Serum Free Light Chain Analysis and Urine Immunofixation Electrophoresis in Patients with Multiple Myeloma

Mohammad R. Nowrousian,[1] Dieter Brandhorst,[1] Christiane Sammet,[1] Michaela Kellert,[1] Rainer Daniels,[1] Philipp Schuett,[1] Miriam Poser,[1] Siemke Mueller,[1] Peter Ebeling,[1] Anja Welt,[1] Arthur R. Bradwell,[2,3] Ulrike Buttkereit,[1] Bertram Opalka,[1] Michael Flasshove,[1] Thomas Moritz,[1] and Siegfried Seeber[1]

Abstract

Purpose: Retrospective studies have shown that immunoassays measuring free light chains (FLC) in serum are useful for diagnosis and monitoring of multiple myeloma. This study prospectively evaluates the use of FLC assays and, for the first time, investigates the relationship between serum FLC concentrations and the presence and detectability of Bence Jones (BJ) proteins in the urine.

Patients and Methods: Three hundred seventy-eight paired samples of serum and urine were tested from 82 patients during the course of their disease. The sensitivities of serum FLC analysis and urine immunofixation electrophoresis (IFE) in detecting monoclonal FLC were compared. Serum FLC concentrations required for producing BJ proteins detected by IFE were determined.

Results: Abnormal FLC were present in 54% of serum samples compared with 25% by urine tests. In abnormal serum samples for κ or λ, the sensitivity of IFE to detect the respective BJ proteins in urine were 51% and 35% and the median serum FLC concentrations required to produce detectable BJ proteins were 113 and 278 mg/L. Renal excretions of monoclonal FLC increased with serum concentrations, but excretions significantly decreased at high serum concentrations combined with renal dysfunction.

Conclusion: Serum FLC assays are significantly more sensitive for detecting monoclonal FLC than urine IFE analysis. They also have the advantage of FLC quantification and are more reliable for monitoring disease course and response to treatment.

Assays that detect monoclonal immunoglobulin free light chain (FLC) are important in the diagnosis and monitoring of light-chain-only myeloma [Bence Jones (BJ) myeloma; refs. 1, 2], nonsecretory myeloma (3), and AL amyloidosis (4, 5). They may also be useful in patients producing intact immunoglobulins. Approximately 50% of such patients produce monoclonal light chains in the urine and it was recently shown that >95% of these patients have monoclonal serum FLC at clinical presentation (6). This is a consequence of the capacity of the kidneys to catabolize up to 30 g/d of FLC, thereby restricting their excretion to detectable levels in the urine (7).

Mead et al. (6) suggested that the potential advantages of measuring FLC in the serum are 3-fold. First is the short serum half-life of 2 to 6 hours compared with ~21 days for IgG. Patients monitored with IgG show a slower response of myeloma protein to therapy than patients monitored with FLC. Second, there is no correlation between the production rates of the different molecules so that some patients produce quite high levels of FLC but relatively small amounts of intact immunoglobulins. In these patients, serum FLC may be more easily measured and more accurate for patient monitoring than weak IgG bands on electrophoresis gels. Third, some patients are in complete remission by conventional electrophoresis tests but have abnormal serum FLC (8). Raised concentrations could be an indication for further therapy in the absence of other abnormalities.

The purpose of this prospective study was (*a*) to compare the sensitivities of serum FLC assays and urine IFE analysis in detecting and monitoring monoclonal FLC in patients with multiple myeloma and (*b*) to investigate the relationship between serum FLC concentrations and the presence and detectability of BJ proteins in the urine.

Patients and Methods

Patients. The patients studied were those attending the University Cancer Center of Essen for routine management of multiple myeloma. Eighty-two sequential patients with all types of multiple myeloma were investigated. These comprised 62 patients with intact monoclonal immunoglobulins, 17 with light-chain monoclonal proteins only (BJ myeloma) and three with nonsecretory myeloma. The patients were monitored at presentation and during the course of their disease

Authors' Affiliations: [1]Department of Internal Medicine (Cancer Research), West German Cancer Center, University Hospital of Essen Medical School, Essen, Germany; [2]Division of Immunology and Infection, University of Birmingham; and [3]The Binding Site Ltd., Birmingham, United Kingdom

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Requests for reprints: Mohammad R. Nowrousian, Department of Internal Medicine (Cancer Research), West German Cancer Center, University Hospital of Essen Medical School, Hufelandstrasse 55, 45122 Essen, Germany. Phone: 49-201-723-3127; Fax: 49-201-723-5984; E-mail: nowrousian@uni-essen.de.

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with repeated measurements of serum and 24-hour urine monoclonal proteins and serum and 24-hour urine FLC.

Measurements of serum and urine monoclonal proteins and FLC were done on samples taken for the routine testing of patients and all patients had provided informed consent for the use of their samples for this study.

Sample selection and assays. All measurements were made on fresh serum and 24-hour urine samples taken at the time of patient attendance at the clinic. Tests were done for serum and urine protein electrophoresis and IFE. FLC tests were done on all serum and 24-hour urine samples. Results in serum were compared with FLC reference ranges from 282 normal individuals ages 20 to 90 years (95% range 3.3-19.4 mg/L for κ and 5.7-26.3 mg/L for λ; diagnostic range for κ/λ ratios 0.26-1.65). Using these reference ranges, the immunoassay used had a sensitivity of 97% and a specificity of 100% (9). FLC κ/λ ratios in urine were compared with reference values of 66 healthy persons ages 18 to 54 years (95% confidence interval, 0.46-4.0; ref. 10).

Assay methods and statistical methods. The serum and urine FLC concentrations were measured nephelometrically using a recently introduced automated immunoassay on the Beckman/Coulter (Krefeld, Germany) IMMAGE II (10). Total serum and urine proteins were measured photometrically (Hitachi-Roche 917-Analyser, Mannheim, Gemany) and the concentrations of albumin and other protein fractions were calculated by their proportions in agarose gel electrophoresis using scanning densitometry (the Paragon electrophoresis systems; Beckman/Coulter). All serum and urine gels were also checked visually for the presence of clonal bands. Urine samples were 10-fold concentrated before they were used for electrophoretic tests. BJ proteins were identified by interpretation of IFE (the Paragon electrophoresis system, Beckman/Coulter). Serum creatinine concentrations were determined using an enzymatic color test (Hitachi-Roche 904/911: CAN 063) with reference ranges of 0.67 to 1.17 mg/dL (59-104 μmol/L) for males and 0.51 to 0.95 mg/dL (45-84 μmol/L) for females (11). Creatinine values above the upper limits of the reference ranges were considered abnormal.

