Association of Serum Ig Free Light Chains with Mortality and ESRD among Patients with Nondialysis-Dependent CKD

James Ritchie, Lakhvir K. Assi,† Anne Burmeister,† Richard Hoefield,* Paul Cockwell,‡§ and Philip A. Kalra**

Abstract

Background and objectives High levels of serum polyclonal combined Ig free light chains are associated with inflammation and decreased excretory kidney function, and they are an independent risk factor for mortality. Whether combined Ig free light chain predicted mortality and progression to ESRD in a stages 3–5 CKD cohort was assessed.

Design, setting, participants, & measurements This was a prospective cohort study of 872 patients with stages 3–5 CKD (nondialysis) recruited into the Chronic Renal Insufficiency Standards Implementation Study. Patients were recruited to the Chronic Renal Insufficiency Standards Implementation Study in an unselected manner from secondary care nephrology clinics between 2004 and 2010. Combined Ig free light chain was measured at recruitment and analyzed by quartiles. The cohort was followed up for a median of 41.4 months (interquartile range =28.3–68.0 months). Cox regression analysis was undertaken to determine the variables associated with mortality and progression to ESRD.

Results Combined Ig free light chain quartiles were <49.4, 49.4–68.8, 68.9–100.7, and >100.7 mg/L. An independent association with death and progression to ESRD was associated with the third and fourth combined Ig free light chain quartiles (quartile 3: death: hazard ratio, 1.49; 95% confidence interval, 1.02 to 2.18; P=0.04; ESRD: hazard ratio, 1.72; 95% confidence interval, 1.0 to 2.97; P=0.05; quartile 4: death: hazard ratio, 1.99; 95% confidence interval, 1.34 to 2.93; P<0.001; ESRD: hazard ratio, 3.73; 95% confidence interval, 2.1 to 6.3; P<0.001). The other independent risk factors were (*1*) preexisting cardiovascular disease, age >65 years old, and eGFR=15–30 ml/min per 1.73 m^2 for death and (*2*) age ≤65 years old, eGFR<30 ml/min per 1.73 m^2, urinary protein-to-creatinine ratio >30 mg/mmol, and serum phosphate level >4.65 mg/dl for progression to ESRD.

Conclusions An elevated serum combined Ig free light chain level is an independent risk factor for mortality and progression to ESRD in patients with stages 3–5 CKD managed in secondary care.

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*Department of Renal Medicine, Salford Royal National Health Service Foundation Trust, Salford, United Kingdom; †The Binding Site Group Ltd., Birmingham, United Kingdom; ‡Department of Nephrology, Queen Elizabeth Hospital, Birmingham, United Kingdom; and §Division of Immunity and Infection, College of Medical and Dental Science, University of Birmingham, Birmingham, United Kingdom

Correspondence: Professor Philip A. Kalra, Department of Renal Medicine, Salford Royal National Health Service Foundation Trust, Salford, M6 8HD, UK. Email: philip.kalra@srft.nhs.uk

Introduction

CKD is associated with increased mortality (1). This mortality risk is related, in part, to the severity of renal impairment and albuminuria (2,3). However, a substantial component of the risk associated with CKD may be caused by nontraditional risk factors, such as inflammation, and biomarkers produced and retained as a consequence of inflammation and impaired renal function associate with higher risk (4–7). These biomarkers may have important use in increasing the sensitivity of risk stratification of CKD (8) in addition to identifying pathophysiologic processes that may contribute to poor outcomes in CKD.

Serum Ig light chains (LCs), which are independent of the intact Ig molecule and referred to as free LCs (FLCs), fulfill criteria for assessment as nontraditional biomarkers in CKD (9): the two LC isotypes (monomeric κ and dimeric λ) are produced in excess in chronic inflammation (10) and with molecular masses of 22.5 and 45 kD, respectively, also accumulate in renal impairment (11).

The recent development of assays that can accurately quantify serum FLC levels below the normal range has recently led to clinical studies that have assessed the relationship between polyclonal serum FLC levels and mortality (12,13). These studies used serum FLC levels calculated as a summation of the two LC isotypes to produce a single combined FLC (cFLC) level. Collectively, the studies provide evidence of an independent association between an elevated cFLC level and a higher mortality risk.

To date, three studies have assessed the relationship between FLC and mortality risk in patients with CKD. They all show an association between elevated FLC and mortality (14–16). However, the predictive strength of this association has not been defined, and there have been no studies of the relationship between cFLC and risk of progression to ESRD as well as mortality in a

secondary care cohort comprising patients with stages 3–5 predialysis CKD. The previous studies of cFLC in patients with moderate to advanced CKD represented relatively small patient numbers and a low prevalence of patients with diabetes (14,15). The largest and most recent study included patients with preserved renal function and did not consider progression of renal failure (16).

The purpose of this study was to use the Chronic Renal Insufficiency Standards Implementation Study (CRISIS), which is a well established prospective secondary care CKD cohort with detailed inception data and long follow-up, to explore the relationship between cFLC and clinical outcomes in stages 3–5 CKD. The outcomes comprised (*1*) all-cause mortality and (2) progression to ESRD. The analysis incorporated bioclinical data of variables known to be associated with the outcomes of interest.

