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DOI:

[10.1371/journal.pone.0197043](https://doi.org/10.1371/journal.pone.0197043)

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Document Version

Publisher's PDF, also known as Version of record

Citation for published version (Harvard):

Fenton, A, Jesky, MD, Webster, R, Stringer, SJ, Yadav, P, Chapple, I, Dasgupta, I, Harding, SJ, Ferro, CJ & Cockwell, P 2018, 'Association between urinary free light chains and progression to end stage renal disease in chronic kidney disease', *PLoS ONE*, vol. 13, no. 5, e0197043. <https://doi.org/10.1371/journal.pone.0197043>

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RESEARCH ARTICLE

Association between urinary free light chains and progression to end stage renal disease in chronic kidney disease

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OPEN ACCESS

Citation: Fenton A, Jesky MD, Webster R, Stringer SJ, Yadav P, Chapple I, et al. (2018) Association between urinary free light chains and progression to end stage renal disease in chronic kidney disease. PLoS ONE 13(5): e0197043. <https://doi.org/10.1371/journal.pone.0197043>

Editor: Petter Bjornstad, University of Colorado Denver School of Medicine, UNITED STATES

Received: October 25, 2017

Accepted: April 25, 2018

Published: May 9, 2018

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Data Availability Statement: Due to ethical restrictions by the National Research Ethics Service (NRES) Committee West Midlands-South Birmingham, data cannot be shared publicly and is subject to adherence to existing ethics approval. Interested, qualified researchers can request the data by contacting Penelope Gregory, REC Manager, at NRESCommittee.westmidlands-southbirmingham@nhs.net.

Funding: The Jabbs Foundation provided funding towards the RIISC study. The Jabbs Foundation

Abstract

Background

Patients with chronic kidney disease (CKD) are at an increased risk of developing end-stage renal disease (ESRD). We assessed for the first time whether urinary free light chains (FLC) are independently associated with risk of ESRD in patients with CKD, and whether they offer incremental value in risk stratification.

Materials and methods

We measured urinary FLCs in 556 patients with CKD from a prospective cohort study. The association between urinary kappa/creatinine (KCR) and lambda/creatinine (LCR) ratios and development of ESRD was assessed by competing-risks regression (to account for the competing risk of death). The change in C-statistic and integrated discrimination improvement were used to assess the incremental value of adding KCR or LCR to the Kidney Failure Risk Equation (KFRE).

Results

136 participants developed ESRD during a median follow-up time of 51 months. Significant associations between KCR and LCR and risk of ESRD became non-significant after adjustment for estimated glomerular filtration rate (eGFR) and albumin/creatinine ratio (ACR), although having a KCR or LCR >75th centile remained independently associated with risk of ESRD. Neither KCR nor LCR as continuous or categorical variables provided incremental value when added to the KFRE for estimating risk of ESRD at two years.

had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The Binding Site, Birmingham, provided support in the form of salaries for author SJH and the measurement of urinary and serum free light chains for the cohort. SJH played a role in study conceptualization and revision of the draft, but did not receive fees, bonuses, or other benefits for the work described in the manuscript. The Binding Site did not have any additional role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: SJH is employed by The Binding Site, Birmingham. This does not alter the authors' adherence to PLOS ONE policies on sharing data and materials.

Conclusions

Urinary FLCs have an association with progression to ESRD in patients with CKD which appears to be explained to a degree by their correlation with eGFR and ACR. Levels above the 75th centile do have an independent association with ESRD, but do not improve upon a current model for risk stratification.

Introduction

Patients with chronic kidney disease (CKD) are at an increased risk of adverse health outcomes including progression to end-stage renal disease (ESRD) and early mortality [1–6]. Risk prediction models can be used to estimate an individual's risk of ESRD, incorporating prognostic factors such as age, gender, glomerular filtration rate (GFR), and level of albuminuria [7–9]. There is significant interest in identifying further prognostic factors, including urinary proteins in addition to albumin, with a view to enhancing prognostic models and identifying potential targets for novel interventions.

During the synthesis of intact immunoglobulins, light chains are produced in excess of heavy chains, resulting in the release into the circulation of approximately 500 mg per day of unbound free light chains (FLC) [10]. As monomeric FLCs (usually kappa) weigh ~25 kDa, and dimeric FLCs (usually lambda) weigh ~50 kDa, they are filtered at the glomerulus and then reabsorbed and metabolised in the proximal tubule [11]. Although small amounts of FLC may be present from mucosal secretion from the urinary tract, the presence of significant FLC in the urine implies either concentrations in the proximal tubule greater than can be reabsorbed, as may occur with excess monoclonal FLC production in plasma cell dyscrasias, or renal disease with glomerular hyperfiltration and/or tubular dysfunction [12].

Previous work has demonstrated that urinary FLC concentration increases as estimated GFR (eGFR) decreases, and urinary FLC may be more sensitive than albuminuria as a marker of early CKD [13]. In patients with type 2 diabetes mellitus (DM), raised urinary concentrations of FLC are detectable prior to the development of increased albuminuria [14, 15]. Urinary FLC levels have also been shown to correlate with disease activity in IgA nephropathy and lupus nephritis [16, 17].

Our objectives were to assess, for the first time, whether urinary FLC are independently associated with risk of ESRD in patients with CKD, and whether measuring urinary FLC improves upon an established model for risk stratification.

Materials and methods

We used data and samples from the Renal Impairment in Secondary Care (RIISC) Study (ClinicalTrials.gov: NCT01722383). The RIISC study is a prospective cohort study of patients with CKD and the detailed methodology has been published previously [18].

In brief, patients in nephrology clinics at two renal centres were invited to participate if they had high-risk CKD, defined as an eGFR < 30 mL/min/1.73m², or an eGFR 30–59 mL/min/1.73m² with a decline of ≥ 5 mL/min/1.73m² over a year or ≥ 10 mL/min/1.73m² over 5 years or an ACR ≥ 70 mg/mmol on three occasions. Patients were excluded if they had received immunosuppression for immune-mediated renal disease or if they had started renal replacement therapy (RRT, i.e. dialysis or kidney transplant). For this analysis, we also excluded patients with a monoclonal gammopathy i.e. monoclonal gammopathy of undetermined

significance (MGUS), including patients with an abnormal serum FLC isotype and a serum kappa/lambda (κ/λ) FLC ratio outside of the renal reference range (0.37–3.1) [13, 19], or a known diagnosis of myeloma, AL amyloidosis, or other monoclonal gammopathy of renal significance. Participants were recruited between October 2010 and December 2015 and followed up until they started RRT or died. The primary outcome of interest was progression to ESRD, which was defined as the initiation of RRT and was captured using both centres' local databases of patients who have started dialysis or received a kidney transplant. Outcomes were captured up to 31 January 2017 and patients who had not reached a study end-point were censored on this date. Ethical approval was granted by the South Birmingham Research Ethics Committee (reference: 10/H1207/6). All patients provided written consent, and the study was conducted in accordance with the Declaration of Helsinki.

Laboratory analyses

Serum and urine were processed immediately after collection according to pre-defined standard operating procedures and stored at -80°C until analysis.

Urinary FLCs were measured by turbidimetry on a Roche Modular P analyser using the Freelite™ immunoassay (The Binding Site Group Ltd, Birmingham, UK). To correct for variations in urine concentration, urinary FLCs were divided by urine creatinine concentration to give urinary FLC/creatinine (FLC/Cr) ratios in mg/mmol, i.e. a kappa/creatinine ratio (KCR) and a lambda/creatinine ratio (LCR). Serum creatinine measurements were performed on a Roche Modular analyser using a rate-blanked and compensated Jaffe reaction, and eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [20]. Serum kappa (κ) and lambda (λ) FLC concentrations were measured by nephelometry on a Dade-Behring BN™ II System (Siemens AG, Erlangen, Germany) using the Freelite™ assay. Urine ACR was measured using a Roche Hitachi 702 analyser. Other biochemistry testing was performed by the local clinical laboratories in accordance with the current standard of care.