Results were presented as scatter diagrams showing serum FLC κ/λ ratios related to reference values for the absence (0.26-1.65) or presence of κ (>1.65) or λ (<0.26) monoclonal proteins. Correlations between serum and urine FLC concentrations were also shown and comparisons were made for 24-hour urinary excretions and 24-hour clearances, both for κ and λ, between various groups of samples. Twenty-four-hour clearances were defined as the ratios of 24-hour urinary excretions to the respective serum concentrations for κ or λ using the following formula: 24-hour clearance (L/24 hours) = urine FLC (mg/24 hours) / serum FLC (mg/L). The FLC clearance represents the volume of plasma that contained the measured FLC excreted in the urine per unit time.

Comparisons of the different measurements were made using a two-tailed Mann-Whitney *U* test and presented as table or graphically as dot diagrams, including medians and the full ranges of the data. Differences between proportions were tested using the χ^2 test. Associations between various variables were evaluated using bivariate correlation procedures. Given the complexity of the samples, all analyses were considered valid if the results were comparable using aggregated data, as well as unweighted and weighted data for overrepresented observations.

Results

Sensitivity of serum free light chain assays and urine immunofixation electrophoresis tests for identifying production of Bence Jones proteins. Three hundred seventy-eight paired samples of serum and 24-hour urine were investigated from 82 patients during the course of their disease. The serum concentrations of the FLC and the distribution of the κ/λ ratios and the associations of these ratios with positive or negative urine tests and vice versa are given in Fig. 1. In the 378 serum samples, 173 (46%) were normal (sFLC−) and 205 (54%) were abnormal (sFLC+) for FLC κ/λ ratios, 98 of κ, and 107 of λ type (Fig. 1A). By comparison, in the corresponding urine samples, 282 (75%) were normal (uBJP−) by IFE for BJ proteins and 96 (25%) were abnormal (uBJP+). Of the 205 sFLC+ samples, 118 (58%) had no urine BJ proteins (sFLC+uBJP−) compared with only 9 (5%) of 173 urine samples that contained BJ proteins but were normal for serum FLC κ/λ ratios (sFLC−uBJP+; $P < 0.0001$; Fig. 1B). The nine discordant samples were eight of κ and one of λ type originating from four and one patients, respectively.

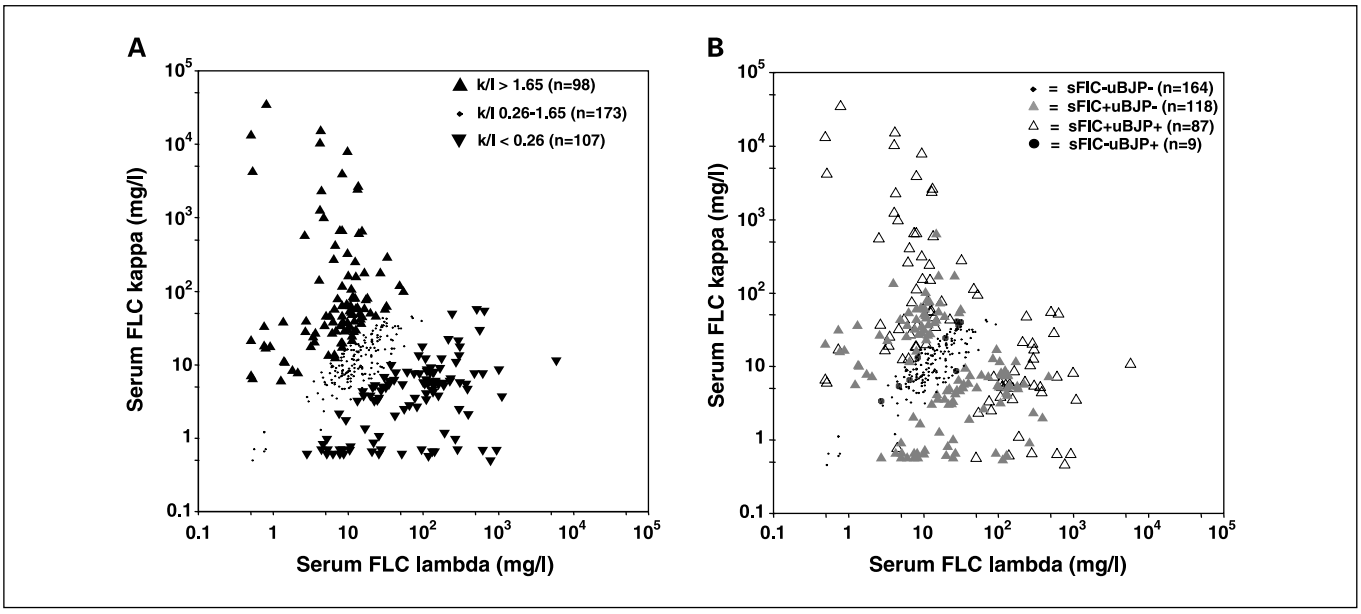

Fig. 1. Serum FLC concentrations and the distribution of κ/λ ratios in the 378 paired serum and urine samples evaluated. *A,* serum FLC ratios related to reference values for the absence (0.26-1.65) or presence of κ (>1.65) or λ (<0.26) monoclonal proteins. *B,* serum FLC ratios related to sFLC+ associated with positive (uBJP+) or negative (uBJP−) urine IFE analysis and negative serum FLC ratios (sFLC−) associated with uBJP+ or uBJP−. Of the 378 serum samples, 205 (54%) had abnormal FLC κ/λ ratios (sFLC+), 98 for κ, and 107 for λ (*A*). Of the 205 sFLC+ samples, 58% were uBJP− and 42% uBJP+. Of the 173 sFLC− samples, only 5% were uBJP+ ($P < 0.0001$; *B*).