Materials and Methods

Study Design

In total, 919 individuals with stages 3–5 CKD were recruited as part of a prospective observational study to investigate adverse outcomes in kidney disease (CRISIS; Salford National Health Service Foundation Trust). Patients were enrolled between April 21, 2004 and November 24, 2010. The study has previously been described (17,18). In brief, patients already under follow-up for or referred for management of CKD in secondary care nephrology clinics were invited to take part in the study. All patients had demographic (age, sex, and ethnicity), clinical (BP and comorbidities), and laboratory data (renal function, proteinuria, hemoglobin, inflammation, and cholesterol) collected at baseline and annual follow-up visits. When the CRISIS was established, erythrocyte sedimentation rate (ESR) data were collected, with C-reactive protein (CRP) data subsequently added. Because baseline ESR data were more complete, this marker of inflammation was used in this analysis. The patients consented for follow-up annually for 10 years from recruitment or until death. The primary study end points of the CRISIS are death and progression to ESRD. Patients could also leave the study through withdrawal of consent or loss to follow-up. The CKD stage at recruitment was assigned by eGFR as recommended by Kidney Disease Outcome Quality Initiative 2002 Guidelines (19). eGFR was obtained using the four-variable Modification of Diet in Renal Disease equation (20). Ethical approval was granted by the Salford and Trafford Local Research Ethics Committee. All eligible patients provided written consent in accordance with the Declaration of Helsinki.

Laboratory Analyses

Blood samples used in this analysis were collected at patient recruitment. Serum samples were processed and stored at −80°C until analysis for κFLC and λFLC by turbidimetry using the Freelite Assay (The Binding Site Group Ltd., Birmingham, UK) on a SPAPLUS Analyzer (The Binding Site Group Ltd.). For the purpose of data analysis, κFLC and λFLC were summated to give a cFLC concentration. The reference range used for normal cFLC levels is 9.3–43.3 mg/L (13).

Patients with a paraprotein were excluded from additional analysis (n=47); paraproteinemia was defined as the presence of an abnormal FLC (κ/λ) ratio using the renal reference range (0.37–3.1) (11) and an elevation of the involved LC and/or positive serum protein electrophoresis (SPE) and immunofixation (IFE) result. SPE and IFE were assayed using the Sebia Hydrasys System (SPE: Hydragel 54 $\beta1$–$\beta2$; IFE: Hydragel 4 IF; Sebia, Evry, France) following the manufacturers' guidelines.

Biochemistry results from clinical laboratories performed in accordance with the current standard of care were collected from electronic patient records as described previously (17). Creatinine measurements were performed on a Roche Modular Analyzer using a blank rated and compensated Jaffe reaction. Normal reference intervals for each variable were defined by the testing laboratory. Where protein-to-creatinine ratios were not available, these were estimated from measurement of 24-hour proteinuria assuming that a protein-to-creatinine ratio of 100 mg/mmol is approximately 1 g per 24-hour proteinuria (21).

Study Outcomes

Patient mortality was captured through electronic patient records and the Office of National Statistics. Causes of death were classified from death certificates according to the International Classification of Diseases, 10th edition and comprised the following groups: cardiovascular, infection, cancer, renal complications, and other causes.

Progression to ESRD was defined as the initiation of RRT (chronic dialysis or renal transplant) or reaching an eGFR of ≤9 ml/min per 1.73 m^2 (the mean level of kidney function at which dialysis is initiated in the United Kingdom) (22). Patients who had an eGFR≤9 ml/min per 1.73 m^2 at recruitment or did not have a follow-up eGFR result recorded were not included in the progression analysis (n=50).

Statistical Analyses

Statistical analysis was performed using SPSS, version 21.0 (IBM, Armonk, NY). Only patients with a κ/λ ratio within the renal reference range were included (0.37–3.1). Differences in variables between patients who did and did not reach the study outcomes were determined by Mann–Whitney U test for continuous variables or chi-squared test for categorical variables. P values <0.05 were considered to be significant. For the survival analyses, variables that were not normally distributed were analyzed on a natural logarithmic scale. Correlation analyses were performed using Pearson's R; values were defined as very weak (R=0–0.19), weak (R=0.2–0.39), moderate (R=0.4–0.59), strong (R=0.6–0.79), and very strong (R>0.8) (23).