Blood pressure

Blood pressure (BP) was measured using the BpTRU automated device (BpTRU Medical Devices, Coquitlam, BC, Canada) which obtains six BP readings after a five-minute rest period. The systolic (SBP) and diastolic BP (DBP) are derived from the mean of the second to sixth readings, and this method has been reported to be comparable to the mean daytime BP from 24-hour ambulatory BP monitoring [21]. Mean arterial pressure (MAP) was calculated as $((2 \times \text{DBP}) + \text{SBP}) \div 3$.

Statistical analyses

Statistical analyses were performed using SPSS Statistics 24 (Armonk, NY: IBM Corp, 2016) and Stata 15 (College Station, TX: StataCorp, 2017). Baseline characteristics are presented as a frequency and percentage for categorical variables, and a median and interquartile range (IQR) for continuous variables.

Urinary FLC/Cr ratios were log-transformed prior to parametric testing. Their associations with other continuous baseline variables were examined by scatter plots and Pearson's correlation coefficients. Correlation coefficients of 0.2, 0.5, and 0.8 were considered weak, moderate, and strong, respectively [22]. To assess their association with categorical variables, between-group differences were tested for using the Mann-Whitney U (two groups) and Kruskal-Wallis (three or more groups) tests. Multiple linear regression was used to further assess the influence

of serum FLC on urinary FLC/Cr ratios after adjustment for other significant determinants of urinary FLC/Cr ratios (eGFR, ACR, DM, and renal diagnosis).

The associations between urinary FLC/Cr and progression to ESRD were assessed in univariable and multivariable regression models. As 75 (13.5%) participants died without ESRD, we used competing-risks regression based on the proportional subhazards model of Fine and Gray [23], which allowed us to handle mortality as a competing event (simply censoring at death in standard Cox regression models would have made the incorrect assumption that the censored deceased participants remained at risk of developing ESRD). In the multivariable models, we adjusted for confounding factors known to be associated with CKD progression: eGFR (model 1); ACR (model 2); eGFR and ACR (model 3); age, gender, ethnicity, renal diagnosis, MAP, eGFR, and ACR (model 4). Subhazard ratios (SHR) are presented with 95% confidence intervals (CI), which for continuous variables represented the risk of ESRD associated with an increase of one standard deviation (SD). Urinary FLC/Cr ratios were analysed as both full-range continuous variables and as categorical variables (comparing above versus below the 75th centiles). Competing-risks models were also built to include restricted cubic splines of urinary FLC/Cr ratios to assess for non-linear associations between urinary FLC/Cr ratios and risk of ESRD. Kaplan-Meier curves were plotted to show the cumulative incidence of ESRD by quartiles of urinary FLC/Cr.

To examine whether urinary FLC/Cr provide any incremental value in risk stratification, we chose the four-variable 'Kidney Failure Risk Equation' (KFRE) [9] as the baseline model for comparison, which estimates an individual's risk of ESRD at two and five years. Binary logistic regression models were fitted for the outcome of ESRD at two years. The baseline model contained only the KFRE-calculated two-year risk of ESRD, calculated as:

$$1 - 0.9832^{e^{(-0.2201 \times (\text{age}/10 - 7.036) + 0.2467 \times (\text{male} - 0.5642) - 0.5567 \times (\text{eGFR}/5 - 7.222) + 0.4510 \times (\log\text{ACR} - 5.137))}}$$

(the four-variable, non-North America, two-year risk equation from eAppendix 2 of [24]; ACR was converted to mg/g before being entered into the model by dividing by 0.113).

KCR and LCR (separately) were added as continuous and categorical predictors to the baseline KFRE model and the models compared. Overall model performance was estimated by R^2 (Nagelkerke, a version of the Cox & Snell R^2 adjusted to cover the full range from 0 to 1 [25]), discrimination was assessed by the C-statistic, and calibration by the Hosmer-Lemeshow goodness-of-fit test. The incremental value of adding KCR or LCR to the baseline model was assessed by the change in C-statistic and by the reclassification measure Integrated Discrimination Index (IDI). The IDI is a measure of the extent to which adding a new marker to a model correctly revises upward the predicted risk of individuals who experience an event and correctly revises downward the predicted risk of individuals who do not experience an event [26, 27].

Results

Urinary FLCs were measured in 636 participants of the RIISC study. We excluded 41 patients with a monoclonal gammopathy (21 with a serum κ/λ FLC ratio outside the renal reference range, 15 with MGUS, and 5 with multiple myeloma), and also excluded 39 patients with urinary FLC or creatinine results above or below the limits of detection so that accurate FLC/Cr ratios could be calculated. Therefore, 556 participants were included for analysis with a median follow-up time of 51 (IQR 46–60) months.

Baseline characteristics

The baseline characteristics of the study population are shown in Table 1. The cohort had a median age of 64 (IQR 51–76) years, were 63% male, and 68% were of White ethnicity. The

Table 1. Baseline characteristics of the study population (N = 556).

Characteristic	Median (IQR) or N (%)	Completeness of data (%)
Age (years)	64 (51–76)	100
Male gender	351 (63.1)	100
Ethnicity		100
White	380 (68.3)	
South Asian	117 (21.0)	
Black	56 (10.1)	
Other	3 (0.5)	
Co-morbidities		100
Diabetes mellitus	196 (35.3)	
Ischaemic heart disease	120 (21.6)	
Cerebrovascular disease	53 (9.5)	
Peripheral vascular disease	53 (9.5)	
COPD	57 (10.3)	
Malignancy	71 (12.8)	
Renal diagnosis		90
Ischaemic/hypertensive	145 (28.9)	
Glomerulonephritis	72 (14.3)	
Diabetic kidney disease	65 (12.9)	
Polycystic kidney disease	29 (5.8)	
Interstitial nephropathy	29 (5.8)	
Reflux nephropathy	12 (2.4)	
Other/uncertain	150 (29.9)	
Estimated GFR (mL/min/1.73m²)	25 (19–34)	98
Urinary ACR (mg/mmol)	28.1 (5.7–103.2)	92
Systolic blood pressure (mmHg)	128 (116–144)	99
Diastolic blood pressure (mmHg)	76 (68–85)	99
Serum κ FLC (mg/L)	44.9 (29.5–67.0)	99
Serum λ FLC (mg/L)	32.5 (23.4–47.0)	99
Urinary KCR (mg/mmol)	14.6 (7.1–27.7)	100
Urinary LCR (mg/mmol)	2.1 (1.0–5.1)	100

Continuous variables are expressed as a median and interquartile range, and categorical variables are expressed as a frequency and percentage.

Abbreviations: ACR = albumin/creatinine ratio; COPD = chronic obstructive pulmonary disease; FLC = free light chains; GFR = glomerular filtration rate; IQR = interquartile range; KCR = kappa/creatinine ratio; LCR = lambda/creatinine ratio.

<https://doi.org/10.1371/journal.pone.0197043.t001>

most common aetiologies of CKD were ischaemic/hypertensive nephropathy (28.9%), glomerulonephritis (14.3%), and diabetic kidney disease (12.9%). Median eGFR was 25 (IQR 19–34) mL/min/1.73m² and median ACR was 28.1 (IQR 5.8–103.2) mg/mmol. Median urinary KCR was 14.6 (IQR 7.1–27.7) mg/mmol and median LCR was 2.1 (IQR 1.0–5.1) mg/mmol.

Urinary FLC/Cr associations

The associations between urinary KCR and LCR with other baseline variables are shown in Table 2. For comparison, the associations with ACR are also given. Urinary FLC/Cr ratios had moderate positive correlations with serum FLC (sFLC), weak-to-moderate positive correlations with ACR, and weak negative correlations with eGFR. The urinary FLC/Cr ratios, unlike ACR, increased significantly with worsening CKD stage.