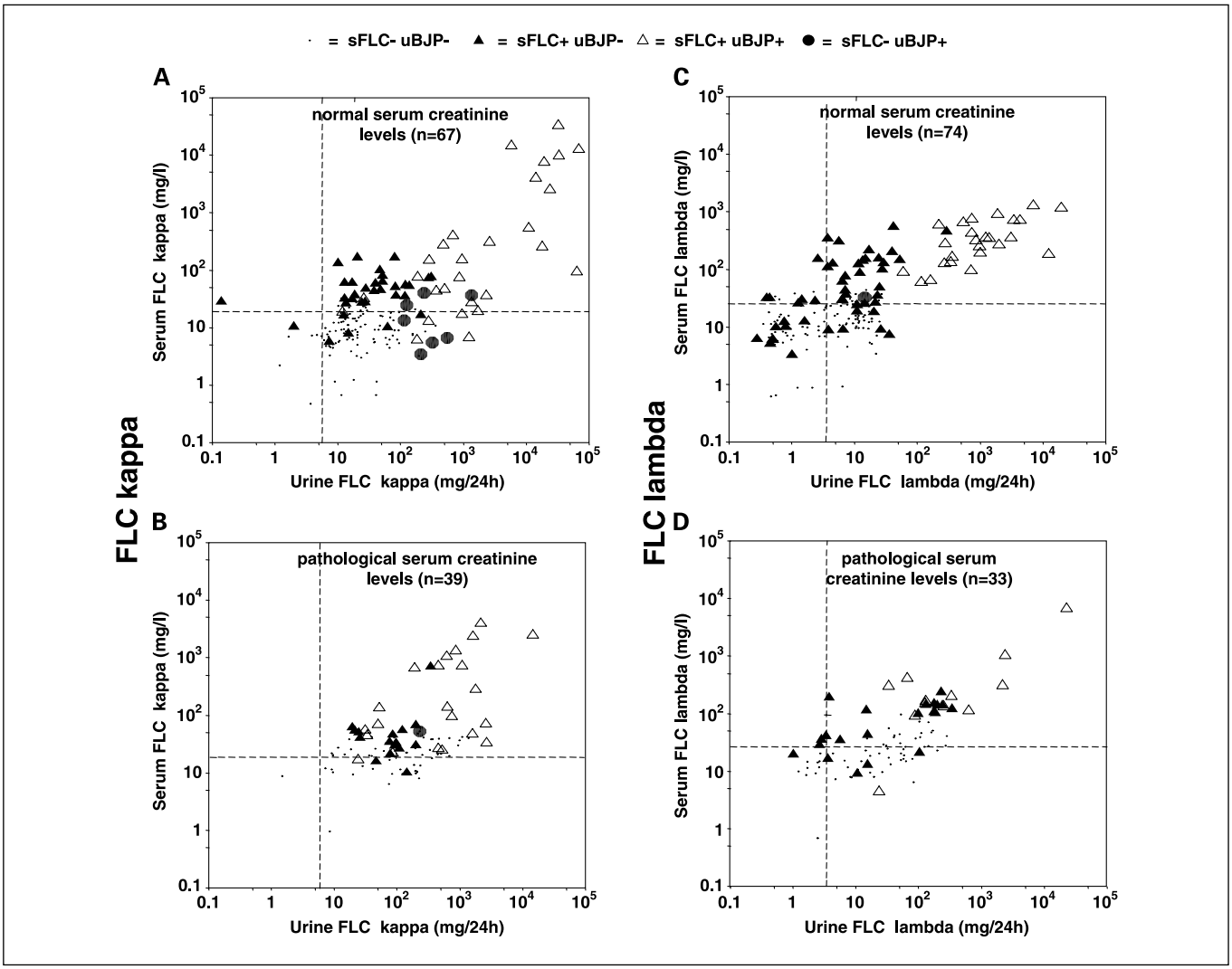

Fig. 2. Correlations between serum FLC concentrations for κ or λ and their respective urine FLC 24-hour excretions in relation to the results of serum FLC κ/λ ratios and urine IFE tests, presented separately for samples with normal (Crea−, *A* and *C*) or increased (Crea+, *B* and *D*) serum creatinine levels (>1.17 mg/dL for males and >0.95 mg/dL for females). Horizontal and vertical dotted lines, upper limits of the reference ranges for FLC in serum and urine, respectively. sFLC−, negative serum FLC κ/λ ratios; sFLC+, positive serum FLC κ/λ ratios; uBJP−, negative urine IFE tests; uBJP+, positive urine IFE tests. For FLC κ, serum concentrations and 24-hour urinary excretions significantly correlated in sFLC+uBJP+ samples both for Crea− and Crea+, whereas sFLC+uBJP− samples showed only a weak correlation in their Crea− subset. For FLC λ, the two variables significantly correlated in sFLC+uBJP+ and sFLC+uBJP− samples, both for Crea− and Crea+. For correlation coefficients and the respective *P* values in various groups of samples, see text.

Similar results were obtained when all samples from patients with BJ proteinuria at presentation were compared. Of the 280 samples, 157 (56%) were sFLC+, but 92 (34%) uBJP+. Of the 157 sFLC+ samples, 74 (48%) were sFLC+uBJP− compared with only 9 (7%) of 123 urine samples that were sFLC−uBJP+ ($P < 0.0001$). These were the same discordant samples mentioned above.

Samples from patients with initially proved BJ proteinuria and currently positive for both serum as well as urine FLC κ/λ ratios were used as positive control to evaluate the analytic sensitivity of urine IFE tests. Of the 64 κ and 47 λ samples, 42 (66%) and 35 (75%) showed positive urine IFE tests, respectively, and 22 (34%) and 12 (25%) did not.

Serum levels of free light chain required to produce overflow leading to Bence Jones proteins detectable by urine immunofixation electrophoresis analysis. In the whole group of samples, serum concentrations of FLC κ or λ significantly correlated with the respective 24-hour urinary excretions. Considerable differences, however, were observed when subsets of samples were evaluated (Fig. 2). For FLC κ, serum concentrations and 24-hour urinary excretions significantly correlated in sFLC+uBJP+ samples, both for normal (Crea−) and abnormal (Crea+) serum creatinine levels ($r = 0.675$, $P = 0.0001$ and $r = 0.51$, $P = 0.008$, respectively), whereas sFLC+uBJP− samples showed only a weak correlation ($r = 0.366$, $P = 0.02$) in their Crea− subset (Fig. 2A and B). For FLC λ, serum concentrations and 24-hour urinary excretions significantly correlated in sFLC+uBJP+ and sFLC+uBJP− samples, both in those associated with Crea− ($r = 0.631$, $P = 0.001$ and $r = 0.509$, $P = 0.0001$, respectively) or Crea+ ($r = 0.524$, $P = 0.04$ and $r = 0.599$, $P = 0.002$, respectively; Fig. 2C and D).

