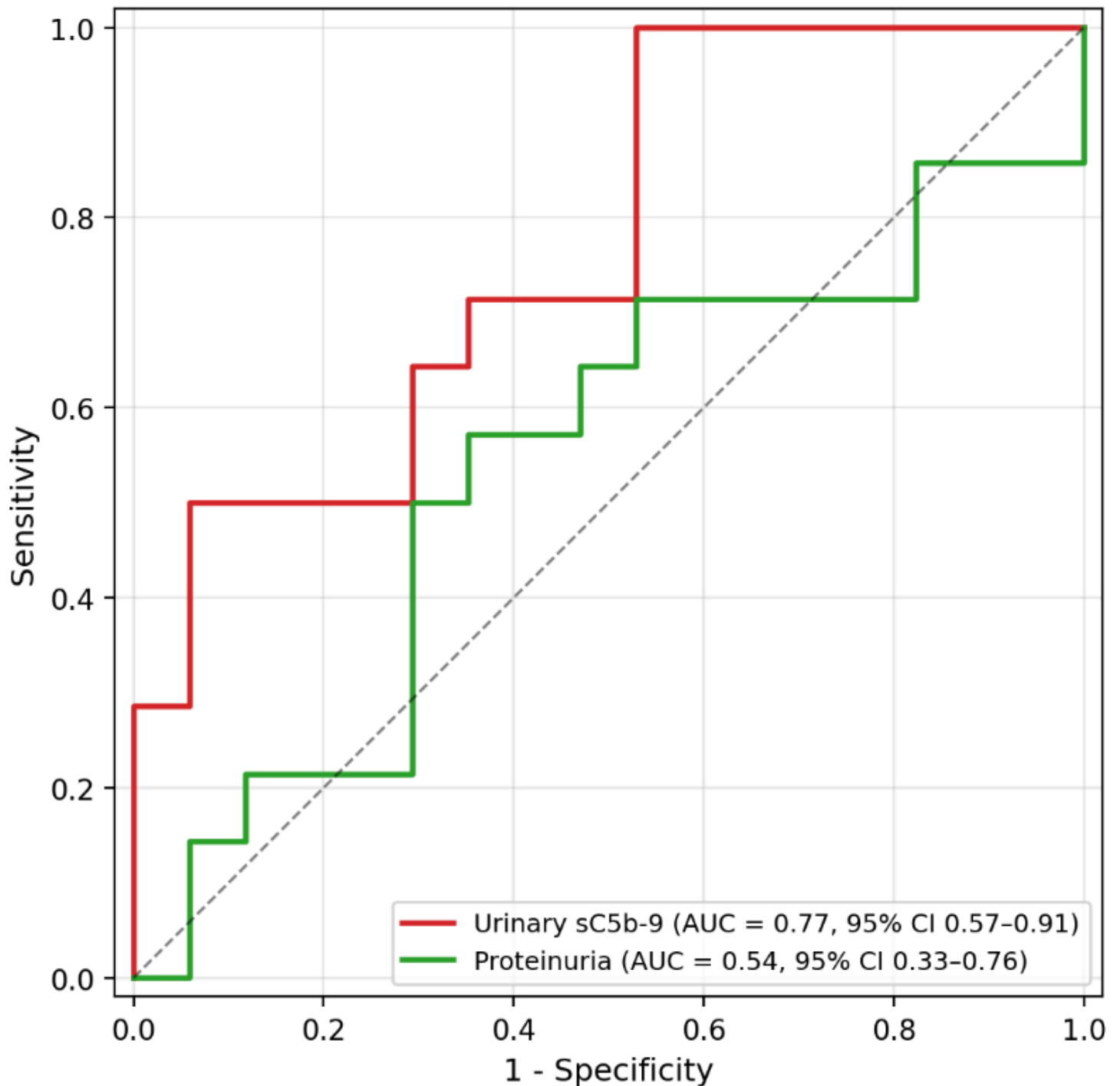


Figure 3

Discriminative performance of urinary sC5b-9 and proteinuria for the identification of above-median intratubular C5b-9 deposition, assessed by receiver operating characteristic (ROC) analysis. Areas under the curve (AUC) are reported with 95% confidence intervals derived from 2000 bootstrap resamples. The dashed diagonal denotes non-informative discrimination (AUC = 0.50). Urinary sC5b-9 was normalised to urinary creatinine ($\mu\text{g}/\text{mmol}$) and undetectable values were assigned the assay limit of detection (0.1 ng/mL). $n = 31$.

Supplementary Files

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