Table 2. Associations between urinary free light chain/creatinine ratios and albumin/creatinine ratio with other baseline variables.

Variable	Urinary kappa/creatinine ratio		Urinary lambda/creatinine ratio		Urinary albumin/creatinine ratio	
	Median (IQR) or <i>r</i>	<i>P</i>	Median (IQR) or <i>r</i>	<i>P</i>	Median (IQR) or <i>r</i>	<i>P</i>
Age	0.109	0.010	0.012	0.77	-0.321	<0.0001
Gender		0.028		0.007		0.038
Male	16.2 (7.4–29.3)		2.3 (1.1–5.2)		32.5 (7.3–111.3)	
Female	12.3 (6.3–26.5)		1.9 (0.7–4.3)		20.0 (4.4–83.2)	
Ethnicity		0.007		0.001		<0.0001
White	13.1 (7.1–24.5)		1.9 (1.0–4.3)		16.9 (4.2–76.4)	
South Asian	20.0 (8.4–37.2)		3.4 (1.5–7.9)		78.2 (22.8–156.6)	
Black	12.9 (4.0–28.9)		1.8 (0.6–5.2)		39.0 (9.5–88.7)	
Other	12.7 (8.3–39.9)		2.0 (1.9–7.4)		237.3 (187.1–302.4)	
Co-morbidities						
Diabetes Mellitus		<0.0001		0.001		0.28
Yes	18.7 (8.1–34.9)		2.9 (1.2–6.4)		23.8 (4.2–86.2)	
No	12.6 (6.6–24.3)		1.8 (0.9–4.3)		29.3 (6.9–108.7)	
Cardiovascular disease		0.37		0.89		0.048
Yes	16.3 (7.1–30.3)		2.2 (1.0–5.2)		22.6 (3.7–82.6)	
No	14.3 (7.1–27.3)		2.0 (1.0–5.0)		29.1 (6.9–117.0)	
Malignancy		0.46		0.35		0.015
Yes	16.0 (7.5–25.9)		1.8 (1.1–3.6)		12.0 (2.9–83.0)	
No	14.3 (7.0–27.8)		2.2 (1.0–5.1)		29.9 (6.5–106.6)	
Renal diagnosis		<0.0001		<0.0001		<0.0001
Ischaemic/hypertensive	13.6 (7.3–27.7)		2.1 (1.0–4.7)		12.4 (2.6–56.0)	
Glomerulonephritis	8.2 (4.9–17.2)		1.4 (0.8–2.7)		70.5 (30.4–159.3)	
Diabetic kidney disease	20.9 (10.4–51.8)		4.7 (1.6–8.1)		64.9 (23.0–237.3)	
Polycystic kidney disease	12.0 (5.5–19.0)		1.5 (0.5–3.1)		10.2 (6.1–18.7)	
Interstitial nephropathy	15.0 (8.1–27.3)		3.1 (1.1–5.0)		10.4 (3.6–35.0)	
Reflux nephropathy	8.2 (3.7–22.8)		1.8 (0.6–3.9)		87.8 (29.3–141.0)	
Other/uncertain	16.1 (7.4–31.0)		2.3 (1.0–5.7)		32.9 (6.9–113.5)	
Estimated GFR	-0.387	<0.0001	-0.340	<0.0001	0.100	0.027
CKD Stage		<0.0001		<0.0001		0.22
G3a	8.6 (4.4–14.0)		1.3 (0.4–2.9)		23.0 (5.1–136.8)	
G3b	12.6 (6.3–22.5)		1.8 (0.8–3.7)		27.9 (7.0–119.8)	
G4	15.2 (7.5–27.8)		2.1 (1.0–5.1)		19.9 (4.4–78.0)	
G5	29.5 (16.3–50.9)		5.3 (2.5–11.0)		33.2 (10.1–117.1)	
Urinary ACR	0.400	<0.0001	0.516	<0.0001	N/A	
Systolic blood pressure	0.184	<0.0001	0.179	<0.0001	0.227	<0.0001
Diastolic blood pressure	0.079	0.06	0.111	0.009	0.231	<0.0001
Serum κ FLC	0.513	<0.0001	0.479	<0.0001	0.175	<0.0001
Serum λ FLC	0.494	<0.0001	0.563	<0.0001	0.221	<0.0001
Urinary KCR	N/A		0.925	<0.0001	0.400	<0.0001
Urinary LCR	0.925	<0.0001	N/A		0.516	<0.0001

Associations with continuous variables are expressed as Pearson's *r* (after log transformation of both variables) and its corresponding *P*. For categorical variables, median and interquartile ranges are shown with between-group differences assessed using the Mann-Whitney U or Kruskal-Wallis tests.

Abbreviations: ACR = albumin/creatinine ratio; CKD = chronic kidney disease; FLC = free light chains; GFR = glomerular filtration rate; IQR = interquartile range; KCR = kappa/creatinine ratio; LCR = lambda/creatinine ratio.

<https://doi.org/10.1371/journal.pone.0197043.t002>

Urinary FLC/Cr ratios were significantly higher in males, those of South Asian ethnicity, and those with diabetic kidney disease, and significantly lower in those with glomerulonephritis and polycystic kidney disease. However, these associations were no longer significant after adjustment for ACR and eGFR. Patients with DM as a comorbidity had significantly higher urinary FLC/Cr ratios, even after adjustment for eGFR, ACR, and serum FLC.

Scatter plots of the relationship between urinary FLC/Cr ratios with eGFR, ACR, and serum FLC, are shown in Figs 1, 2 and 3, respectively. Urinary FLC/Cr ratios were most strongly correlated with serum FLC; after adjustment for eGFR, ACR, DM, and renal diagnosis, a 10% higher serum kappa was associated with a 4.8% (3.4–6.2%) higher urinary KCR, and a 10% higher serum lambda was associated with a 7.5% (5.8–9.3%) higher urinary LCR.

Progression to ESRD

During follow-up, 136 (24.5%) participants progressed to ESRD. A higher KCR and LCR were both associated with a significantly increased risk of ESRD. The association between urinary FLC/Cr ratios and risk of ESRD approximated a linear relationship, although there was a smaller increase in risk per unit increase in urinary FLC/Cr ratio at the lower and upper extremes of the range, as shown in Fig 4. A one SD higher KCR was associated with a HR of 1.58 (1.40–1.79) and a one SD higher LCR was associated with a HR of 1.47 (1.31–1.66). A urinary FLC/Cr ratio above the 75th centile was associated with HRs of 3.18 (2.26–4.47) for KCR and 3.67 (2.62–5.16) for LCR. The cumulative incidence of ESRD by quartiles of urinary FLC/Cr are shown in Fig 5. The univariable associations between other baseline variables and risk of ESRD are shown in Table 3.

Multivariable models incorporating urinary FLC/Cr ratios are shown in Table 4. Urinary KCR and LCR both remained significantly associated with risk of ESRD after adjustment for either eGFR (model 1) or ACR (model 2). However, after adjustment for both eGFR and ACR (model 3), including models incorporating age, sex, ethnicity, renal diagnosis, MAP, eGFR, and ACR (model 4), urinary KCR and LCR as continuous or spline predictor variables were not significantly associated with risk of ESRD. The significant association between urinary FLC/Cr ratios above the 75th centile and risk of ESRD did however remain in models 3 and 4.

When these analyses were repeated in participants with a urinary ACR < 30 mg/mmol ($N = 265$), we found that higher urinary FLC/Cr ratios, as either continuous or categorical variables, were associated with a significantly increased risk of ESRD on univariable analysis, but the associations became non-significant after adjustment for eGFR.