To further evaluate the relationship between serum concentrations and 24-hour urinary excretions of FLC, absolute values of these two variables were compared in relation to the type of

FLC, the results of FLC κ/λ ratios, and urine IFE analysis as well as renal function. The 24-hour FLC clearances were also compared.

Of the 98 sFLC+ samples for κ, 48 (49%) were of sFLC+uBJP− and 50 (51%) of sFLC+uBJP+ type. The median serum κ concentrations in the two groups of samples were 40 mg/L (range 6-710) and 113 mg/L (range 7-39,500; $P = 0.001$), respectively, indicating an almost 3-fold greater median value, which was approximately six times the upper limit of the reference range (3.3-19.4 mg/L) for samples with positive urine IFE analysis. sFLC+uBJP− and sFLC+uBJP+ samples also significantly differed in their Crea− or Crea+ subsets. The respective median serum κ concentrations were 48 and 134 mg/L ($P = 0.015$) and 31 and 103 mg/L ($P = 0.007$; Fig. 3A and B) and the respective median urinary excretions were 32 and 1,277 mg/24 hours ($P < 0.0001$) and 89 and 635 mg/24 hours ($P = 0.002$; Fig. 3C and D). Urinary excretions, however, were significantly higher in sFLC+uBJP− samples with Crea+ than Crea− (89 versus 32 mg/24 hours; $P = 0.013$), whereas sFLC+uBJP+ samples showed a trend toward a significant decrease with Crea+ (1,277 versus 635 mg/24 hours; $P = 0.053$). Comparisons of the 24-hour clearances for FLC κ showed an almost 11-fold greater median value in sFLC+uBJP+ than sFLC+uBJP− samples with Crea− (7.58 versus 0.7 L/24 hours, $P = 0.006$; Table 1). Such a difference, however, was not observed in the respective Crea+ subsets. In these subgroups, the median 24-hour clearance was significantly higher in sFLC+uBJP− (3.06 versus 0.7, $P = 0.006$), but significantly lower in sFLC+uBJP+ samples (1.65 versus 7.58 L/24 hours, $P = 0.04$) when they were compared with the corresponding Crea− subgroups.

Of the 107 sFLC+ samples for λ, 70 (65%) were of sFLC+uBJP− and 37 (35%) of sFLC+uBJP+ type. Serum creatinine values were available in 106 of 107 samples. The median serum λ concentrations in sFLC+uBJP− and sFLC+uBJP+

