

Quantitative Assessment of Serum and Urinary Polyclonal Free Light Chains in Patients with Chronic Kidney Disease

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Background and objectives: Monoclonal free light chains (FLC) frequently cause kidney disease in patients with plasma cell dyscrasias. Polyclonal FLC, however, have not been assessed in patients with chronic kidney disease (CKD) yet could potentially play an important pathologic role. This study describes for the first time polyclonal FLC in patients with CKD.

Design, setting, participants, & measurements: A sensitive, quantitative immunoassay was used to analyze serum and urinary polyclonal FLC in 688 patients with CKD of various causes.

Results: Serum κ and λ FLC concentrations increased progressively with CKD stage (both $P < 0.001$) and strongly correlated with markers of renal function, including cystatin-C (κ : $R = 0.8$, $P < 0.01$; and λ : $R = 0.79$, $P < 0.01$). Urinary FLC concentrations varied significantly between disease groups (κ : $P < 0.001$; λ : $P < 0.005$) and also rose significantly with increasing CKD stage (both FLC $P < 0.0001$). Urinary FLC concentrations were positively correlated with their corresponding serum concentration (κ : $R = 0.63$; λ : $R = 0.65$; both $P < 0.001$) and urinary albumin creatinine ratio (κ : $R = 0.58$; λ : $R = 0.65$; both $P < 0.001$). The proportion of patients with abnormally high urinary FLC concentrations rose with both the CKD stage and the severity of albuminuria.

Conclusions: This study demonstrates significant abnormalities of serum and urinary polyclonal FLC in patients with CKD. These data provide the basis for studies that assess the contribution of polyclonal FLC to progressive renal injury and systemic inflammation in patients with kidney disease.

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Monoclonal free light chains (FLC) frequently cause kidney disease in patients with plasma cell dyscrasias (1–4). Until recently, FLC have predominantly been measured in the urine as Bence-Jones proteins (5). This reflected technical limitations precluding sensitive measurement of FLC in the serum. The recent introduction of highly sensitive, quantitative immunoassays have enabled the measurement of serum FLC to become a routine part of the screening and monitoring of plasma cell dyscrasias (6–8).

Despite the widely known nephrotoxicity of monoclonal FLC, there has been no detailed assessment of polyclonal FLC in patients with chronic kidney disease (CKD). During synthesis of intact Ig by plasma cells, FLC are produced in excess of heavy chains (9). These excess polyclonal FLC are released into the serum, from where they are rapidly removed by the kidneys with a half-life of 2 to 6 h (10). In patients with CKD, however, as the GFR reduces, the renal clearance of polyclonal FLC decreases and serum concentrations rise. It is possible that these rising concentrations of polyclonal FLC may result in the

same progressive renal pathologies that are frequently associated with monoclonal FLC. In addition, the known systemic toxicity of monoclonal FLC indicates that elevated polyclonal FLC may have further biologic relevance in CKD, but this hypothesis has not been previously explored, and accurate quantification of FLC in patients with CKD is lacking. The aim of this study was to assess quantitatively polyclonal FLC in patients with CKD, describing for the first time the relationship of serum and urinary FLC concentrations to renal function and albuminuria.

Materials and Methods

The study was approved by the Solihull and South Birmingham Research Ethics Committees and the Research and Development Department of University Hospital Birmingham NHS Foundation Trust. All patients gave informed, written consent.

Study Design and Participants

Serum samples were initially assessed in 756 patients with stages 1 through 5 CKD (predialysis) and 46 long-term dialysis patients. For the predialysis CKD group, archived serum from 370 patients who were enrolled in the Chronic Renal Impairment in Birmingham (CRIB) cohort (11) were retrospectively analyzed, and 386 patients were recruited prospectively from clinics at our center between January and September 2006. For the dialysis group, 24 peritoneal dialysis (PD) patients and 22 hemodialysis (HD) patients were studied, with samples taken im-

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mediately predialysis from HD patients. All serum samples were initially screened for monoclonal proteins by serum protein electrophoresis (SPE) and quantification of κ and λ FLC with calculation of the FLC ratio. When a monoclonal band was suggested on SPE or the FLC ratio was outside the normal range, the presence of a monoclonal protein was confirmed by immunofixation electrophoresis and these patients were excluded from further evaluation. Urinary FLC were assessed in the prospective population of patients with CKD ($n = 386$).