Urinary FLC/Cr in risk stratification

After excluding those who died without ESRD within two years ($N = 34$) and those with less than two years of follow-up ($N = 58$), 464 participants had data on ESRD at two years, of whom 60 (12.9%) had reached ESRD. Measures of model performance for the prediction of ESRD at two years are shown in Table 5, including the incremental value of adding urinary FLC/Cr ratios to KFRE. The baseline model containing only KFRE had a strong predictive ability for ESRD at two years (C-statistic 0.89 [95% CI 0.84–0.94]) and was well calibrated (Hosmer-Lemeshow statistic 12.19, $P = 0.14$). None of the KFRE + KCR or KFRE + LCR models had a significant change in the C-statistic, suggesting no improvement in discrimination between those who did and did not develop ESRD, nor any significant improvement in reclassification of risk based on the IDI. Similarly, the addition of splines of KCR and LCR to the KFRE model did not improve the C-statistic or show any improvement in discrimination based on the IDI.

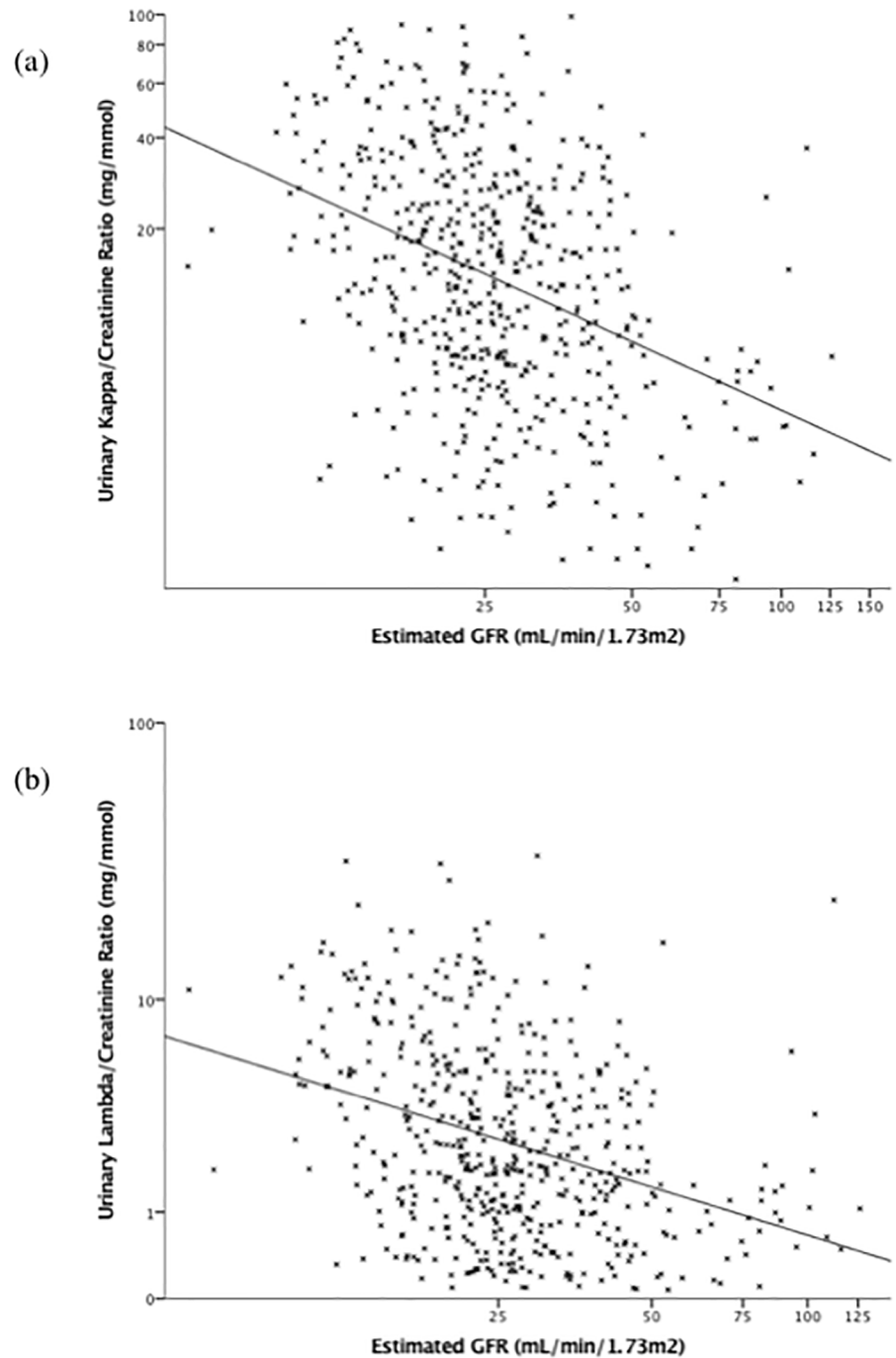


Fig 1. Scatter plots of urinary kappa/creatinine ratio (a) and lambda/creatinine ratio (b) by estimated glomerular filtration rate. Scales are logarithmic. Abbreviations: GFR = glomerular filtration rate.

<https://doi.org/10.1371/journal.pone.0197043.g001>

Discussion

There is significant interest in identifying bioclinical prognostic factors in CKD that might enhance our current models for risk stratification and potentially identify novel therapeutic targets. This is to the best of our knowledge the first study to evaluate whether there is an

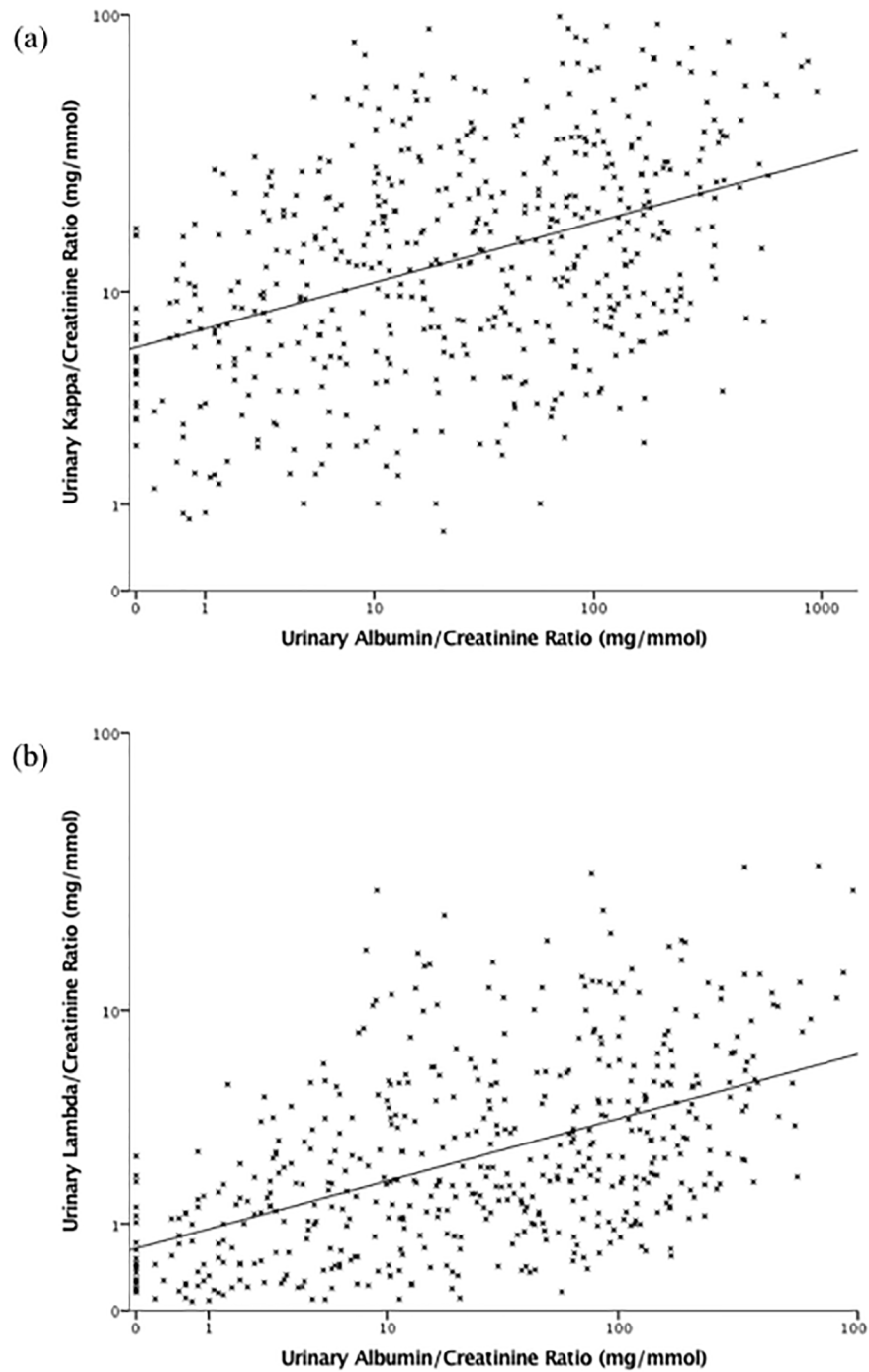


Fig 2. Scatter plots of urinary kappa/creatinine ratio (a) and lambda/creatinine ratio (b) by urinary albumin/creatinine ratio. Scales are logarithmic.

