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Serum Reference Intervals and Diagnostic Ranges for Free κ and Free λ Immunoglobulin Light Chains: Relative Sensitivity for Detection of Monoclonal Light Chains

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Background: **The detection of monoclonal free light chains (FLCs) is an important diagnostic aid for a variety of monoclonal gammopathies and is especially important in light-chain diseases, such as light-chain myeloma, primary systemic amyloidosis, and light-chain-deposition disease. These diseases are more prevalent in the elderly, and assays to detect and quantify abnormal amounts of FLCs require reference intervals that include elderly donors.**

Methods: **We used an automated immunoassay for FLCs and sera from a population 21–90 years of age. We used the calculated reference and diagnostic intervals to compare FLC results with those obtained by immunofixation (IFE) to detect low concentrations of monoclonal κ and λ FLCs in the sera of patients with monoclonal gammopathies.**

Results: **Serum κ and λ FLCs increased with population age, with an apparent change for those >80 years. This trend was lost when the FLC concentration was normalized to cystatin C concentration. The ratio of κ FLC to λ FLC (FLC K/L) did not exhibit an age-dependent trend. The diagnostic interval for FLC K/L was 0.26–1.65. The 95% reference interval for κ FLC was 3.3–19.4 mg/L, and that for λ FLC was 5.7–26.3 mg/L. Detection and quantification of monoclonal FLCs by nephelometry were more sensitive than IFE in serum samples from patients with primary systemic amyloidosis and light-chain-deposition disease.**

Conclusions: **Reference and diagnostic intervals for serum FLCs have been developed for use with a new, automated immunoassay that makes the detection and quantification of monoclonal FLCs easier and more sensitive than with current methods. The serum FLC assay complements IFE and allows quantification of FLCs in light-chain-disease patients who have no detectable serum or urine M-spike.**

Monoclonal gammopathies are characterized by the clonal expansion of plasma cells. The monoclonal immunoglobulin secreted by these cells is an indicator of clonal proliferation and can be quantitatively measured to monitor disease course *(1)*. The monoclonal gammopathies include multiple myeloma (MM),[3] light-chain myeloma, Waldenstrom macroglobulinemia, nonsecretory myeloma (NSMM), smoldering multiple myeloma, monoclonal gammopathy of undetermined significance, primary systemic amyloidosis (AL), and light-chain-deposition disease (LCDD) *(2)*. The monoclonal light-chain diseases (light-chain myeloma, AL, and LCDD) and NSMM often do not have sufficiently high concentrations of serum monoclonal light chains to be detected by serum protein electrophoresis (SPEP) or immunofixation (IFE) *(3)*. In addition, when IFE detects a monoclonal light chain, the amount of protein may be too low to be quantified and monitored by SPEP.

Sensitive nephelometric assays that are specific for κ

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[3] Nonstandard abbreviations: MM, multiple myeloma; NSMM, nonsecretory multiple myeloma; AL, primary systemic amyloidosis; LCDD, light-chain-deposition disease; SPEP, serum protein electrophoresis; IFE, immunofixation; FLC, free light chain; FLC K/L, ratio of κ FLC to λ FLC; PPV, positive predictive value; and NPV, negative predictive value.

and λ free light chains (FLCs) but that do not recognize light chains bound to immunoglobulin heavy chains have recently been described *(4)*. These automated assays are reported to be more sensitive than IFE for the detection of monoclonal FLCs. The nephelometric assays were used to evaluate serum samples from NSMM patients whose serum and urine samples were negative for FLCs by IFE, and these assays detected excess serum κ or λ FLCs in 19 of 28 patients *(5)*. In a subset of NSMM patients, serial serum samples were analyzed for FLCs, whose quantities were correlated with disease activity.

Because IFE does not quantify FLCs and is not sufficiently sensitive to detect small amounts of monoclonal FLCs in all patients with light-chain plasma-cell dyscrasias, it is important to evaluate the utility of FLC assays to diagnose and monitor the light-chain diseases. These diseases affect mainly the elderly, and we designed this study to determine the 95% reference intervals of κ and λ FLCs, as well as the diagnostic interval for the ratio of κ FLC to λ FLC (FLC K/L) in a population 21–90 years of age. In addition, we applied these intervals to a group of patients with light-chain diseases and compared the ability of the nephelometric assay with that of the IFE assay to detect abnormal light chains.