The CKD serum samples were compared with a published control population ($n = 282$) (12). Urine samples, from healthy volunteers without albuminuria (assessed by albumin/creatinine ratio [ACR]) were used to create a control population for urinary FLC ($n = 104$).

Sample Handling and Laboratory Assessment

The CRIB samples were stored at -80°C until analysis; previous work has demonstrated the stability of FLC over many years (13). Serum creatinine had previously been analyzed at the time of collection. Samples from the prospective cohort were immediately analyzed for serum and urinary creatinine and urinary albumin. Aliquots were stored at 4°C until analysis for serum monoclonal proteins, and a separate aliquot was frozen until analysis for cystatin C.

Serum and urine κ and λ FLC concentrations were measured by nephelometry, on a Dade-Behring BN II Analyser, using particle-enhanced, high-specificity, homogeneous immunoassays (Freelite; The Binding Site, Birmingham, UK) (6). The assay sensitivity was <1 mg/L (12). The normal serum reference ranges used have previously been reported and were as follows: κ 3.3 to 19.4 mg/L; λ 5.7 to 26.3 mg/L; ratio 0.26 to 1.65 (12). Urine samples were analyzed undiluted, and FLC results were corrected for urinary creatinine to give an FLC/creatinine ratio. SPE and immunofixation were undertaken using the Sebia Hydragel 15/30 Protein kit and the Hydragel 4 Immunofixation PE kit on the Hydrasys system (Sebia, Lisses, France).

Serum creatinine was measured using the Roche Modular Analyser (Roche Diagnostics, Newhaven, UK); the normal range was 50 to $125\mu\text{mol/L}$. Cystatin C was measured using a nephelometric immunoassay (The Binding Site); the normal range was 0.55 to 1.44 mg/L. Estimated GFR (eGFR) were calculated using the creatinine-based Cockcroft-Gault equation (14).

Serum Polyclonal FLC in Patients with CKD and End-Stage Renal Failure

Median serum FLC concentrations and $\kappa:\lambda$ ratios were calculated for the control and study populations. In the study population, median κ and λ concentrations and $\kappa:\lambda$ ratios were calculated for each CKD stage. Correlations of κ and λ concentrations with markers of renal function were determined (serum creatinine, eGFR, and cystatin C).

Urinary Polyclonal FLC in Control Population and Patients with CKD

Reference ranges for urinary κ /creatinine and λ /creatinine ratios (KCR and LCR, respectively) were defined from the control population. Median urinary KCR and LCR were calculated for the study population as a whole and each CKD stage. The proportion of patients with CKD and abnormal urinary FLC was assessed for each CKD stage and the degree of albuminuria (urinary ACR groups: <2 , 2 to 10, 10 to 20, and >20). Correlations of urinary FLC/creatinine ratios with serum concentrations of FLC and the urinary ACR were determined.

Statistical Analysis

SPSS 14.0 (SPSS, Chicago, IL) was used to assess statistical significance. All data were nonparametric and presented as medians with

ranges; compared using the Mann-Whitney U test, $P < 0.05$ was considered significant. The Kruskal-Wallis test was used to determine the significance of variations in results between CKD stages and diagnostic groups. Spearman correlations were undertaken to give correlation coefficients; significance was assumed at $P < 0.01$.

Results

Study Populations

After exclusion for monoclonal proteins ($n = 68$; 9%), polyclonal FLC were analyzed in 334 samples from the CRIB cohort and 354 samples from the prospective cohort (Figure 1). Urine analysis was undertaken on samples from 338 patients in the prospective cohort. The study population demographics, renal diagnoses, and biochemical parameters are presented in Table 1.

Serum Polyclonal FLC and Renal Function

The serum polyclonal FLC concentrations for the study population are presented in Table 1. Both κ and λ FLC concentrations were significantly greater in patients with CKD than in the control population: κ 43.8 (3 to 251) versus 7.3 (3.3 to 19.4) and λ 38 (1 to 251) versus 12.4 (5.7 to 26.3; both $P < 0.001$). Furthermore, concentrations of serum FLC rose progressively through each CKD stage (both $P < 0.0001$; Figure 2A). Serum κ and λ concentrations correlated significantly with all markers of renal function studied (Table 2). The strongest correlations were with cystatin C (κ : $R = 0.80$, $P < 0.01$; and λ : $R = 0.79$, $P < 0.01$).