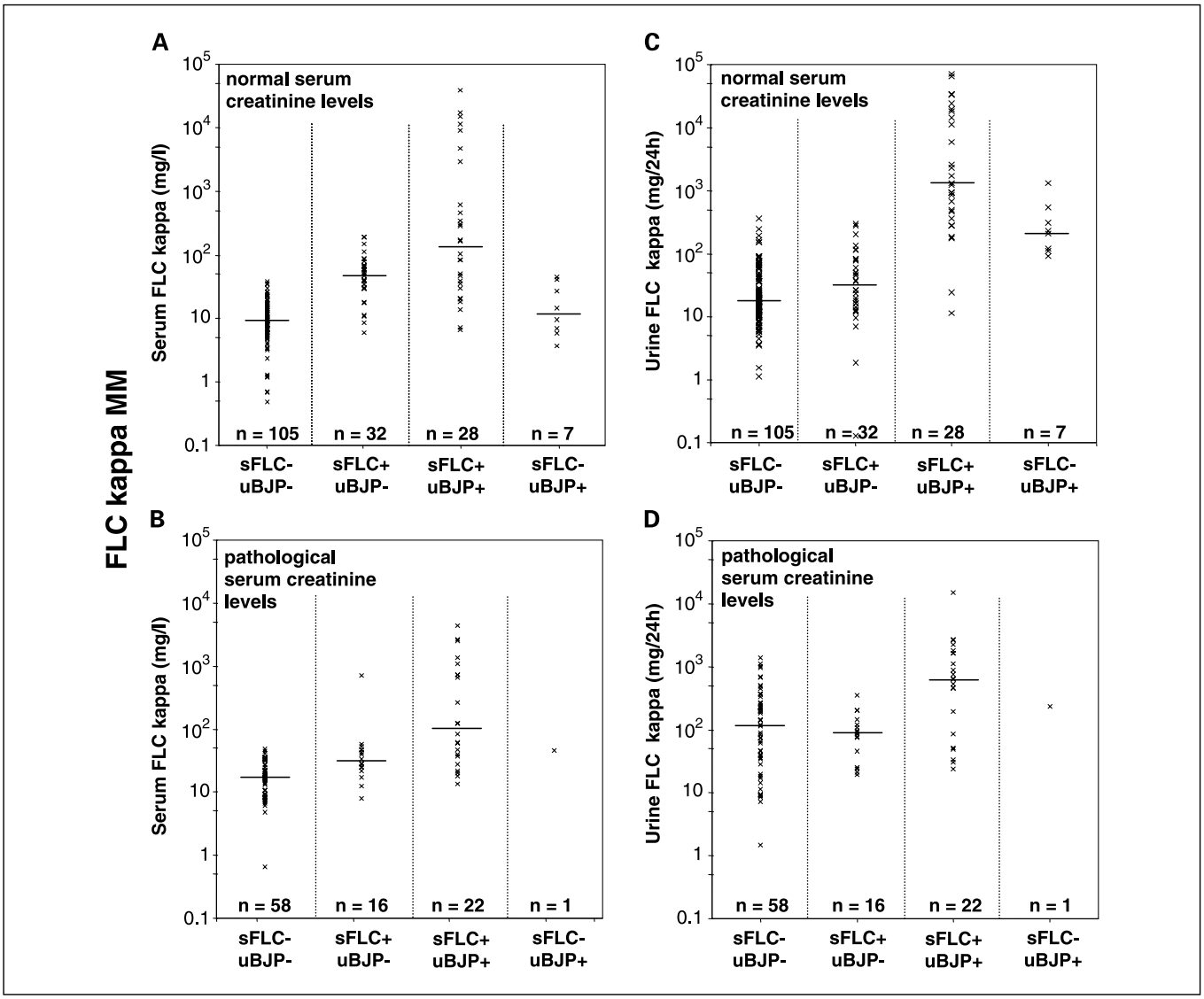

Fig. 3. Median values and distributions of serum concentrations and 24-hour urinary excretions of FLC κ in relation to normal (Crea−, *A* and *C*) or increased (Crea+, *B* and *D*) serum creatinine levels (>1.17 mg/dL for males and >0.95 mg/dL for females) and the results of serum FLC κ/λ ratios and urine IFE tests. sFLC+uBJP− and sFLC+uBJP+ samples significantly differed in serum concentrations and urinary excretions both in Crea+ and Crea− subgroups. Urinary excretions, however, were significantly higher in sFLC+uBJP− samples with Crea+ than Crea−. In sFLC+uBJP+ samples, in contrast, urinary excretions showed a trend toward a significant decrease with Crea+. In sFLC−uBJP+ samples, urinary excretions were significantly higher than in sFLC−uBJP− samples, although the two groups had comparable serum concentrations.

samples were 44 mg/L (range 3-561) and 278 mg/L (range 5-7,060; $P = 0.0001$), respectively, indicating an almost 6-fold greater median value, ~2.5-fold greater than for κ and ~11 times the upper limit of the reference range (5.7-26.3) for samples with positive urine IFE analysis. sFLC+uBJP− and sFLC+uBJP+ samples also significantly differed in their Crea− and Crea+ subsets. The respective median serum λ concentrations were 31 and 313 mg/L ($P < 0.0001$) and 111 and 202 mg/L ($P = 0.004$; Fig. 4A and B) and the respective median urinary excretions were 1,116 and 12 mg/24 hours ($P < 0.0001$) and 189 and 15 mg/24 hours ($P = 0.009$; Fig. 4C and D). In the sFLC+uBJP− samples, however, urinary excretions showed an increase of borderline significance between Crea− and Crea+ subgroups (13 versus 15 mg/24 hours, $P = 0.064$), whereas in sFLC+uBJP+ samples, there was a significant decrease (1,116 versus 189 mg/24 hours, $P = 0.019$). Similar results were obtained when 24-hour FLC clearances for λ were compared (Table 1). In Crea− subsets, the median 24-hour clearance was almost 16-fold greater in sFLC+uBJP+ than sFLC+uBJP− samples (3.77 versus 0.24, $P = 0.0001$). In Crea+ subsets, however, the difference was only of borderline significance (1.57 versus 0.86, $P = 0.058$). In addition, in these subsets, the 24-hour clearance was significantly lower in sFLC+uBJP+ samples than in the respective samples with Crea− (1.57 versus 3.77 L/24 hours, $P = 0.016$).

In the nine samples of sFLC-uBJP+ type, eight of κ type, and one of λ type, serum FLC κ/λ ratios were at the border of the 95% reference interval (0.3-1.2) with a median of 1.18 (range 0.95-1.48) for κ samples and a value of 0.3 for the λ sample (Fig. 1B). Additionally, all κ samples had pathologic urine FLC κ/λ ratios with a range of 5.1-93.6 (median 18.42), confirming the finding of BJ proteinuria by IFE analysis. The λ sample had a borderline value of 3.5 (95% reference interval, 0.37-4). Except for one κ specimen, all samples were associated with normal serum creatinine levels (Figs. 3 and 4). The serum concentrations of the eight κ samples ranged between 3.7 and 44.4 mg/L (median 11.9) and the serum concentration of the λ sample was 31.6 mg/L (Figs. 3 and 4). The respective values for 24-hour urinary excretions were 90.9-1,336 mg (median 218.1) and 25.7 mg. The median 24-hour urinary excretion of κ in sFLC−uBJP+ samples was almost 12-fold greater than in sFLC−uBJP− samples as negative control ($P = 0.0067$), although the two sample groups had comparable serum concentrations (Fig. 3). In addition, in sFLC−uBJP+ samples, the median 24-hour FLC clearance of κ was 33.15 and, thus, the highest among the various sample groups in this study (Table 1).

Impact of renal function on the excretion of polyclonal free light chains. One hundred sixty-four samples were of sFLC−uBJP− type. Serum creatinine levels were available for 163 of 164 samples. These samples were used to evaluate the relationship between renal function and the 24-hour urinary excretions of polyclonal FLC, both for κ as well as λ. One hundred five samples were Crea− and 58 were Crea+. In the first group of samples, the median serum concentration for FLC κ was 9 mg/L (range 0.5-38) compared with 17 mg/L (range 0.7-48) in the second ($P = 0.001$; Fig. 3). The respective urinary excretions were 18 mg/24 hours (range 1.1-359.1) and 118 mg/24 hours (range 1.5-1397.8; $P = 0.001$), showing an almost 6-fold increase in median urinary excretion of polyclonal FLC κ with increasing creatinine levels to values above the reference range. The median 24-hour FLC clearance was almost 4-fold greater in Crea+ samples (1.75 versus 7.79, $P = 0.0001$; Table 1). For λ FLC, the median serum concentration in Crea− samples was 12 mg/L (0.6-43) compared with 23 mg/L (range 0.8-108) in Crea+ ones ($P = 0.001$; Fig. 4). The respective urinary excretions were 10 mg/24 hours (range 0.8-48) and 32 mg/24

Table 1. Twenty-four-hour FLC clearances for κ and λ in relation to normal (Crea−) or pathologically increased (Crea+) serum creatinine levels (>1.17 mg/dL for males and >0.95 mg/dL for females) and the results of serum FLC assays and urine IFE tests

Isotype	Sample groups	Normal serum creatinine FLC clearance (L/24 h)		Abnormal serum creatinine FLC clearance (L/24 h)	
		n	Median (range)	*n*	Median (range)
κ MM	FLC−BJP−	105	1.75 (0.21-57.06)*	58	7.79 (0.22-42.89)*
	FLC+BJP−	32	0.70 (0.004-11.47)†**	16	3.06 (0.36-18.27)††
	FLC+BJP+	28	7.58 (0.34-634.95)‡**	22	1.65 (0.29-99.65)††
	FLC−BJP+	7	33.15 (4.52-76.44)	1	—
λ MM	FLC−BJP−	105	0.71 (0.04-13.91)§	58	1.04 (0.10-11.08)§
	FLC+BJP−	48	0.24 (0.02-8.39)‖‡‡	21	0.86 (0.02-4.65)‖§§
	FLC+BJP+	25	3.77 (0.52-70.27)¶‡‡	12	1.57 (0.10-6.49)¶§§
	FLC−BJP+	1	—	—	—