<https://doi.org/10.1371/journal.pone.0197043.g002>

independent association between urinary FLCs and risk of ESRD in patients with CKD. We utilised a cohort of patients who were prospectively recruited with (i) a high risk of CKD progression; (ii) extended follow-up; (iii) a hard end-point; and (iv) a high proportion progressing to the end-point.

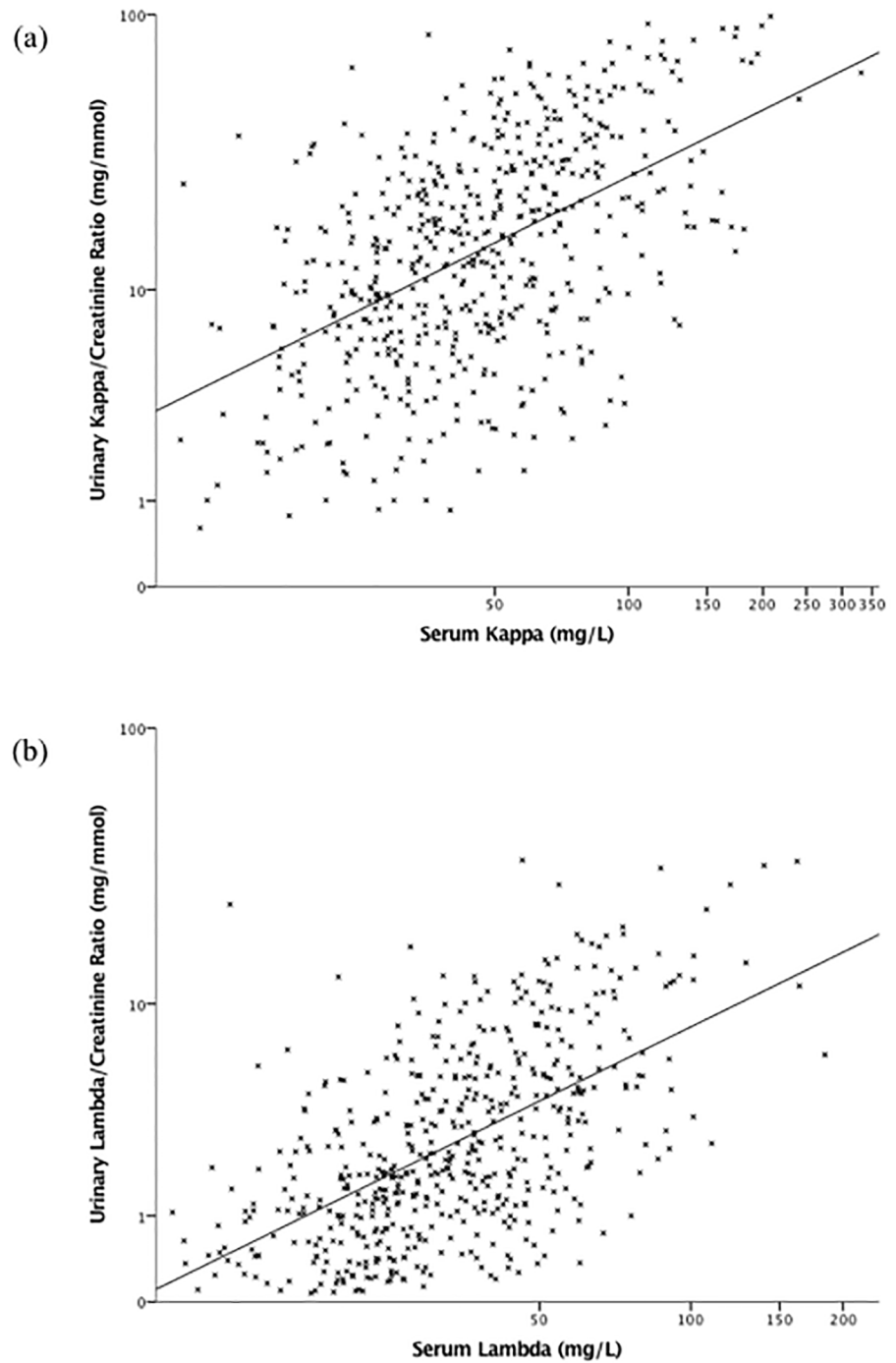


Fig 3. Scatter plots of urinary kappa/creatinine ratio by serum kappa (a) and urinary lambda/creatinine ratio by serum lambda (b). Scales are logarithmic.

<https://doi.org/10.1371/journal.pone.0197043.g003>

There was a negative correlation between eGFR and urinary FLC/Cr ratios, i.e. FLC/Cr ratios increase as GFR falls, consistent with previously published data [13]. We hypothesise that in patients with CKD, as nephrons are lost, there is hyperfiltration of the remaining functional glomeruli and increased concentrations of FLC in the glomerular filtrate that exceeds