Patients on both HD and PD had significantly raised serum FLC concentrations compared with the control population (Figure 2B). Furthermore, in both of these groups, concentrations were greater than predialysis patients with stage 5 CKD (both $P < 0.01$): HD: κ 130 mg/L (53 to 408), λ 109 mg/L (51 to 470); PD: κ 119 mg/L (30 to 266), λ 121 mg/L (36 to 263); CKD stage 5: κ 80.3 mg/L (23 to 223), λ 63.7 mg/L (19 to 210).

The median $\kappa:\lambda$ ratio for the whole CKD population was significantly greater than that of the control population: 1.12

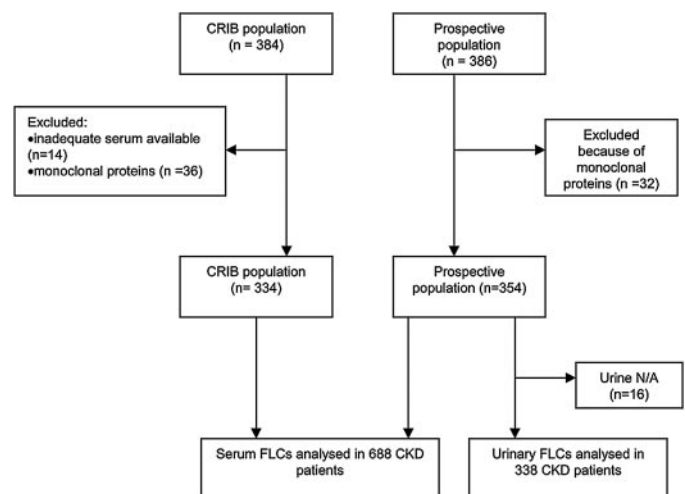


Figure 1. Schematic of chronic kidney disease (CKD) populations assessed. N/A, not available.

Table 1. Serum and urinary FLC concentrations by renal diagnosis^a

Renal Diagnoses	N (Urinary)	Age	Serum Creatinine (mmol/L)	eGFR (ml/min per 1.73 m ²)	Serum κ (mg/L)	Serum λ (mg/L)	Urinary KCR ^b	Urinary LCR ^b
Whole CKD population	688 (338)	64.00 (17.00 to 96.00)	225.00 (56.00 to 875.00)	29.40 (6.00 to 128.00)	43.80 (3.00 to 251.00)	38.00 (1.00 to 251.00)	8.60 (0.09 to 198)	1.50 (0.05 to 77.40)
Nondiabetic glomerular nephropathies	158 (71)	59.00 (18.00 to 86.00)	217.00 (56.00 to 875.00)	34.90 (6.00 to 128.00)	39.70 (6.70 to 223.00)	37.80 (1.00 to 146.00)	5.60 (0.09 to 74.00)	1.10 (0.06 to 26.20)
Diabetic nephropathy	73 (41)	67.00 (33.00 to 84.00)	287.00 (66.00 to 833.00)	25.00 (7.00 to 85.00)	64.00 (12.00 to 246.00) ^c	55.00 (15.00 to 251.00) ^c	20.90 (0.17 to 159.00) ^c	4.60 (0.05 to 77.40) ^c
Ischemic nephropathy/renovascular disease	112 (62)	74.00 (29.00 to 86.00)	213.00 (88.00 to 792.00)	28.00 (7.00 to 70.00)	39.50 (13.00 to 176.00)	35.90 (1.50 to 156.00)	9.30 (0.17 to 77.00)	1.78 (0.05 to 77.40)
Chronic pyelonephritis/renal calculi	62 (31)	61.00 (17.00 to 86.00)	241.00 (69.00 to 746.00)	26.80 (6.00 to 111.00)	41.80 (8.00 to 178.00)	42.70 (9.30 to 153.00)	9.90 (0.11 to 91.00)	1.70 (0.06 to 24.50)
Hereditary nephropathies	48 (19)	51.50 (21.00 to 80.00)	278.00 (89.00 to 831.00)	28.00 (6.00 to 79.00)	47.00 (8.00 to 111.00)	36.00 (10.00 to 87.00)	6.40 (0.16 to 36.80)	0.80 (0.02 to 8.90)
Other diagnoses ^d	235 (114)	64.00 (19.00 to 96.00)	203.00 (66.00 to 820.00)	31.30 (6.00 to 116.00)	31.00 (3.00 to 251.00)	35.40 (1.00 to 210.00)	7.40 (0.25 to 198.00)	1.34 (0.06 to 50.20)