Participants and Methods

PARTICIPANTS

Fresh sera from 127 healthy donors 21–62 years of age [68 (54%) women and 59 (46%) men] were obtained from a pool of donors who had typical FLC values (Mayo Clinic). Frozen sera from 155 donors 51–90 years of age [78 (50%) women and 77 (50%) men] were obtained from the serum bank of an epidemiologic study that surveyed the incidence of monoclonal gammopathies in Olmsted County, MN. These samples were used under a minimum-risk protocol approved by the Mayo Clinic Institutional Review Board. Both fresh and frozen sera were used in the study to obtain a sampling from a wide age range. For donors 51–62 years of age, there were 25 fresh samples and 47 frozen samples. No significant differences in κ FLC content, λ FLC content, or FLC K/L were found between the 25 fresh and 47 frozen samples in this overlapping age group. All sera were assessed by SPEP and IFE for an M-spike or a restricted migration pattern that would suggest the presence of a monoclonal protein. No abnormalities were detected.

Twenty-five polyclonal hypergammaglobulinemia serum samples were obtained from the clinical electrophoresis laboratory. As determined by SPEP, all 25 sera had increased γ-globulin concentrations of 18–39 g/L. None of these sera contained a monoclonal protein as determined by IFE.

The clinical laboratory also identified 47 serum samples that had given equivocal IFE results. These samples were either determined to have a monoclonal light chain after multiple IFE assays at different sample dilutions or were from patients who were serum negative but urine positive for a monoclonal light chain. These 47 frozen sera had been collected from AL, LCDD, or MM patients: 24 samples were from patients with a monoclonal κ light chain and 23 from patients with a monoclonal λ light chain.

Sera from 19 patients with LCDD were obtained from the Dysproteinemia Clinic frozen serum bank. These LCDD serum samples were selected to represent patients with (*a*) monoclonal light chains detected by IFE in the serum, (*b*) monoclonal light chains detected by IFE in the urine but not the serum, or (*c*) a monoclonal population of bone marrow plasma cells but no IFE-detectable light chains in either serum or urine.

ANALYTIC METHODS

SPEP was performed on agarose gels with the Helena REP system (Helena Laboratories). Ponceau S stain was used to visualize the proteins, and the stained gels were scanned with a Helena Cliniscan 3 scanner. Serum total protein was determined with biuret reagent on a Hitachi 747 analyzer (Boehringer-Mannheim Corp.). The serum total protein value multiplied by the percentage of protein migrating in the gamma region was used to quantify the gamma fraction.

IFE was performed with a Sebia HYDRAGEL 4IF reagent set on a Sebia HYDRASYS electrophoresis system and agarose gels. The IFE assay used antisera against γ, α, μ, κ, and λ to fix specific proteins after electrophoretic separation, and precipitated protein was visualized with acid-violet stain. The detection limits of the IFE assay for monoclonal proteins are 25–50 mg/L, depending on the position of the monoclonal protein band and the content of polyclonal immunoglobulin. Any samples that exhibited monoclonal light-chain staining but no corresponding monoclonal heavy-chain staining were also analyzed by the Ouchterlony method for δ and ε reactivity. This assay has a detection limit of 40 mg/L for IgD and IgE. When either δ or ε was detected, the IFE was repeated with antisera to κ, λ, δ, and ε to detect a monoclonal IgD or IgE protein.

Nephelometry was performed on a Dade-Behring BNII. Quantification of total κ, total λ, and cystatin C used antibodies from Dade-Behring. FLCs were quantified with FREELITE™ reagent sets from The Binding Site Ltd. These FLC assays use sheep antisera coated on polystyrene latex particles and are enhanced by the addition of polyethylene glycol to the reaction. Saline-diluted serum samples that contained either a monoclonal κ or a λ light chain were used to test assay linearity. We performed 20 replicate tests on polyclonal sera with typical FLC values to determine the CV of the assay. The κ FLC assay was linear to a minimum value of 0.5 mg/L; at 0.7 mg/L, the intraassay CV was 7.9%; and at 14 mg/L, the interassay CV was 8.7%. The λ FLC assay was linear to a minimum value of 0.6 mg/L; at 0.9 mg/L, the intraassay CV was 10%; and at 32 mg/L, the interassay CV was 7%.