Survival analysis was performed using the Cox proportional hazard test. Censoring occurred on patient withdrawal from the study or at their last attended clinic appointment before July 10, 2012. All potentially relevant recorded variables (Table 1) were analyzed in a categorical form, defined by using either the upper or the lower limit of the clinically defined normal range, or defined by using the median value for the population when a normal range was not applicable (for example, where biomarkers [*e.g.*, cFLC] are renally cleared and therefore, have higher serum levels than is normally seen in patients without renal insufficiency). Variables significant to P<0.10 by univariate analysis were included in a multivariate analysis. This was performed using a forward likelihood ratio method for variable

Table 1. Patient demographics

Variable	All Patients (n=872)	cFLC Quartile 1 (<49.4 mg/L)	cFLC Quartile 2 (49.4–68.8 mg/L)	cFLC Quartile 3 (68.9–100.7 mg/L)	cFLC Quartile 4 (>100.7 mg/L)	P Value
Age, yr	66 (55–74)	64 (54–71)	65.7 (54–74.75)	66 (56–74)	68 (56–76.45)	0.14
Men	61.7%	n=119 (54.6%)	n=137 (62.8%)	n=132 (60.6%)	n=150 (68.8%)	<0.001
Primary renal disease						
Diabetes	15.4%	n=17 (7.8%)	n=35 (16.1%)	n=48 (22%)	n=42 (19.3%)	<0.001
ADPKD	7.0%	n=8 (3.7%)	n=18 (8.3%)	n=13 (6%)	n=10 (4.6%)	
Vascular	22.5%	n=57 (26.1%)	n=67 (30.7%)	n=60 (27.5%)	n=59 (27.1%)	
GN	19.4%	n=12 (5.5%)	n=9 (4.1%)	n=10 (4.6%)	n=6 (2.8%)	
Pyelonephrtitis	8.0%	n=18 (8.3%)	n=15 (6.9%)	n=18 (8.3%)	n=15 (6.9%)	
Other	14.0%	n=58 (26.6%)	n=49 (22.5%)	n=39 (17.9%)	n=61 (28%)	
Unknown	13.7%	n=48 (22%)	n=25 (11.5%)	n=30 (13.8%)	n=25 (11.5%)	
CVD						
Yes	n=75 (34.4%)	n=97 (44.5%)	n=95 (43.6%)	n=101 (46.3%)	n=75 (34.4%)	<0.001
Diabetes						
Yes	n=51 (23.4%)	n=62 (28.4%)	n=88 (40.4%)	n=79 (36.2%)	n=51 (23.4%)	<0.001
Smoking history						
Never	n=77 (35.3%)	n=73 (33.5%)	n=74 (33.9%)	n=59 (27.1%)	n=77 (35.3%)	<0.001
Former	n=122 (56%)	n=119 (54.6%)	n=112 (51.4%)	n=121 (55.5%)	n=122 (56%)	
Current	n=19 (8.7%)	n=26 (11.9%)	n=31 (14.2%)	n=35 (16.1%)	n=19 (8.7%)	
Systolic BP (mmHg)	134 (122–150)	131 (121.5–145.5)	132 (120.75–144)	138 (123–152)	136 (122–152)	0.09
Diastolic BP (mmHg)	75 (66–82)	78 (68.5–83)	76 (68–80)	72 (62–81)	74 (66–82)	0.04
eGFR (ml/min per 1.73 m^2)	30 (21–41)	42.94 (33.48–49.08)	33.22 (25.72–42.36)	25.8 (19.54–35.08)	19.99 (15.43–27.51)	<0.001
ΔeGFR (ml/min per 1.73 m^2 per yr)	−1.16 (−2.77–0.19)	−0.07 (−1.78–1.8)	−0.99 (−2.29–0.2)	−1.63 (−2.85 to −0.04)	−1.9 (−3.75 to −0.56)	<0.001
Phosphate (mg/dl)	3.50 (2.73–4.03)	3.28 (2.94–3.72)	3.47 (3.07–3.96)	3.62 (3.10–4.15)	3.75 (3.25–4.34)	<0.001
Corrected calcium (mg/dl)	9.10 (8.82–9.42)	9.18 (8.86–9.46)	9.14 (8.90–9.42)	9.06 (8.74–9.38)	9.02 (8.74–9.34)	0.05
Parathyroid hormone (mg/L)	63 (37–112)	43 (24–68)	54 (34–90.5)	74 (44.25–121.75)	97 (51–166)	<0.001
Urea (mg/dl)	37.8 (28.3–51.8)	28.2 (22.9–35.2)	35.6 (28–45.4)	44.5 (32.6–56.5)	48.9 (38.2–66.3)	<0.001
Sodium (mmol/L)	141 (139–143)	141 (140–143)	141 (139–142)	141 (139–142)	141 (139–143)	0.08
Potassium (mmol/L)	4.8 (4.4–5.2)	4.7 (4.3–5.07)	4.8 (4.4–5.2)	4.8 (4.4–5.3)	5 (4.5–5.4)	<0.001
Total random cholesterol (mg/dl)	166 (139–189)	174 (155–201)	170 (147–193)	159 (135–184)	155 (128–182)	<0.001
Hemoglobin (g/L)	124 (114–136)	130 (118.25–139.75)	126 (117–137)	123 (113–133.75)	118 (109–128)	<0.001
Albumin (g/L)	43 (40–45)	44 (42–46)	43 (41–45)	43 (40–45)	42 (39–43.75)	<0.001
PCr (mg/mmol)	25.4 (12.3–69.2)	20 (10–37.5)	30 (10–70)	40 (20–107.5)	80 (30–167)	0.01
ESR (mm/h)	22 (11–40)	13 (8–27)	19 (11–35)	24 (14–39)	33 (18–54)	<0.001
κFLC (mg/L)	37.4 (25.6–52.6)	21.05 (17.33–23.64)	31.41 (28.13–34.47)	45.17 (40.17–49.42)	69.45 (58.48–83.19)	<0.001
λFLC (mg/L)	32.1 (23.3–45.8)	19.23 (15.91–22.19)	27.31 (24.28–30.44)	38.2 (33.71–41.91)	62.13 (52.25–77.24)	<0.001