^aData are medians (range). CKD, chronic kidney disease; FLC, free light chain; KCR, κ/creatinine ratio; LCR, λ/creatinine ratio.

^bKCR and LCR varied significantly between diagnostic groups.

^cPatients with diabetes had significantly greater serum and urinary FLC concentrations compared with other disease groups, all $P < 0.05$ (across CKD stages).

^dOther diagnoses include unspecified proteinuria and hematuria, single kidney, bilateral small kidneys, interstitial nephritis and renal malignancies.

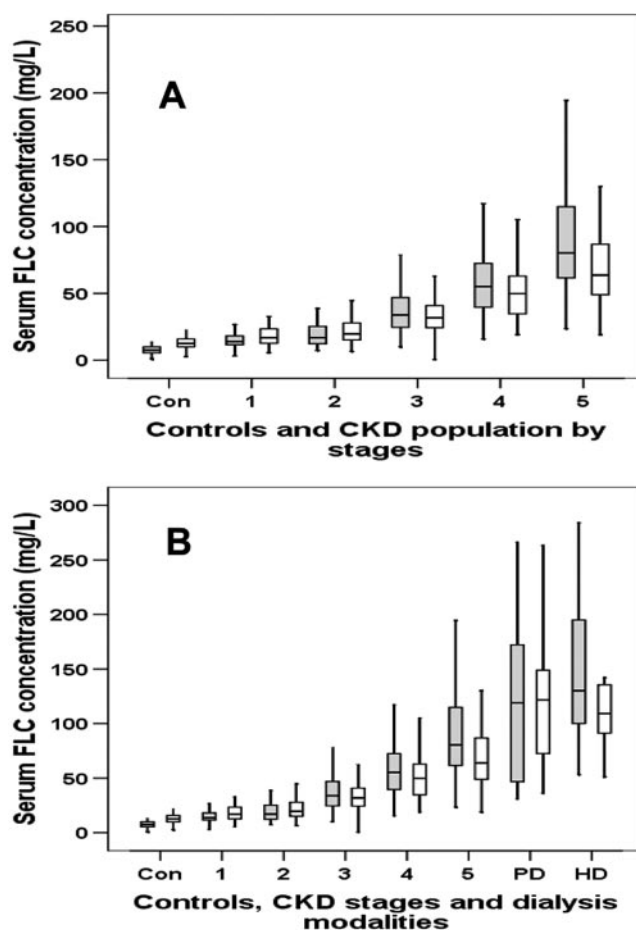


Figure 2. Serum free light chain (FLC) concentrations in patients with CKD. (A) Both κ (■) and λ (□) FLC increased progressively with each CKD stage (both $P < 0.0001$). (B) Patients who required renal replacement therapy had serum FLC concentrations significantly higher than patients with stage 5 CKD (both $P < 0.001$). There were no significant differences in serum concentrations of FLC between the peritoneal dialysis (PD) and hemodialysis (HD) populations: κ, $P < 0.09$; λ, $P < 0.5$. Data presented as box plots with whiskers; solid lines denote median values.

Table 2. Spearman correlation coefficients for CKD polyclonal serum FLC with serum creatinine, eGFR, and cystatin C^a

Parameter	Serum κ Concentration	Serum λ Concentration	Ratio
Creatinine	0.78	0.73	0.36
Cystatin C	0.80	0.79	0.28
eGFR	-0.72	-0.66	-0.34

^aAll correlations significant at the 0.01 level (two-tailed). eGFR, estimated GFR.