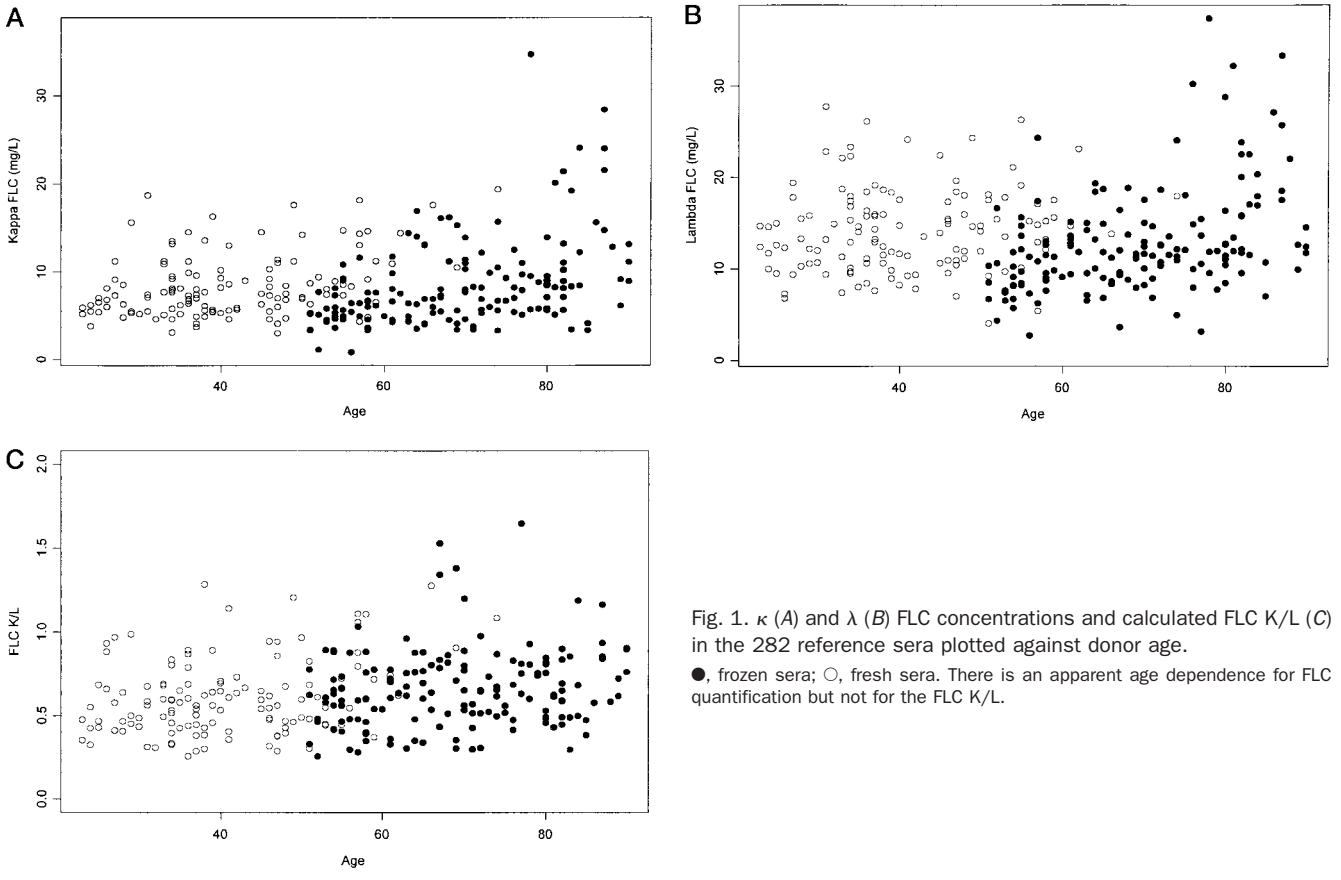

Fig. 1. κ (*A*) and λ (*B*) FLC concentrations and calculated FLC K/L (*C*) in the 282 reference sera plotted against donor age.