Table 1. (Continued)

Variable	All Patients (n=872)	cFLC Quartile 1 (<49.4 mg/L)	cFLC Quartile 2 (49.4–68.8 mg/L)	cFLC Quartile 3 (68.9–100.7 mg/L)	cFLC Quartile 4 (>100.7 mg/L)	P Value
κ\λFLC ratio	1.12 (0.95–1.32)	1.08 (0.94–1.22)	1.13 (0.97–1.37)	1.15 (1.02–1.37)	1.12 (0.9–1.35)	<0.001
cFLC (mg/L)	68.9 (49.4–100.9)	41.45 (33.97–45.5)	58.97 (53.8–64.38)	81.95 (75.21–90.1)	128.15 (113.35–157.09)	<0.001

Forty-four patients were not included in the progression analysis, because they had already reached the end point of GFR<9 ml/min per 1.73m^2 at recruitment into the study; six patients did not have ΔGFR/RRT status recorded. Values for continuous variables are presented as medians (interquartile ranges). Where protein-to-creatinine ratio (PCr) data were not available, PCr was estimated from 24-h proteinuria on the basis of the assumption that a PCr of 100 mg/mmol is approximately 1 g per 24-h proteinuria. GN includes vasculitis. ADPKD, adult dominant polycystic kidney disease; CVD, cardiovascular disease (defined as angina pectoris, myocardial infarction, percutaneous or surgical coronary revascularization, stroke, or peripheral vascular disease); ESR, erythrocyte sedimentation rate; FLC, free light chain; cFLC, combined free light chain.

inclusion. Overall survival and progression to ESRD were assessed by Kaplan–Meier analysis; significance was determined using the log-rank test. To better consider the discriminatory value of cFLC in these analyses, patient outcomes were considered in relation to cFLC quartiles. Finally, preliminary sensitivity analyses were performed comparing the area under the receiver operating curve between eGFR and cFLC levels for each end point.

The association between cFLC and each cause of death was investigated by cross-tabulation using chi-squared analysis.

Results

Patient Demographics

A population of 872 patients with CKD stages 3–5 (CKD stage 3a, n=159; CKD stage 3b, n=279; CKD stage 4, n=349; and CKD stage 5, n=85) was available for analysis. Median follow-up time was 41.4 months (interquartile range [IQR]=28.3–68.0), with a maximum follow-up period of 117.9 months. Complete follow-up data were available for 87% of scheduled visits. Patients with missing data were significantly older (age: 65.3±13.7 versus 62.9±13.7 years old), had more preserved renal function (eGFR=34.0±13 versus 30.2±12 ml/min per 1.72 m^2), and had lower BP (131±28/72±16 versus 133±35/75±20 mmHg). Where follow-up data were missing, electronic health records still allowed mortality and RRT events to be captured.

At recruitment, the population had a median age of 66 years old (IQR=55–74) and consisted of 62% men (Table 1). Median eGFR was 30 ml/min per 1.73 m^2 (IQR=21–41). Diabetes was present in 32% of patients, and 42% of patients had a prior history of cardiovascular disease (CVD) defined as angina pectoris, myocardial infarction, percutaneous or surgical coronary revascularization, stroke, or peripheral vascular disease. The median cFLC concentration for the population was 68.9 mg/L (IQR=49.4–100.9), which was greater than the median previously reported in the general population (median=28 mg/L; normal range=9.3–43.3 mg/L) (13). The κ/λ FLC ratio for the CKD population was also higher compared with the general population (median=1.12 versus 0.8) (13). Complete baseline data are presented in Table 1.

Two hundred eighty-seven patients (33%) died during follow-up, with a median survival time of 30 months (IQR=16.1–48.4). Of the patients who died, 78 (27%) progressed to ESRD before death. Fifty-five patients (19%) died within the first 12 months of follow-up. In 822 patients with available data, 202 patients (25%) progressed to ESRD, with a median time to ESRD of 36.2 months (IQR=19.0–53.3); 64 patients progressed to ESRD within the first 12 months of follow-up.

The patients who died were generally older, were men, were smokers, had diabetes and/or a history of CVD, and had more advanced CKD. Comparatively, the patients who progressed to ESRD were younger, with no significant baseline differences in sex, diabetic status, or history of CVD. Patients who progressed to ESRD had significantly poorer renal function at recruitment (median GFR=21 versus 34 ml/min per 1.73 m^2; P<0.001) as well as greater annual decline in eGFR (−2.93 versus −0.54 ml/1.73 m^2 per year; P<0.001). Baseline cFLC concentrations were higher in both patients who died (median =87.0 versus

63.4 mg/L; $P<0.001$) and patients who reached ESRD (median =94.2 versus 59.7 mg/L; $P<0.001$).

cFLC on a natural logarithmic scale correlated moderately with eGFR ($R=-0.56$, $P<0.001$) but not with age ($R=0.06$, $P=0.08$) (Table 2). cFLC also displayed a weak correlation with phosphate, parathyroid hormone, urea, cholesterol, hemoglobin, albumin, and ESR (all $P<0.001$) (Table 2). There was a very weak correlation with CRP ($R=0.15$, $P<0.001$).