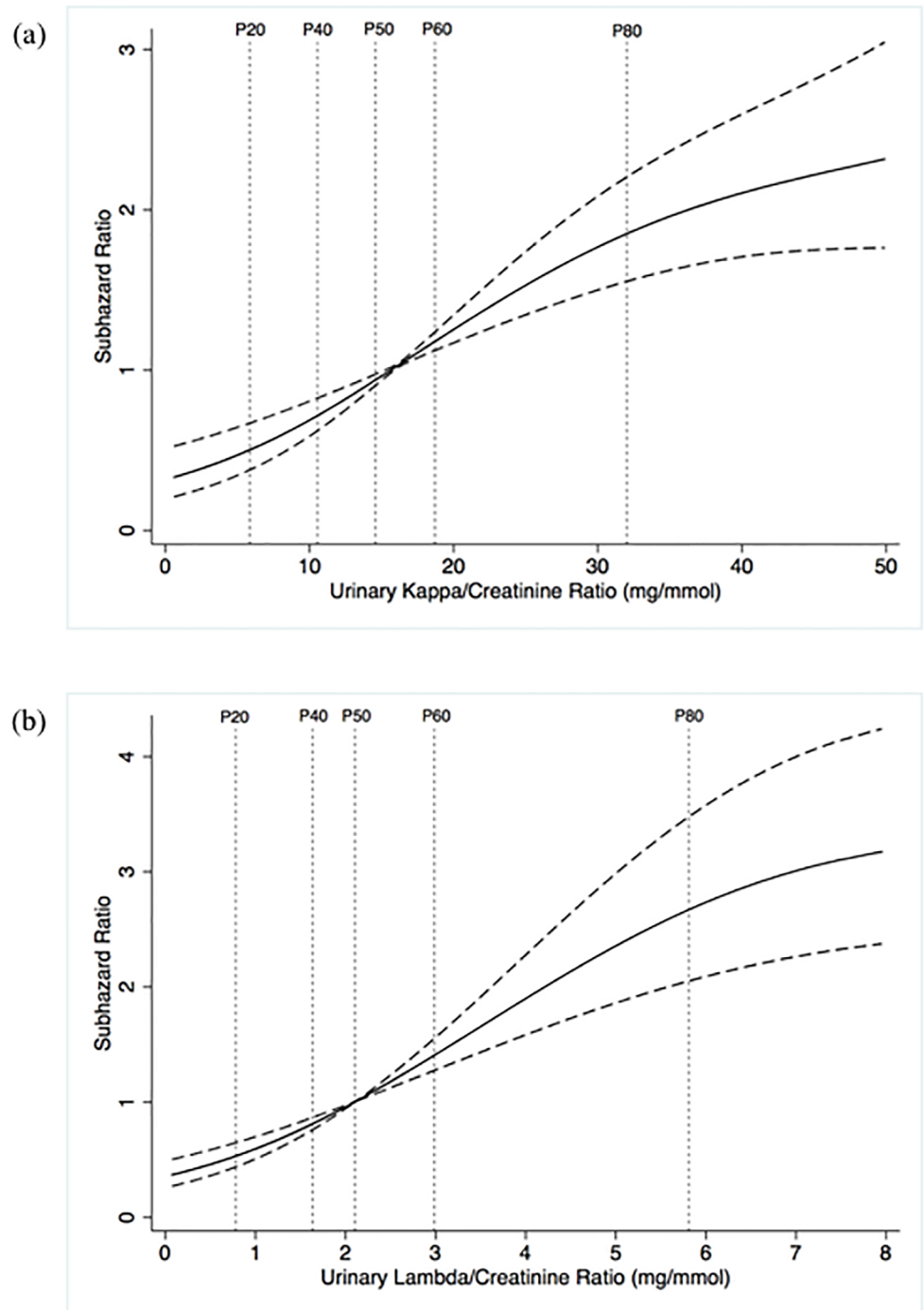


Fig 4. Restricted cubic splines of the subhazard ratio (solid line, with 95% confidence intervals shown as interrupted lines) of end stage renal disease by urinary kappa/creatinine ratio (a) and urinary lambda/creatinine ratio (b), adjusted for the competing risk of death. Percentiles are shown as vertical dotted lines. Abbreviations: P = percentile.

<https://doi.org/10.1371/journal.pone.0197043.g004>

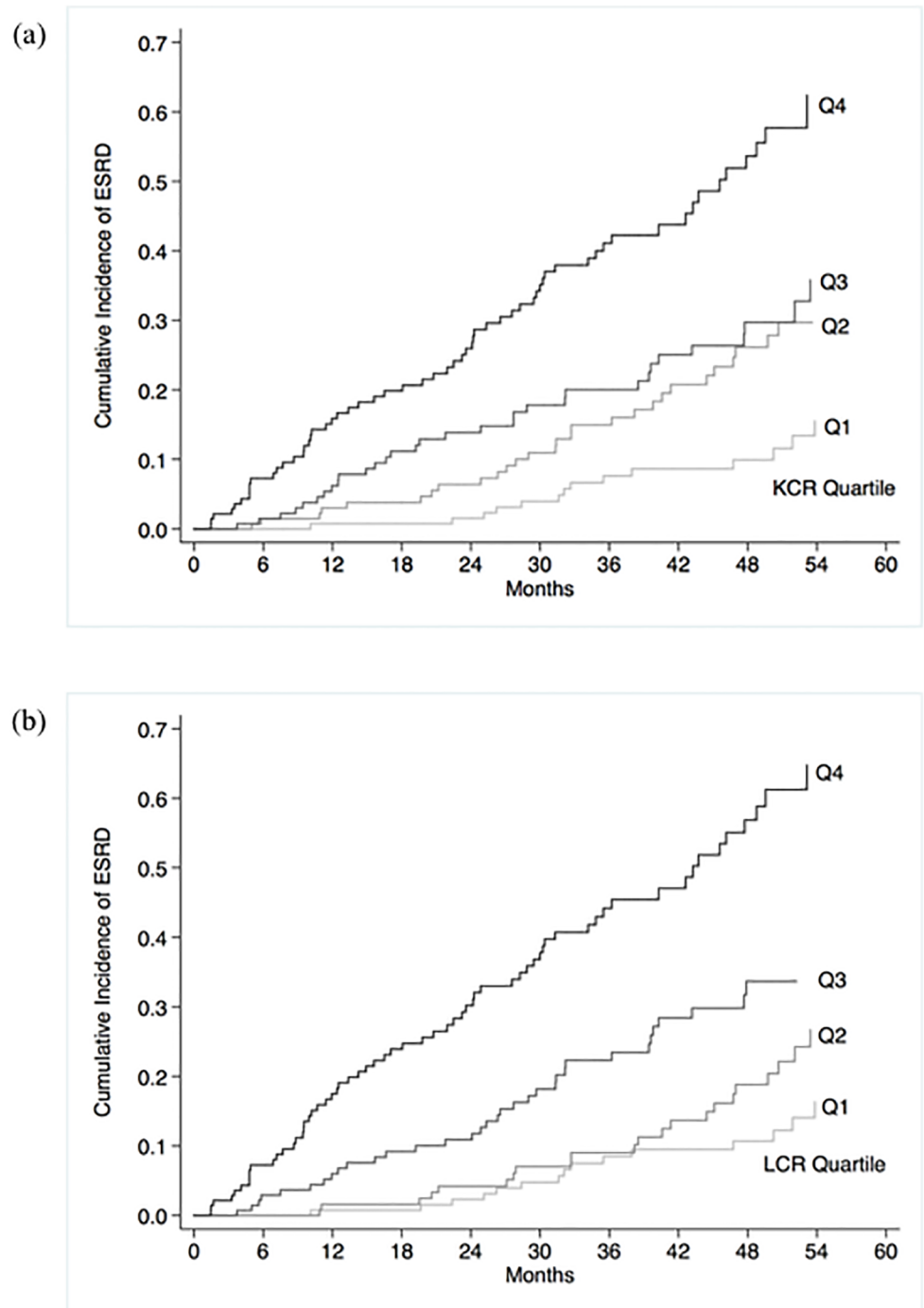


Fig 5. Cumulative incidence of end stage renal disease by quartiles of urinary kappa/creatinine ratio (a) and lambda/creatinine ratio (b). Abbreviations: ESRD = end stage renal disease; KCR = kappa/creatinine ratio; LCR = lambda/creatinine ratio; Q = quartile.

<https://doi.org/10.1371/journal.pone.0197043.g005>

the capacity of the proximal tubule to reabsorb and metabolise them. Tubular dysfunction with decreased uptake of FLC may also contribute to increasing urinary FLC/Cr ratios.

There was also a positive correlation between urinary FLC/Cr ratios and ACR. Regardless of the underlying cause of CKD, glomerular damage (associated with albuminuria and possibly

Table 3. Subhazard ratios with 95% confidence intervals in univariable competing-risk models for risk of end stage renal disease accounting for the competing risk of death.