NOTE: Twenty-four-hour FLC clearance was calculated as the ratio of 24-hour urinary excretion for κ or λ to the respective serum concentration using the following formula: 24-hour clearance (L/24 hours) = urine FLC (mg/24 hours) / serum FLC (mg/L). The FLC clearance represents the volume of plasma that contained the measured FLC excreted in the urine per unit time. In sFLC+uBJP+ samples, the 24-hour clearances were significantly higher than in sFLC+uBJP− samples, both for κ and λ, when they were associated with Crea−. In sFLC+uBJP− samples, the 24-hour clearances were significantly higher in samples with Crea+ than Crea−, but only for κ. In sFLC+uBJP+ samples, the 24 clearances were significantly lower in Crea+ than Crea− samples, both for κ and λ. In sFLC−uBJP− samples, there were significantly higher 24-hour clearances in Crea+ than Crea− samples, both for κ and λ. The group of sFLC−uBJP+ samples had the highest 24-hour clearances for FLC κ observed. Abbreviations: MM, multiple myeloma; sFLC−, negative serum FLC κ/λ ratios; sFLC+, positive serum FLC κ/λ ratios; uBJP−, negative urine IFE tests; uBJP+, positive urine IFE tests.

* versus *, $P = 0.001$; † versus †, $P = 0.006$; ‡ versus ‡, $P = 0.044$; § versus §, $P = 0.035$; ‖ versus ‖, $P = 0.257$; ¶ versus ¶, $P = 0.016$; ** versus **, $P = 0.0001$; †† versus ††, $P = 0.609$; ‡‡ versus ‡‡, $P = 0.0001$; §§ versus §§, $P = 0.0578$.

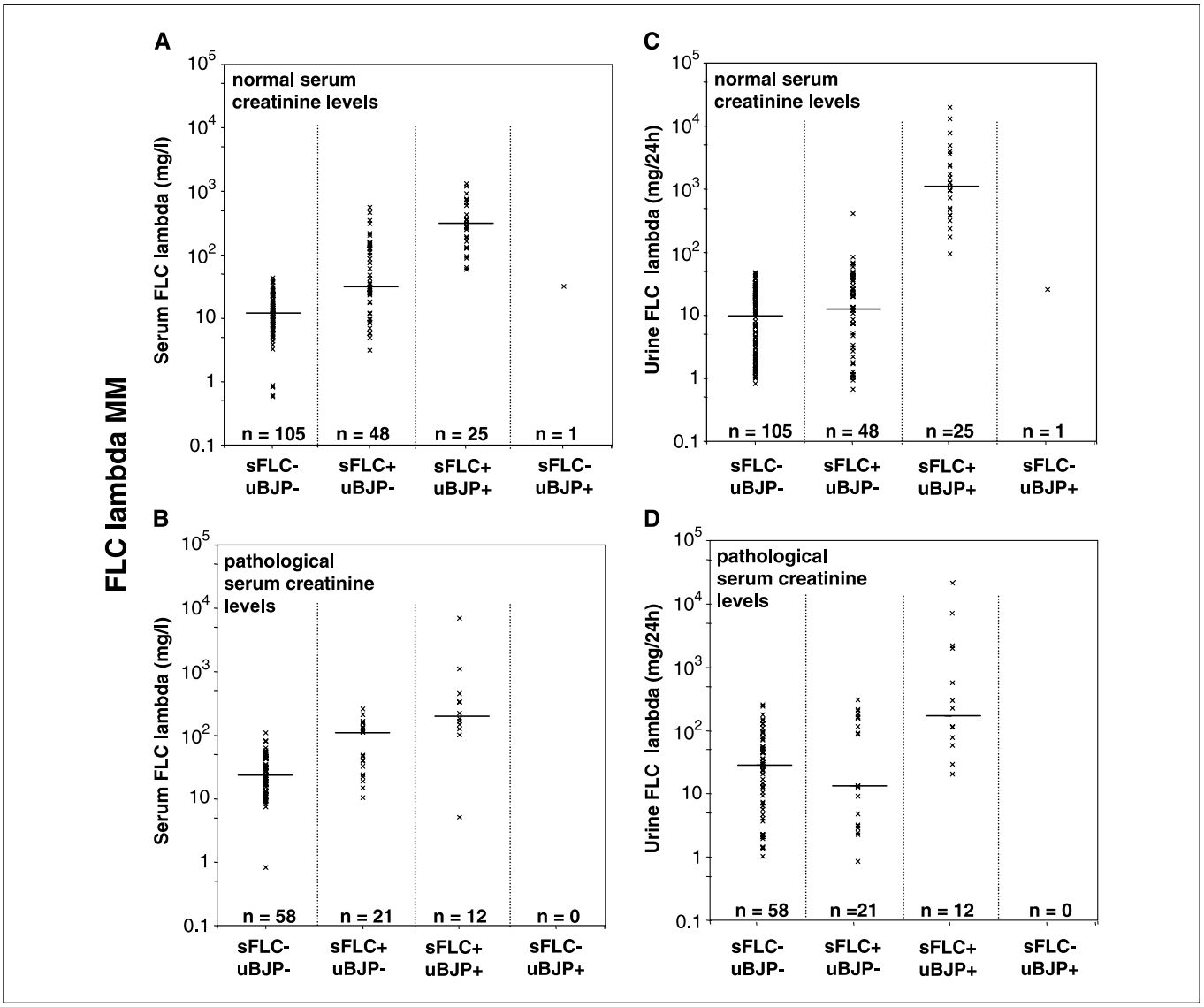

Fig. 4. Median values and distributions of serum concentrations and 24-hour urinary excretions of FLC λ in relation to normal (Crea−, *A* and *C*) or increased (Crea+, *B* and *D*) serum creatinine levels (>1.17 mg/dL for males, >0.95 mg/dL for females) and the results of serum FLC κ/λ ratios and urine IFE tests. sFLC+uBJP− and sFLC+uBJP+ samples significantly differed in serum concentrations and urinary excretions both in Crea+ and Crea− subgroups. Urinary excretions, however, showed increased values of borderline significance in sFLC+uBJP− samples with Crea+ than Crea−. In sFLC+uBJP+ samples, in contrast, there was a significant decrease with Crea+.

hours (range 1-280; $P = 0.001$), indicating a median increase of ~3-fold. The increase in median 24-hour clearance was almost 2-fold (0.71 versus 1.04, $P = 0.035$; Table 1).