Univariate Analyses

cFLC showed a significant association with both death (hazard ratio [HR], 2.11 per one log-unit higher; 95% confidence interval [95% CI], 1.71 to 2.63; $P<0.001$) and progression to ESRD (HR, 4.39 per one log-unit higher, 95% CI, 3.51 to 5.50; $P<0.001$). Univariate analysis of categorical variables confirmed the association between elevated concentrations of cFLC with patient mortality and progression (Table 3). Patients with cFLC>68.9 mg/L were two times as likely to die during the study follow-up compared with patients with cFLC concentrations ≤68.9 mg/L (HR, 2.20; 95% CI, 1.72 to 2.81; $P<0.001$). Risk increased in relation to the lower quartile of cFLC. For death, cFLC had a significantly higher area under the curve than eGFR (Supplemental Figure 1). Similarly, cFLC>68.9 mg/L was associated with an approximately 6-fold higher risk of progression to ESRD (HR, 5.98; 95% CI, 4.25 to 8.42; $P<0.001$). Again, the risk for ESRD increased with cFLC level, with the greatest risk for ESRD associated with the upper quartile of cFLC (HR, 8.74; 95% CI, 5.85 to 13.06; $P<0.001$). However, this was not associated with a significant increase in area under the curve compared with eGFR (Supplemental Figure 2). Other variables significantly associated with both end points included age >65 years old, systolic BP >130 mmHg, eGFR<30 ml/min per 1.73 m^2, parathyroid hormone >65 mg/L, total cholesterol <193 mg/dl, and hemoglobin <130 g/L. The median survival and progression to ESRD times were significantly decreased in patients with cFLC>68.9 mg/L (Figure 1).

Multivariate Analyses

Results of multivariate analyses of categorical variables associated with reduced survival and progression to ESRD are shown in Tables 4 and 5, respectively. cFLC remained independently associated with both outcomes. Because of the known associations of age with patient survival and ESRD, the regression models were repeated with and without age as a covariate. In an age-adjusted model, elevated cFLC values remained independently associated with risk for death (quartile 3: HR, 1.49; 95% CI, 1.02 to 2.18; $P=0.04$; quartile 4: HR, 1.99; 95% CI, 1.34 to 2.93; $P<0.001$) and progression to ESRD (quartile 3: HR, 1.72; 95% CI, 1.00 to 2.97; $P=0.05$; quartile 4: HR, 3.73; 95% CI, 2.10 to 6.30; $P<0.001$).

Table 2. Correlation analyses of combined free light chains and clinically significant variables

Variable	Pearson *R*	*P* Value
Age	0.06	0.08
eGFR	−0.56	<0.001
Phosphate	0.26	<0.001
Potassium	0.15	<0.001
Sodium	−0.08	0.02
PTH[a]	0.37	<0.001
Urea[a]	0.46	<0.001
Total cholesterol	−0.23	<0.001
Hemoglobin	−0.27	<0.001
Albumin	−0.24	<0.001
ESR[a]	0.33	<0.001

PTH, parathyroid hormone; ESR, erythrocyte sedimentation rate.
[a]Combined free light chain, PTH, urea, and ESR analyzed on a natural log scale.

Rate of eGFR Loss

In this study, the median rate of eGFR loss was 1.56 ml/min per 1.73 m^2 per year (IQR=−2.78–0.19). Patients with higher cFLC quartiles had more rapid rates of eGFR loss. Median annual rate of change in eGFR increased from −0.07 ml/min per 1.73 m^2 per year (IQR=−1.78–1.8) in the lowest quartile to −0.99 ml/min per 1.73 m^2 per year (IQR=−2.29–0.2) in quartile 2, −1.63 ml/min per 1.73 m^2 per year (IQR=−2.85 to −0.04) in quartile 3, and −1.90 ml/min per 1.73 m^2 per year (IQR=−3.75 to −0.56) in quartile 4 ($P<0.001$). The proportion of patients with an annual rate of eGFR loss above the 75th percentile increased in each quartile of cFLC (quartile 1–4: 12%, 18%, 24%, and 32%, respectively; $P<0.01$).

Associations between cFLC and Different Causes of Death

Cause of death data were available for 282 of 287 patients (98%). Deaths were grouped into cardiovascular ($n=117$), infectious causes ($n=64$), cancer ($n=33$), renal complications ($n=28$), and other ($n=40$). A significantly higher proportion of patients with cFLC concentrations >68.9 mg/L died because of an infection (61% versus 39%; $P=0.02$).

Discussion

This study of a prospective secondary care cohort of patients with stages 3–5 CKD showed a strong independent relationship between high cFLC levels and outcomes of mortality and progression to ESRD. In a categorical multivariate analysis, we used values above the upper median level of FLC as high for this cohort.