●, frozen sera; ○, fresh sera. There is an apparent age dependence for FLC quantification but not for the FLC K/L.

STATISTICAL ANALYSIS

The SAS and S-PLUS statistical software packages were used to perform the analyses and create graphs. Reference intervals were determined with a method suggested by O'Brien and Dyck *(6)*. This method accounts for differences in means and the variability across age and sex groups. Linear regression analysis was used to adjust for significant differences in means across age or sex groups *(6)*. Significance was defined as both $P < 0.05$ and $R^2 > 0.10$. For variables with a means adjustment, variability was adjusted separately for positive and negative residuals from the first regression, again only when significant. *Z*-scores were then created for each datum by subtracting the fitted value from the first regression and dividing the fitted value (corresponding to the appropriate second regression), depending on whether the residual from the first regression was positive or negative. The 2.5 and 97.5 percentiles were then determined from the *Z*-scores. These percentiles were back-transformed to the units of measure by reversing the process that had created the *Z*-scores, which produced reference intervals stratified by age and/or sex when significant.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were estimated for the FLC K/L on the basis of both the central 95% interval and a diagnostic range that captured 100% of the test data. Accuracy was calculated as the proportion of individuals classified correctly. Confidence intervals were calculated according to the exact binomial distribution for sensitivity, specificity, and accuracy and by bootstrap for PPV and NPV. We calculated both PPV and NPV after assuming a 15% prevalence of monoclonal proteins in the samples submitted for monoclonal protein studies.

Results

The γ fraction, total κ, and total λ were quantified for the 282 reference serum samples (data not shown). There was no age or sex dependence in samples from donors between the ages of 21 and 90 years. The median and central 95% interval for the γ fraction were 13.1 and 7.9–19.3 g/L, respectively. The γ fraction should be mostly IgG, and approximately one-third of its mass should be immunoglobulin light chain (two-thirds κ and one-third λ) bound to the γ heavy chain. The total κ content was 2.52 g/L (1.55–3.78 g/L), and the total λ content was 1.43 g/L (0.89–2.03 g/L). The ratio of total κ to total λ (K/L) had a median value of 1.78 and a central 95% interval of 1.30–2.52.

Quantification of κ and λ FLCs showed a trend of increasing values with increasing age (Fig. 1, A and B). There was no relationship between FLC and sex. The κ and λ FLC values showed an increase that was most apparent for those >80 years of age (Table 1). Although the data for the FLCs tended to increase with increasing

Table 1. Median κ FLC, λ FLC, and FLC K/L values by decade.[a]

Age, years	κ FLC, mg/L	λ FLC, mg/L	FLC K/L
20–29	6.3	12.4	0.49
30–39	7.2	13.6	0.55
40–49	7.5	12.8	0.58
50–59	6.4	11.3	0.59
60–69	6.9	11.8	0.70
70–79	8.0	11.9	0.65
80–90	9.1	15.1	0.64

[a] κ FLC and λ FLCs were measured for the 282 reference serum samples, and the FLC K/L was calculated.

Table 2. FLC reference intervals and diagnostic ranges.[a]

	95% reference interval	Diagnostic range
κ FLC	3.3–19.4	
λ FLC	5.7–26.3	
FLC K/L	0.3–1.2	0.26–1.65

[a] The reference intervals for the 282 reference serum samples were calculated as the central 95% interval. The FLC K/L diagnostic range includes 100% of the reference population.

age, this increase was not significant. The *P* value and R^2 result for κ FLC vs age were 0.004 and 0.06, respectively, whereas for λ FLC, the respective values were 0.06 and 0.03. A central 95% reference interval was defined without regard to age or sex (Table 2). FLC K/L did not vary with age (Fig. 1C). Cystatin C concentrations showed a relationship with age similar to that between κ and λ FLC concentrations and age (Fig. 2A). When the FLC results were expressed as a ratio of FLC to cystatin C, the trend with age was no longer apparent (Fig. 2, B and C). When the FLC results were expressed as a ratio of FLC to creatinine, the age dependence was reduced but not eliminated (data not shown).