Variable	Subhazard ratio (95% CI)	P
Age	0.75 (0.65–0.87)	<0.0001
Gender		
Male	0.97 (0.68–1.36)	0.84
Female	1.04 (0.73–1.46)	0.84
Ethnicity		
White	0.66 (0.47–0.93)	0.017
South Asian	1.50 (1.03–2.19)	0.036
Black	1.38 (0.82–2.31)	0.23
Comorbidities		
Diabetes mellitus	0.90 (0.63–1.30)	0.58
Ischaemic heart disease	0.69 (0.44–1.08)	0.10
Cerebrovascular disease	1.18 (0.69–2.01)	0.55
Peripheral vascular disease	0.73 (0.37–1.43)	0.36
COPD	0.57 (0.29–1.13)	0.11
Malignancy	0.61 (0.34–1.09)	0.10
Renal diagnosis		
Ischaemic/hypertensive	0.99 (0.67–1.45)	0.95
Glomerulonephritis	0.85 (0.51–1.41)	0.52
Diabetic kidney disease	1.64 (0.99–2.72)	0.06
Polycystic kidney disease	2.22 (1.34–3.69)	0.002
Interstitial nephropathy	0.71 (0.32–1.59)	0.40
Reflux nephropathy	0.30 (0.05–1.94)	0.21
Other/uncertain	0.90 (0.62–1.32)	0.60
Estimated GFR	0.25 (0.17–0.38)	<0.0001
Urinary ACR	1.38 (1.20–1.58)	<0.0001
Systolic blood pressure	1.28 (1.09–1.49)	0.002
Diastolic blood pressure	1.24 (1.04–1.48)	0.015
Serum κ FLC	1.67 (1.49–1.88)	<0.0001
Serum λ FLC	1.57 (1.40–1.78)	<0.0001
Serum κ+λ FLC	1.69 (1.50–1.91)	<0.0001
Urinary KCR		
Per +1 SD	1.58 (1.40–1.79)	<0.0001
> 27.7 mg/mmol	3.18 (2.26–4.47)	<0.0001
Urinary LCR		
Per +1 SD	1.47 (1.31–1.66)	<0.0001
> 5.1 mg/mmol	3.67 (2.62–5.16)	<0.0001

Urinary KCR and LCR were analysed as both a continuous variable (per +1 SD) and a categorical variable (above versus below the 75th centile). Subhazard ratios for continuous variables represent the risk associated with an increase of one standard deviation.

Abbreviations: ACR = albumin/creatinine ratio; CI = confidence interval; COPD = chronic obstructive pulmonary disease; FLC = free light chains; GFR = glomerular filtration rate; KCR = kappa/creatinine ratio; LCR = lambda/creatinine ratio; SD = standard deviation; SHR = subhazard ratio

<https://doi.org/10.1371/journal.pone.0197043.t003>

Table 4. Subhazard ratios with 95% confidence intervals for urinary free light chain/creatinine ratios as continuous and categorical (above 75th centile) predictors in multivariable competing risk models for end-stage renal disease, adjusted for the competing risk of death.

Model	Subhazard Ratio (95% Confidence Interval)			
	Kappa/creatinine ratio		Lambda/creatinine ratio	
	Per +1 SD	> 27.7 mg/mmol	Per +1 SD	> 5.1 mg/mmol
1 ^a	1.35 (1.19–1.55)	2.22 (1.55–3.18)	1.30 (1.14–1.49)	2.56 (1.78–3.68)
2 ^b	1.43 (1.23–1.65)	2.55 (1.71–3.80)	1.32 (1.14–1.54)	2.79 (1.85–4.21)
3 ^c	1.14 (0.96–1.36)	1.61 (1.05–2.49)	0.99 (0.78–1.24)	1.65 (1.05–2.60)
4 ^d	1.22 (1.00–1.50)	1.74 (1.12–2.71)	1.00 (0.78–1.27)	2.05 (1.26–3.33)

Bold values have a *P* value < 0.05.

^aAdjusted for eGFR.

^bAdjusted for urinary ACR.

^cAdjusted for eGFR and urinary ACR.

^dAdjusted for age, sex, ethnicity, renal diagnosis, MAP, eGFR, and urinary ACR.

Abbreviations: ACR = albumin/creatinine ratio; eGFR = estimated glomerular filtration rate; FLC = free light chains; MAP = mean arterial pressure; SD = standard deviation.

<https://doi.org/10.1371/journal.pone.0197043.t004>

with increased filtration of FLCs) often coexists with tubulointerstitial fibrosis (and thus possibly reduced tubular FLC reabsorption). The correlation between urinary albumin and FLC is therefore not unexpected.

Adjustment for the confounding relationships with eGFR and ACR significantly attenuated the association between FLC/Cr ratios as continuous predictor variables and risk of ESRD, which became non-significant. The lack of an independent association may reflect their lack of specificity for renal damage: urinary FLC/Cr ratios correlated most strongly with serum FLC, suggesting that systemic inflammation may be an important determinant of urinary levels, and urinary FLC concentration may also be influenced by mucosal secretion in the urinary tract, although data do not exist on how much this pathway contributes.

Despite the lack of an independent association between FLC/Cr ratios and progression to ESRD when analysed as full-range continuous variables, our multivariable analyses did show

Table 5. Logistic regression models for the prediction of end stage renal disease at two years, with measures of model performance and the incremental value of adding kappa/creatinine ratio (KCR) or lambda/creatinine ratio (LCR) to a baseline model containing the kidney failure risk equation (KFRE).

Statistic	Model				
	Kidney Failure Risk Equation (KFRE)	KFRE + KCR		KFRE + LCR	
		Per +1 SD	> 27.6 mg/mmol	Per +1 SD	> 5.0 mg/mmol
Odds ratio (95% CI)					
KFRE	1.10 (1.08–1.13)	1.10 (1.07–1.13)	1.10 (1.07–1.13)	1.10 (1.07–1.13)	1.09 (1.07–1.12)
KCR or LCR		1.05 (0.71–1.55)	1.54 (0.67–3.50)	1.16 (0.75–1.77)	2.67 (1.19–6.03)
R² (Nagelkerke)	0.436	0.437	0.440	0.438	0.457
Hosmer-Lemeshow (P)	12.19 (0.14)	12.37 (0.14)	9.44 (0.31)	11.71 (0.16)	7.07 (0.53)
C-statistic (95% CI)	0.89 (0.84–0.94)	0.89 (0.84–0.94)	0.89 (0.84–0.94)	0.89 (0.83–0.94)	0.90 (0.85–0.95)
Δ C-statistic		0.00 (-0.01–0.01)	0.00 (-0.02–0.02)	0.00 (-0.01–0.01)	0.01 (-0.01–0.03)
IDI		0.00 (-0.01–0.02)	0.00 (-0.01–0.02)	0.00 (-0.01–0.02)	0.00 (-0.01–0.04)

Models were fitted with LCR and KCR as both continuous predictors (per +1 SD) and with a categorical cut-off above the 75th centile.

Abbreviations: CI = confidence interval; IDI = integrated discrimination improvement; KCR = kappa/creatinine ratio; KFRE = kidney failure risk equation; LCR = lambda/creatinine ratio; SD = standard deviation.

<https://doi.org/10.1371/journal.pone.0197043.t005>

that urinary FLC/Cr ratios above the 75th centile were independently associated with a significantly increased risk of ESRD. It is possible that this is due to a nephrotoxic effect of FLCs, i.e. those with the highest levels in the urine may be more likely to sustain FLC-induced tubular damage, thus increasing their risk of CKD progression and ESRD. A nephrotoxic effect of FLCs has been proposed as a possible explanation for an independent association between serum FLC levels and progression to ESRD in patients with CKD, although the published data on the relationship between serum FLC and progression to ESRD are inconsistent [28, 29]. Alternatively, the highest levels may be present in patients who have glomerular hyperfiltration and/or tubular dysfunction, both factors that are associated with accelerated progression of CKD. It is also possible that the association reflects a residual confounding effect of kidney function that has not been fully accounted for by adjustment for creatinine-based eGFR.

Adding urinary FLC/Cr ratios to an established model for the estimation of two-year risk of ESRD did not improve model performance in our cohort of patients with advanced kidney disease. However, having previously been shown to be detectable prior to the development of albuminuria, the use of urinary FLCs to stratify risk in early CKD could be examined in other CKD cohorts with populations made up of less severe CKD.

The strength of this study is that it included a well-characterised cohort of patients with prospective follow-up and a significant number of outcome events. Limitations were that it was an observational study without mechanistic data, and we lacked a separate cohort to validate our findings.

In conclusion, urinary FLCs have an association with progression to ESRD in patients with CKD which appears to be explained largely by their correlation with eGFR and ACR, and the current evidence suggests that measuring urinary FLC does not improve upon current models for risk stratification.