This study used the Freelite assay, which separately measures both isotypes of FLC: κ and λ. For this study, the levels of the individual isotypes were combined to produce a cFLC level. This is in contrast to the use of the assay in the diagnosis and monitoring of paraproteinemia, where the isotypes are used to screen for an LC paraprotein as defined by the presence of an abnormal LC ratio and elevation of the involved isotype (24–26). With an elevated FLC in the absence of a paraprotein, the ratio of the isotypes remains within the normal range, although this range shifts slightly in CKD because of the differential retention of κFLC (11,27).

The association between cFLC and long-term outcomes of ESRD and death has been shown in two previous studies. In an analysis of the Chronic Renal Impairment in Birmingham Study data, there was a weak independent association with polyclonal λFLC in a multivariable model that included troponin and N-terminal pro brain natriuretic peptide (14). However, this cohort included patients with a higher degree of comorbidity and more advanced CKD (mean eGFR=21.9 ml/min per 1.73 m^2) compared with this study population.

Table 3. Results of univariate analyses

Threshold Value	Survival		Progression to ESRD[a]	
	Hazard Ratio (95% Confidence Interval)	*P* Value	Hazard Ratio (95% Confidence Interval)	*P* Value
Age				
>65 yr	4.87 (3.62 to 6.55)	<0.001	0.63 (0.48 to 0.84)	0.001
Sex				
Women	0.74 (0.58 to 0.95)	0.02	1.11 (0.84 to 1.47)	0.47
CVD				
Yes	2.70 (2.12 to 3.43)	<0.001	0.82 (0.62 to 1.09)	0.17
Diabetes				
Yes	1.74 (1.38 to 2.20)	<0.001	1.23 (0.92 to 1.65)	0.16
Smoking history				
Former	1.89 (1.42 to 2.50)	<0.001	1.40 (0.93 to 2.10)	0.11
Current	1.78 (1.22 to 2.61)	0.003	0.92 (0.67 to 1.25)	0.59
Systolic BP				
>130 mmHg	1.33 (1.04 to 1.70)	0.02	1.42 (1.06 to 1.91)	0.02
Diastolic BP				
<80 mmHg	1.50 (1.17 to 1.93)	0.001	0.79 (0.59 to 1.05)	0.10
eGFR (ml/min per 1.73 m^2)				
>30	Referent		Referent	
15–30	1.86 (1.45 to 2.38)	<0.001	4.14 (2.78 to 6.20)	<0.001
<15	1.62 (1.09 to 2.41)	0.02	16.91 (10.90 to 26.31)	<0.001
Phosphate				
>4.65 mg/dl	1.17 (0.81 to 1.69)	0.40	3.44 (2.37 to 4.99)	<0.001
Potassium				
<3.5 mmol/L	0.80 (0.26 to 2.49)	0.70	1.90 (1.39 to 2.61)	<0.001
Sodium				
<135 mmol/L	1.70 (0.93 to 3.11)	0.08	0.80 (0.30 to 2.16)	0.66
Corrected calcium				
<8.4 mg/dl	0.89 (0.53 to 1.50)	0.66	1.89 (1.16 to 3.07)	0.01
Parathyroid hormone				
>65 mg/L	1.45 (1.10 to 1.90)	0.01	2.24 (1.59 to 3.15)	<0.001
Total cholesterol				
<193 mg/dl	1.91 (1.29 to 2.82)	0.001	1.55 (1.02 to 2.36)	0.04
Hemoglobin				
<130 g/L	1.51 (1.17 to 1.94)	0.001	2.13 (1.55 to 2.92)	<0.001
Albumin				
<35 g/L	1.47 (0.82 to 2.61)	0.20	2.25 (1.22 to 4.13)	0.01
PCr				
>30 mg/mmol	1.09 (0.82 to 1.44)	0.54	3.56 (2.27 to 5.57)	<0.001
ESR				
>7 ml/h	1.54 (0.99 to 2.41)	0.06	2.24 (1.22 to 4.13)	0.01
κFLC				
>37.4 mg/L	2.10 (1.64 to 2.67)	<0.001	5.41 (3.88 to 7.56)	<0.001
λFLC				
>32.1 mg/L	2.11 (1.65 to 2.69)	<0.001	5.62 (4.01 to 7.89)	<0.001
cFLC				
>68.9 mg/L	2.20 (1.72 to 2.81)	<0.001	5.98 (4.25 to 8.42)	<0.001
cFLC (mg/L)				
Quartile 1: <49.4	Referent		Referent[a]	
Quartile 2: 49.4–68.8	1.03 (0.69 to 1.53)	0.90	Referent[a]	
Quartile 3: 68.9–100.7	1.87 (1.30 to 2.69)	<0.001	3.42 (2.20 to 5.30)	<0.001
Quartile 4: >100.7	2.62 (1.84 to 3.71)	<0.001	8.74 (5.85 to 13.06)	<0.001

Where PCr data were not available, PCr was estimated from 24-h proteinuria on the basis of the assumption that a PCr of 100 mg/mmol is approximately 1 g per 24-h proteinuria. CVD, cardiovascular disease; PCr, protein-to-creatinine ratio; ESR, erythrocyte sedimentation rate; FLC, free light chain; cFLC, combined free light chain.