(0.37 to 3.1) versus 0.58 (0.26 to 1.65) respectively. In addition, the ratio demonstrated a stepwise increase through the CKD stages ($P < 0.01$; Figure 3A) and remained elevated in patients who received both PD and HD (Figure 3B).

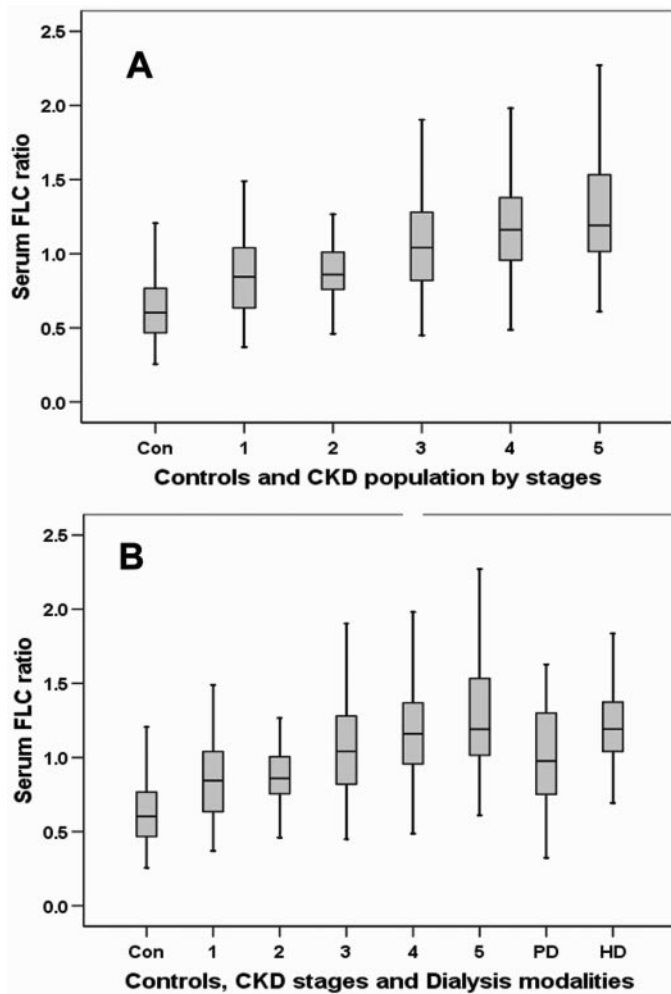


Figure 3. Serum FLC ratio in patients with CKD. (A) The FLC ratio increased progressively through stages 1 through 5 CKD: 0.85 (0.3 to 1.8), 0.86 (0.5 to 1.92), 1.04 (0.45 to 2.3), 1.16 (0.49 to 3), and 1.19 (0.6 to 2.9), respectively ($P < 0.01$). (B) The FLC ratio was also significantly raised in patients who received renal replacement therapy compared with that of the control population: 0.6 (0.26 to 1.65); PD 0.97 (0.32 to 2.35) and HD 1.2 (0.69 to 2.57). Data presented as box plots with whiskers; solid lines denote median values.

Urinary Polyclonal FLC

Urinary FLC were assessed in a control population with normal urinary ACR. The 100% ranges for KCR and LCR in this population were 0.006 to 8.7 (median 0.68) and 0.003 to 0.649 (median 0.06), respectively. The 95% reference ranges were 0.01 to 4.0 and 0.005 to 0.45, respectively.

Urinary KCR and LCR rose progressively through CKD stages (Figure 4) and had a negative correlation with renal function (eGFR: $R = -0.515$, $P < 0.0001$; and $R = -0.522$, $P < 0.0001$, respectively). The proportion of patients with abnormal FLC/creatinine ratios was higher with both increasing CKD stage and albuminuria (Figure 5). There were significant differences in KCR and LCR between various diagnostic groups ($P < 0.005$ and < 0.0001 , respectively; Table 1); however, multivariate analysis demonstrated that this difference was not independent