To further evaluate the relationship between renal function and urinary excretion of polyclonal FLC, serum creatinine concentrations and 24-hour urinary excretions of FLC were correlated in samples from patients with BJ proteinuria at presentation but currently in complete remission (CR) indicated by normal FLC κ/λ ratios in serum and urine, as well as negative urine IFE tests. The results showed a significant log-linear relationship between the two variables both for κ as well as λ FLC (Fig. 5). There was also a significant correlation ($r = 0.927$; $P = 0.001$) between the two types of FLC, indicating that the FLC excreted were polyclonal. Interestingly, urinary excretions of FLC, both for κ as well as λ, increased with increasing serum creatinine concentrations not only above the reference range but also within this range, indicating that renal tubular dysfunctions were present before serum creatinine levels became abnormal. The 24-hour clearances were 1.75 and 7.79 L/24 hours ($P = 0.0001$) for Crea− and Crea+ samples, respectively, for polyclonal FLC κ and 0.71 and 1.04 L/24 hours for FLC λ ($P = 0.035$; Table 1).

Discussion

Several recent studies have evaluated the potential role of serum FLC measurements for the diagnosis and management of patients with multiple myeloma. Using stored samples, those retrospective studies indicated that FLC tests were more sensitive than existing serum and urine electrophoresis tests (1, 3, 5, 12).

The data of this prospective study fully support the findings of the earlier studies. The results from 378 paired serum and 24-hour urine samples evaluated from 82 patients at various

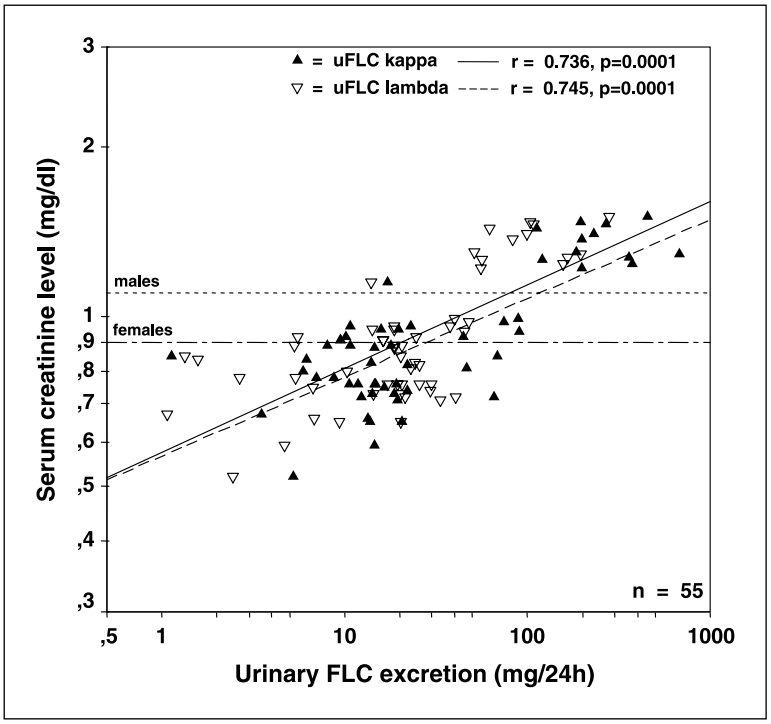

Fig. 5. Correlations between serum creatinine levels and urine 24-hour excretions for polyclonal FLC κ and λ. Samples originated from patients with initially proved BJ proteinuria, but currently in CR. Dotted horizontal lines, upper limits of the reference ranges for serum creatinine levels in males (1.17 mg/dL) and females (0.95 mg/dL). Urine 24-hour excretions and serum creatinine levels showed a strong and highly significant correlation both for κ and λ. This correlation was given not only for increased but also for normal serum creatinine concentrations, showing that urinary excretions of polyclonal FLC were earlier indicators of renal dysfunction than serum creatinine levels.

times of their disease showed that abnormal serum FLC were present in 42% of samples that were negative by urine tests. In contrast, only 5% of samples, mainly of FLC κ, were positive by urine tests but negative by serum analyses. All of these samples had borderline FLC κ/λ ratios (Fig. 1) and eight of nine had pathologic urine FLC κ/λ ratios, indicating that in such cases additional urine tests, either measurements of FLC κ/λ ratio or IFE analysis or both, should be done. One explanation for the presence of these samples may be that the current reference range for FLC κ/λ ratios in serum, particularly for κ, is not quite optimal and should be a little narrower. Other explanations may be the easy leakage of the kidney for FLC κ, subtle tubular dysfunctions with increased FLC excretions, particularly for κ, and the presence of truncated molecules of FLC, such as FLC fragments with inadequate reabsorption in the kidney. FLC fragments are known to occur in patients with multiple myeloma and other plasma cell dyscrasias (13, 14). Of particular interest is the finding that this group of samples had a very high 24-hour FLC clearance for κ, indicating a rapid renal excretion.

The sensitivity of urine IFE tests is methodologically influenced by the type of urine collection, the degree of urine concentration, and the IFE method used, particularly the affinity of polyclonal antibodies used, and the sensitivity of the staining technique. The indicated amount for polyclonal light chain excretion in healthy individuals, however, is ~10 mg/L and, thus, a sensitivity down to this limit has been recommended for IFE tests to evaluate BJ proteins in urine (10, 15). In our study, the lowest urine concentration associated with a monoclonal band identified by IFE tests was 5.4 mg/L (11.34 mg/24-hour urine) for κ and 17.4 mg/L (22.62 mg/24-hour urine) for λ FLC, showing that the above-mentioned criterion was more than met for κ and approximately met for λ FLC (Figs. 3 and 4). Based on these detection limits and using samples from patients with initially proved BJ proteinuria and currently positive both for serum and urine FLC κ/λ ratios, the analytic sensitivity of urine IFE tests found in our study was 66% for κ and 75% for λ FLC. It might be that higher mechanical concentrations of urine than used in our study could have resulted in a higher sensitivity of IFE tests, but highly concentrated urines, particularly in case of multiple myeloma, may be associated with a number of disadvantages, including loss of FLC as low-molecular-weight proteins, increased background, appearance of ladder patterns and prozone effects making interpretation of IFE tests more difficult or requiring subsequent dilution steps. Multiple myeloma is usually accompanied by a wide range of urinary protein excretions, in our study between 0.011 and 22.83 g/24 hours, which makes the use of an appropriate degree of urine concentration difficult and has led to contradictory suggestions regarding this point (13–21).