Acknowledgments

AF would like to acknowledge The Binding Site Ltd (Birmingham, UK) for the funding of his research fellowship.

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References

1. Turin TC, Tonelli M, Manns BJ, Ahmed SB, Ravani P, James M, et al. Lifetime risk of ESRD. *Journal of the American Society of Nephrology: JASN*. 2012; 23(9):1569–78. <https://doi.org/10.1681/ASN.2012020164> PMID: 22904351.
2. Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. *Kidney Int*. 2011; 80(1):17–28.
3. Gansevoort RT, Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, et al. Lower estimated GFR and higher albuminuria are associated with adverse kidney outcomes. A collaborative meta-analysis of general and high-risk population cohorts. *Kidney Int*. 2011; 80(1):93–104.
4. Astor BC, Matsushita K, Gansevoort RT, van der Velde M, Woodward M, Levey AS, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with mortality and end-stage renal disease. A collaborative meta-analysis of kidney disease population cohorts. *Kidney Int*. 2011; 79(12):1331–40.
5. Chronic Kidney Disease Prognosis C, Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, et al. Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: a collaborative meta-analysis. *Lancet*. 2010; 375(9731):2073–81.
6. van der Velde M, Matsushita K, Coresh J, Astor BC, Woodward M, Levey A, et al. Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. *Kidney Int*. 2011; 79(12):1341–52.
7. Tangri N, Kitsios GD, Inker LA, Griffith J, Naimark DM, Walker S, et al. Risk prediction models for patients with chronic kidney disease: a systematic review. *Ann Intern Med*. 2013; 158(8):596–603. <https://doi.org/10.7326/0003-4819-158-8-201304160-00004> PMID: 23588748.
8. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney International Supplements*. 2013; 3(1):1–150.
9. Tangri N, Stevens LA, Griffith J, Tighiouart H, Djurdjev O, Naimark D, et al. A predictive model for progression of chronic kidney disease to kidney failure. *JAMA*. 2011; 305(15):1553–9. <https://doi.org/10.1001/jama.2011.451> PMID: 21482743.
10. Waldmann TA, Strober W, Mogielnicki RP. The renal handling of low molecular weight proteins. II. Disorders of serum protein catabolism in patients with tubular proteinuria, the nephrotic syndrome, or uremia. *J Clin Invest*. 1972; 51(8):2162–74. <https://doi.org/10.1172/JCI107023> PMID: 5054468.
11. Maack T, Johnson V, Kau ST, Figueiredo J, Sigulem D. Renal filtration, transport, and metabolism of low-molecular-weight proteins: a review. *Kidney Int*. 1979; 16(3):251–70. PMID: 393891.
12. Solomon A. Light chains of human immunoglobulins. *Methods Enzymol*. 1985; 116:101–21. PMID: 3937021.
13. Hutchison CA, Harding S, Hewins P, Mead GP, Townsend J, Bradwell AR, et al. Quantitative assessment of serum and urinary polyclonal free light chains in patients with chronic kidney disease. *Clin J Am Soc Nephrol*. 2008; 3(6):1684–90. <https://doi.org/10.2215/CJN.02290508> PMID: 18945993.
14. Hassan SB, Hanna MO. Urinary kappa and lambda immunoglobulin light chains in normoalbuminuric type 2 diabetes mellitus patients. *J Clin Lab Anal*. 2011; 25(4):229–32. <https://doi.org/10.1002/jcla.20463> PMID: 21786323.
15. Hutchison CA, Cockwell P, Harding S, Mead GP, Bradwell AR, Barnett AH. Quantitative assessment of serum and urinary polyclonal free light chains in patients with type II diabetes: an early marker of diabetic kidney disease? *Expert Opin Ther Targets*. 2008; 12(6):667–76. <https://doi.org/10.1517/14728222.12.6.667> PMID: 18479214.
16. Hanaoka M, Gono T, Kawaguchi Y, Uchida K, Koseki Y, Katsumata Y, et al. Urinary free light chain is a potential biomarker for ISN/RPS class III/IV lupus nephritis. *Rheumatology*. 2013; 52(12):2149–57. <https://doi.org/10.1093/rheumatology/ket108> PMID: 23463805.
17. Rocchetti MT, Papale M, d’Apollo AM, Suriano IV, Di Palma AM, Vocino G, et al. Association of urinary laminin G-like 3 and free K light chains with disease activity and histological injury in IgA nephropathy.

- Clinical journal of the American Society of Nephrology: CJASN. 2013; 8(7):1115–25. <https://doi.org/10.2215/CJN.05950612> PMID: 23599406.
18. Stringer S, Sharma P, Dutton M, Jesky M, Ng K, Kaur O, et al. The natural history of, and risk factors for, progressive chronic kidney disease (CKD): the Renal Impairment in Secondary care (RIISC) study; rationale and protocol. *BMC Nephrol.* 2013; 14:95. <https://doi.org/10.1186/1471-2369-14-95> PMID: 23617441.
 19. Hutchison CA, Plant T, Drayson M, Cockwell P, Kountouri M, Basnayake K, et al. Serum free light chain measurement aids the diagnosis of myeloma in patients with severe renal failure. *BMC Nephrol.* 2008; 9:11. <https://doi.org/10.1186/1471-2369-9-11> PMID: 18808676.
 20. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF 3rd, Feldman HI, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med.* 2009; 150(9):604–12. PMID: 19414839.
 21. Brothwell S, Dutton M, Ferro C, Stringer S, Cockwell P. Optimising the accuracy of blood pressure monitoring in chronic kidney disease: the utility of BpTRU. *BMC Nephrol.* 2013; 14:218. <https://doi.org/10.1186/1471-2369-14-218> PMID: 24112304.
 22. Zou KH, Tuncali K, Silverman SG. Correlation and simple linear regression. *Radiology.* 2003; 227(3):617–22. <https://doi.org/10.1148/radiol.2273011499> PMID: 12773666.
 23. Fine JP, Gray RJ. A Proportional Hazards Model for the Subdistribution of a Competing Risk. *Journal of the American Statistical Association.* 1999; 94(446):496–509. <https://doi.org/10.2307/2670170>
 24. Tangri N, Grams ME, Levey AS, Coresh J, Appel LJ, Astor BC, et al. Multinational Assessment of Accuracy of Equations for Predicting Risk of Kidney Failure: A Meta-analysis. *JAMA.* 2016; 315(2):164–74. <https://doi.org/10.1001/jama.2015.18202> PMID: 26757465.
 25. Nagelkerke NJD. A note on a general definition of the coefficient of determination. *Biometrika.* 1991; 78(3):691–2. <https://doi.org/10.1093/biomet/78.3.691>
 26. Pencina MJ, D'Agostino RB Sr., D'Agostino RB Jr., Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Stat Med.* 2008; 27(2):157–72; discussion 207–12. <https://doi.org/10.1002/sim.2929> PMID: 17569110.
 27. Hlatky MA, Greenland P, Arnett DK, Ballantyne CM, Criqui MH, Elkind MS, et al. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. *Circulation.* 2009; 119(17):2408–16. <https://doi.org/10.1161/CIRCULATIONAHA.109.192278> PMID: 19364974.
 28. Ritchie J, Assi LK, Burmeister A, Hoefield R, Cockwell P, Kalra PA. Association of Serum Ig Free Light Chains with Mortality and ESRD among Patients with Nondialysis-Dependent CKD. *Clin J Am Soc Nephrol.* 2015; 10(5):740–9. <https://doi.org/10.2215/CJN.09660914> PMID: 25825483.
 29. Haynes R, Hutchison CA, Emberson J, Dasgupta T, Wheeler DC, Townend JN, et al. Serum free light chains and the risk of ESRD and death in CKD. *Clin J Am Soc Nephrol.* 2011; 6(12):2829–37. <https://doi.org/10.2215/CJN.03350411> PMID: 22034503.