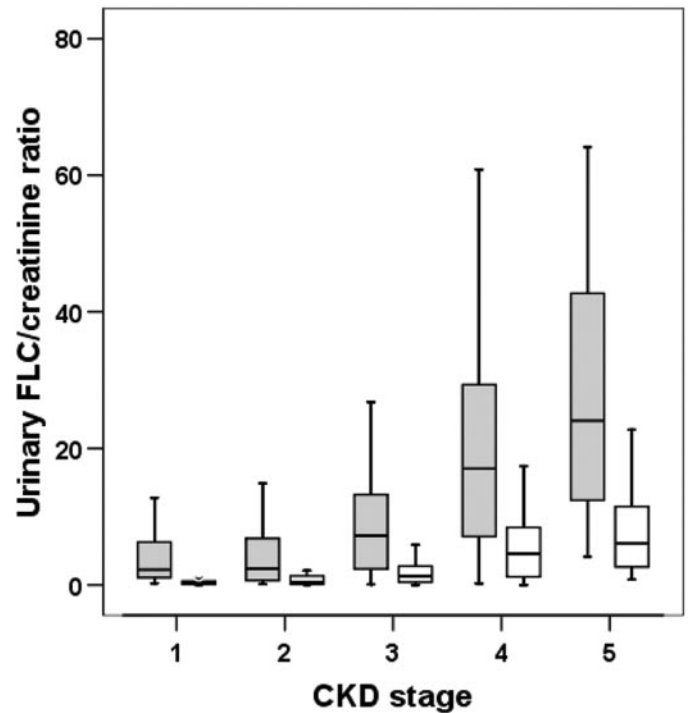


Figure 4. Relationship of urinary FLC/creatinine ratios with CKD stages. Both κ /creatinine ratio (KCR; shaded box plots) and λ /creatinine ratio (LCR; white box plots) increased progressively through each CKD stage; 1, 2.3 (0.3 to 21) and 0.25 (0.03 to 8.7); 2, 2.3 (0.11 to 31) and 0.39 (0.02 to 10); 3, 7.2 (0.1 to 126) and 1.23 (0.01 to 70); 4, 17 (0.22 to 123) and 4.55 (0 to 36.8); and 5, 24 (4.2 to 121) and 6.1 (0.78 to 77), respectively (both $P < 0.0001$). Data presented as box plots with whiskers; solid lines denote median values.

of eGFR and urinary ACR ($P < 0.2$). Urinary KCR and LCR correlated with each other ($R = 0.96$, $P < 0.001$) and their corresponding serum FLC concentrations ($R = 0.55$ and 0.57 respectively, $P < 0.001$ [controlling for ACR]). The correlations of urinary KCR and LCR with urinary ACR varied between the disease groups (Table 3).

Discussion

Historically, FLC have only been quantifiable in patients with plasma cell dyscrasias and then only in the urine as Bence-Jones proteins (5). This was because of the lack of sensitive and specific assays for measuring FLC in the serum. Since 2001, immunoassays that can measure FLC at low concentrations have been available (6), and these are now routinely used in the diagnosis and follow-up of patients with plasma cell dyscrasias (7,8).

Nowrousian *et al.* (15) used these assays to measure polyclonal FLC in patients with multiple myeloma and found that serum and urinary FLC concentrations were increased in those with renal impairment. Using these assays, we have characterized polyclonal FLC, for the first time, in a population of patients with CKD of mixed causes, excluding those with plasma cell dyscrasias. We describe the relationship of serum