Another problem of urine IFE analysis is that this method is only qualitative and not quantitative. In addition, BJ proteins and polyclonal immunoglobulin light chains are metabolized in the kidney by glomerular filtration, proximal renal tubular absorption, and catabolism within the tubular cells. These mechanisms and disease-related as well as treatment-related reductions in glomerular filtration rate and impairments of renal tubular function, both commonly observed in patients with multiple myeloma, affect the excretions of these proteins. As a result, the amounts of BJ proteins in urine not necessarily correlate with their serum concentrations and are, therefore, not directly related to the activity and mass of tumor cells (15).

The present study evaluates for the first time the relationship between serum concentrations of monoclonal FLC and their urinary excretions in relation to renal function and is also the first study to determine serum concentrations of monoclonal FLC required to produce renal overflow and BJ proteinuria detectable by IFE tests. Analysis of paired serum and urine samples associated with normal or pathologically increased

serum creatinine levels showed that for κ producing myelomas, the median serum levels needed to be elevated approximately seven and five times the upper limit of the reference range, respectively, to produce an overflow with sufficiently high urine concentrations to be detected by IFE analysis (Fig. 3). For λ myelomas, the median serum levels associated with normal or pathologic creatinine concentrations had to be elevated ~12 and 8 times the upper limit of the reference range, respectively, before urine IFE analysis became abnormal (Fig. 4). Differences between the amounts of κ and λ excreted can be explained by dimerization of λ molecules limiting their filtration through the glomerular membranes (10). As shown in our study, the 24-hour clearance for κ is ~2.5-fold higher than for λ (Table 1). The present results further show that renal excretions of monoclonal FLC are determined primarily by serum concentrations for λ, and also by serum concentrations, renal function, and, probably, molecular changes for κ. For both, however, renal excretions significantly decrease when high serum concentrations combined with renal dysfunction are present, possibly related to an accelerated renal damage by the overwhelming amounts of FLC. The results also explain the findings of other studies showing that serum concentrations and urinary excretions of monoclonal FLC correlate individually in each patient (2); however, considerable variations occur in the ratios of serum levels to urinary excretions of FLC between patients (22).

This is also the first study to evaluate the relationship between renal function and excretion of polyclonal FLC in patients with BJ proteinuria at presentation, who were currently in CR. The amounts of polyclonal FLC excreted ranged between 1.0 and 682.0 mg/24 hours for κ and 1.0 and 280.2 mg/24 hours for λ, both significantly depending on serum creatinine levels. The amounts of polyclonal FLC excreted can be assumed to be higher in patients with currently detectable BJ proteins because urinary excretions of tubular proteins have been reported to increase with increasing urinary output of BJ proteins (23). Our data further showed that urinary excretion of polyclonal FLC significantly increased with serum creatinine concentrations. This was observed not only for values above the reference range but also within this range, indicating that increased serum creatinine concentrations are late indicators of impaired renal function. Earlier indicators are the amounts of polyclonal FLC excreted in urine, which should also be evaluated in other diseases with the risk of renal failure. The results also show that measurements of FLC in urine have to be recognized with caution because they include both monoclonal FLC and, depending on renal function, varying amounts of polyclonal FLC. A more reliable variable may be the ratio of the excreted amounts of κ and λ FLC. The most reliable variable for evaluating disease activity, however, according to our data, is the direct measurement of serum FLC.

In the present study, 58% of abnormal serum samples from all patients and 48% from patients with BJ proteinuria at presentation were associated with no detectable BJ proteins in the urine, indicating that serum FLC assays were able to detect smaller amounts of disease. This was apparent at diagnosis, as well as during the course of disease. The clinical aim of chemotherapy in patients with multiple myeloma is a maximum effect, usually indicated by a plateau or disappearance of myeloma protein as an essential criterion for achieving CR. Using serum FLC assays, however, CR should be redefined because in some patients with multiple myeloma, BJ proteins disappear during treatment in urine but are still present in serum. Continuation of treatment can reduce the ratios of FLC κ/λ to within the reference range, indicating the disappearance of the monoclonal isotypes in serum. In a study including myeloma patients with normal urine IFE analysis after treatment, persisting abnormal serum FLC κ/λ ratios were found to predict shorter survival (8). Abnormal serum FLC κ/λ ratios were also found to predict disease progression in patients with monoclonal gammopathy of undetermined significance (24). In patients with AL amyloidosis, the outcome of chemotherapy appeared to correlate with the degree of decrease in serum FLC concentrations during treatment (4).

Circulating FLC are present in >95% of patients with multiple myeloma (6) and up to 50% of patients have a decreased creatinine clearance at presentation. Approximately 20% develop progressive renal failure during the course of disease (2). In our study, 33% of all patients had abnormal serum creatinine levels during the course of their disease and 35% of patients who had BJ proteinuria at presentation, but were currently in CR, showed increased urinary excretions of polyclonal FLC as a sign of renal tubular dysfunction. Occasionally, we observed impairment of renal function at serum FLC concentrations that were not associated with detectable BJ proteinuria, and treatment instigation to reduce the serum FLC concentrations had a beneficial effect on renal function.

Conclusion

This prospective study evaluated serum and urine samples from patients with multiple myeloma during the course of their disease. Abnormal serum FLC were detectable in 54% of samples tested compared with 26% by urine IFE analysis. Median serum FLC concentrations associated with negative or positive urine IFE tests were found to be elevated approximately two and six times the upper limit of the reference range for κ and 2 and 11 times for λ, respectively. Based on these results, serum FLC assays are significantly more sensitive in detecting monoclonal FLC than urine IFE analysis. They also have the advantage of FLC quantification over a large concentration range and are more reliable for monitoring course of disease and response to treatment. In cases with borderline serum FLC κ/λ ratios, particularly for κ, urine FLC assays or urine IFE analysis or both may be positive for BJ proteins and, therefore, should be done.

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