[a]For assessment of cFLC quartile in relation to RRT, only two of 218 patients in the lowest quartile reached the end point; therefore, a composite of the lowest two quartiles formed the referent category.

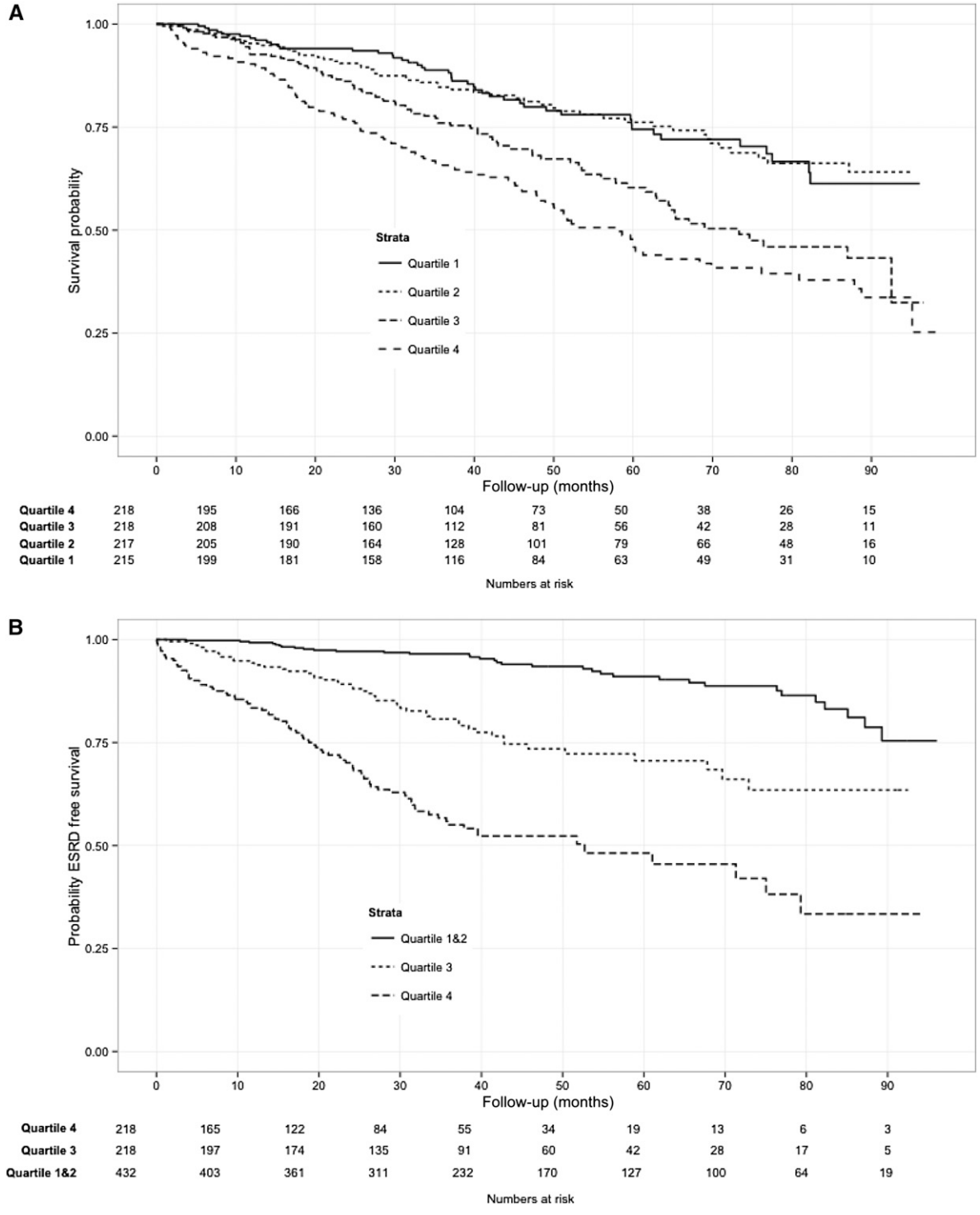

Figure 1. | Kaplan–Meier plots demonstrating increased risk for mortality and progression to ESRD by quartile of combined free light chain (cFLC). The *y* axis shows probability of event-free survival. The *x* axis shows time in months from study recruitment. Survival curves show (A) mortality-free survival and (B) ESRD-free survival divided by quartiles of cFLC. Statistical significance was determined using the Mantel–Cox log-rank test (*P* for both <0.001).

In a more recent study from a different United Kingdom center, 848 patients with CKD, preserved renal function (mean baseline eGFR=45±28 ml/min per 1.73 m^2), and a lower degree of proteinuria (mean albumin-to-creatinine ratio =10 mg/mmol) were followed up for a median of 63 months, and high cFLC levels were an independent risk factor for death, with an HR of 2.71 (95% CI, 1.98 to 3.70) (16).

Previous studies have reported variable associations between cFLC and nontraditional risk factors for poor outcomes in CKD, including CRP and phosphate (14,16).