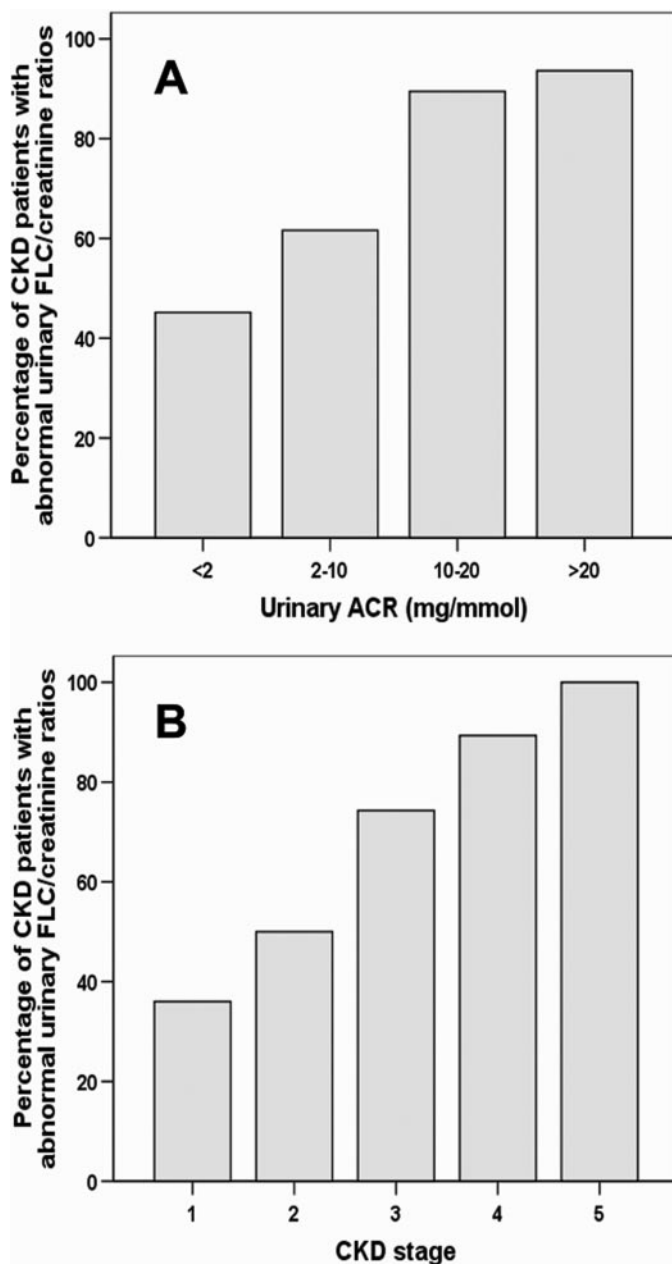


Figure 5. Percentage of patients with abnormal urinary FLC/creatinine ratios according to urinary albumin/creatinine ratio (ACR; A) and CKD stages (B). The percentage of patients with abnormal urinary FLC/creatinine ratios increased with increasing ACR (<2 = 45%; 2 to 10 = 61%; 10 to 20 = 89%; >20 = 93%; $P < 0.001$) and through the CKD stages (1 through 5: 36, 50, 74, 89, and 100%, respectively; $P < 0.001$).

and urinary polyclonal FLC with kidney function and disease states.

In patients with CKD, serum κ and λ FLC showed strong positive correlations with serum creatinine and cystatin C and negative correlations with eGFR. The correlations of both serum FLC were stronger with cystatin C than either creatinine or eGFR. Possible explanations for this are that cystatin C has a molecular weight closer to FLC than creatinine and the preci-

sion of creatinine-based estimation of GFR in patients with moderate renal impairment, which is known to be relatively low (16). There was a stepwise increase in the median serum FLC concentrations with each CKD stage. Moreover, patients on dialysis had significantly higher FLC concentrations than predialysis patients with stage 5 CKD, which emphasizes that current dialysis modalities do not provide adequate clearance of these middle molecular weight proteins (17).

Serum FLC concentrations varied between the different renal pathologies, most notably patients with diabetes had concentrations higher than patients with other diagnoses. A possible explanation for this finding is chronic inflammation, which is well described in patients with diabetes (18,19), because chronic inflammation itself is associated with increased polyclonal FLC (20,21).

κ FLC are produced at approximately twice the rate of λ FLC (22); however, λ FLC more frequently form dimers, which doubles their molecular weight and slows their renal clearance. Consequently, in normal serum, κ FLC concentrations are lower than λ FLC concentrations, resulting in a median serum ratio of 0.58 (normal range 0.26 to 1.65) (12). As the renal clearance of serum FLC is reduced, the reticuloendothelial system becomes an increasingly important route for FLC clearance (9). Because reticuloendothelial clearance is not influenced by the molecular weight of the FLC, the serum half-lives of κ and λ become closer as renal function worsens. Consistent with this, the median FLC ratio increased with CKD stage. Hence, the serum ratio moves toward the higher κ production rate as the GFR decreases. This increased the reference range for the FLC ratio in patients with kidney failure from 0.26 to 1.65 to 0.37 to 3.1. Using this new reference range increased the specificity of the FLC assays for detecting monoclonal FLC production in patients with severe renal failure (23).

Urinary polyclonal FLC concentrations correlated strongly with serum FLC concentrations as might be anticipated because FLC are filtered relatively freely at the glomerulus. Previous population studies, however, reported poor correlations between urinary and serum concentrations of monoclonal FLC (24,25). This is likely to reflect divergent tubular handling of polyclonal and monoclonal FLC. Proximal tubular reabsorption of proteins is mediated by megalin-cubulin receptor endocytosis, which has a limited capacity and differential affinity for individual proteins (26,27). Accordingly, the concentration of a monoclonal FLC in the urine will depend on the serum FLC concentration, the degree of albuminuria (reflective of glomerular damage from the monoclonal protein), the degree of tubular damage (reflective of monoclonal tubular disease), and the affinity of tubular receptors for that individual FLC. In contrast, the urinary concentration of polyclonal FLC in a patient with CKD will depend on the serum FLC concentrations, the degree of albuminuria, the degree of tubular damage (of any cause), and the affinity of the tubules for all FLC (a median value).

The fractional excretion of both κ and λ FLC varied significantly between the various renal disease groups. In particular, patients with diabetic nephropathy and those with ischemic nephropathy had higher fractional excretions than those with nondiabetic glomerular diseases. This may relate to differences

Table 3. Urinary FLC according to disease group^a

Parameter	Serum κ	Serum λ	Urinary ACR	Fractional Excretion ^b
Nondiabetic glomerular nephropathies				
KCR	0.53 ^c	–	0.58 ^c	32.30
LCR	–	0.52 ^c	0.63 ^c	5.40
Diabetic nephropathy				
	0.47 ^c	–	0.22	94.70
	–	0.48 ^c	0.18	30.90
Ischemic nephropathy/renovascular disease				
	0.47 ^c	–	0.09	42.40
	–	0.6 ^c	0.20	9.20

^aCorrelations of urinary FLC/creatinine ratios with corresponding serum FLC concentrations (controlling for albumin/creatinine ratio [ACR]) and urinary ACR (controlling for serum FLC concentrations) according to disease groups.

^bFractional excretion of both urinary FLC varied significantly by disease group.

^cCoefficients significant at the level of 0.01 (two-tailed).

in proximal tubular function between these disease groups. In addition, patients with nondiabetic glomerular diseases did not have very high urinary FLC concentrations, but they did demonstrate a significant correlation between urinary ACR and FLC that was not evident in other disease groups. This may reflect the limited capacity for tubular reabsorption of middle molecules and would be compatible with previous work that demonstrated that the tubules reabsorb albumin in preference to FLC.

This study has demonstrated that as renal function decreases, the serum and urinary concentrations of polyclonal FLC increase up to five-fold in predialysis CKD. Thus, as nephrons are lost, the remaining nephrons are exposed to increasing concentrations of polyclonal FLC. Recent studies demonstrated that monoclonal FLC promote proximal tubular injury by increasing intracellular oxidative stress with the subsequent production of monocyte chemoattractant protein 1 (28). Further work has shown that FLC induce epithelial-mesangial transition (29) and activate mesangial cells to proliferate and increase production of matrix proteins (30). The hypothesis that polyclonal FLC present in increasing concentrations in patients with CKD may be capable of inducing similar injuries is now being investigated. Furthermore, FLC may have a role as systemic uremic toxins. In a series of studies, Cohen *et al.* (31–33) demonstrated that polyclonal FLC, isolated from patients with end-stage renal failure, reduced neutrophil function. This may predispose patients to increased bacterial infections and promote the proinflammatory state characteristic of patients with advanced kidney disease.

Conclusions

This study describes polyclonal FLC in patients with CKD. Serum concentrations of polyclonal FLC correlated closely with all markers of renal function studied. Urinary FLC concentrations varied according to the type of renal disease, CKD stage, and albuminuria. These data provide the basis for studies to assess the contribution of polyclonal FLC to progressive renal injury and systemic inflammation in patients with kidney disease.

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Disclosures

S.H. is an employee and G.P.M. and A.R.B. are directors of The Binding Site Ltd. (Birmingham, UK).

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