Table 4. Results of multivariate analyses showing variables associated with higher risk of mortality

Variable	Not Age Adjusted		Age Adjusted	
	Hazard Ratio (95% Confidence Interval)	*P* Value	Hazard Ratio (95% Confidence Interval)	*P* Value
Age >65 yr	Not included		4.01 (2.95 to 5.45)	<0.001
eGFR (ml/min per 1.73 m^2)				
>30	Referent		Referent	
15–30	1.45 (1.10 to 1.91)	0.01	1.37 (1.04 to 1.80)	0.02
<15	1.16 (0.75 to 1.78)	0.51	1.27 (0.82 to 1.96)	0.27
CVD	2.61 (2.05 to 3.32)	<0.001	1.82 (1.41 to 2.33)	<0.001
cFLC (mg/L)				
Quartile 1: <49.4	Referent		Referent	
Quartile 2: 49.4–68.8	0.87 (0.57 to 1.30)	0.48	1.18 (0.57 to 1.27)	0.42
Quartile 3: 68.9–100.7	1.51 (1.03 to 2.21)	0.03	1.49 (1.02 to 2.18)	0.04
Quartile 4: >100.7	1.97 (1.33 to 2.92)	<0.001	1.99 (1.34 to 2.93)	<0.001

CVD, cardiovascular disease; cFLC, combined free light chain.

Table 5. Results of multivariate analyses showing variables associated with higher risk of ESRD

Variable	Not Age Adjusted		Age Adjusted	
	Hazard Ratio (95% Confidence Interval)	*P* Value	Hazard Ratio (95% Confidence Interval)	*P* Value
Age >65 yr	Not included		0.41 (0.27 to 0.62)	<0.001
eGFR (ml/min per 1.73 m^2)				
>30	Referent		Referent	
15–30	2.38 (1.45 to 3.91)	<0.001	2.58 (1.58 to 4.21)	<0.001
<15	4.35 (2.29 to 8.28)	<0.001	4.52 (2.38 to 8.56)	<0.001
PCr>30 mg/mmol	1.96 (1.20 to 3.12)	0.01	1.77 (1.10 to 2.84)	0.01
Phosphate >4.65 mg/dl	1.70 (0.98 to 2.96)	0.06	1.70 (1.0 to 2.95)	0.05
cFLC (mg/L)				
Quartile 1/2: <68.9	Referent		Referent	
Quartile 3: 68.9–100.7	1.60 (0.92 to 2.76)	0.09	1.72 (1.0 to 2.97)	0.05
Quartile 4: >100.7	3.37 (2.00 to 5.70)	<0.001	3.73 (2.10 to 6.30)	<0.001

When protein-to-creatine ratio (PCr) not available, estimated from 24-h urinary protein on the basis of the assumption that a PCr of 100 mg/mmol is approximately 1 g per 24-h proteinuria. cFLC, combined free light chain.

Notably, in these reports, elevated cFLC was a better indicator of risk over and above CRP. This stronger association of FLC than CRP with clinical outcomes has also been shown in other studies that have used CRP and cFLC (14,15). We have recently shown that the kinetics of CRP and cFLC levels in disease states differ, with CRP levels more closely associated with acute inflammation and FLC associated with chronic inflammation (28). In this study, ESR levels were measured and incorporated into the analyses in preference to CRP because of ESR having a similar correlation with eGFR and stage of CKD (29) and a standard assay being used over the study period. There were associations between ESR and adverse outcomes in the univariate analysis but no associations in the multivariate analysis. FLC may be a more specific assessment of inflammation than ESR, because it is a direct function of adaptive immunity through B-cell lineage production rather than a general marker of inflammation. In a study derived from the Olmsted County cohort, Dispenzieri *et al.* (13) commented on the use of cFLC as a biomarker across a range of mortality risk and made recommendations for additional study.

The strength of the association between cFLC and the outcome parameters considered in this study suggests its potential as a prognostic marker representative of the inflammatory limb of the immune response. There is great interest in developing risk models in kidney disease, particularly with a focus on the enhanced mortality associated with CKD (30–32). Additional studies are now required that assess whether a risk stratification model that incorporates cFLC can direct patient care toward improved patient outcomes.

In this study, there was no relationship between the proportion of deaths from cardiovascular causes and the level of cFLC, consistent with previous reports in a CKD population (16). No difference in the proportion of cancer-related deaths was observed between groups, although absolute numbers of cancers were small, and the study is likely to be underpowered to show the association with death from cancer that was found in other studies.

Whether cFLC represents molecules that are pathogenic when present in excess is uncertain. There is an increasing body of work that suggests that cFLC can activate mast

cells and other circulating cells in a range of autoimmune and inflammatory diseases (33,34). However, the nature of the interactions between polyclonal LCs and specific receptors is uncertain; this area is under investigation.

In summary, this study adds evidence from an established secondary care CKD cohort to show that cFLC is a strong independent outcome marker in CKD. This is consistent with both the mode of production of FLC and the clearance of the molecules. Additional studies are now needed to assess if this biomarker can be used to inform risk stratification and better direct the care of patients with CKD.

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Disclosures

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