The median FLC K/L was 0.59 and was substantially different from the median total K/L of 1.78. This difference has been attributed to the dimerization of λ light chains and the consequently slower clearance compared with that of κ light chains *(5)*. The FLC K/L was not significantly related to age or sex. The FLC K/L central 95% reference interval was 0.3–1.2 (Table 2). By definition, 5% of the general population will have a FLC K/L outside this 95% reference interval. If the FLC K/L is used as a diagnostic test for monoclonal FLCs and the FLC diseases, a 5% false-positive rate is unacceptable. We therefore defined a FLC K/L diagnostic range that included all 282 reference sera tested in this study. The FLC K/L had a 100% range of 0.26–1.65 (Table 2).

The FLC results for 25 serum samples with polyclonal hypergammaglobulinemia are shown in Table 3. Although a majority of the κ and λ FLCs were increased in this group of hypergammaglobulinemia sera, none of the FLC K/L values were abnormal.

The κ and λ FLC values were determined for 47 patient serum samples that had been difficult to immunotype by IFE (Table 4). Twenty-four samples came from patients for whom IFE had detected a monoclonal κ light chain in the urine or who had a history of a previous urine sample that contained a monoclonal κ light chain. The serum IFE assays identified 21 positive (monoclonal κ) and 3 equivocal sera. Two of the patients with an equivocal IFE result for serum monoclonal κ protein had this protein detected in their urine by IFE. The third patient with an equivocal IFE result for serum monoclonal κ protein had no monoclonal κ in the concurrent urine sample. Interestingly, one of the patients with monoclonal serum κ protein, as detected by IFE, had no monoclonal κ protein detected in the concurrent urine by IFE. The κ FLC and the FLC K/L were abnormally increased in all 24 sera. The median κ FLC, median λ FLC, and median FLC K/L were 716 mg/L, 1.2 mg/L, and 539, respectively.

There were also 23 serum samples from patients for whom IFE had detected monoclonal λ light chain protein in the urine or who had a history of monoclonal λ light chain protein detected in urine. The serum IFE assays identified 15 positive and 8 negative sera. Seven of the patients whose sera were λ protein negative by IFE had concurrent urine samples that were positive for monoclonal λ protein by IFE. One patient with a negative serum result by IFE had a concurrent urine sample that was also negative for monoclonal λ protein by IFE. The λ FLC concentration was abnormally increased in 22 sera, and the FLC K/L was abnormally low in 22 sera. The patient with negative FLC λ results also had negative serum and urine results by IFE. As in the κ group, there was one patient whose serum was positive for monoclonal λ by IFE and FLC, but whose urine was λ protein negative by IFE. The median κ FLC, median λ FLC, and median FLC K/L were 4.5 mg/L, 258 mg/L, and 0.019, respectively.

The results for 19 LCDD patients are listed in Table 5. These four patient groups included (*a*) 9 patients whose sera were monoclonal κ positive by IFE; (*b*) 3 whose sera were monoclonal λ positive by IFE; (*c*) 4 whose sera were IFE negative but whose urine samples were positive for monoclonal κ by IFE; and (*d*) 3 whose sera and urine samples were both negative by IFE but whose bone marrow stains were restricted to κ. Among these 19 patients, 12 had a serum monoclonal protein detected by IFE (63%) and 17 had a serum monoclonal protein detected by FLC K/L (89%). Six patients with a negative serum IFE had a positive FLC K/L, and one patient with a positive serum IFE had a negative FLC K/L.

The results from the 25 polyclonal hypergammaglobulinemia sera (Table 3) and 282 reference sera were used to calculate the specificity of FLC K/L, and the results from the 66 patients with AL, MM, or LCDD (Tables 4 and 5) were used to calculate the sensitivity in this selected patient group (Table 6). As expected, use of the diagnostic range for FLC K/L significantly improved the specificity of the FLC assay.

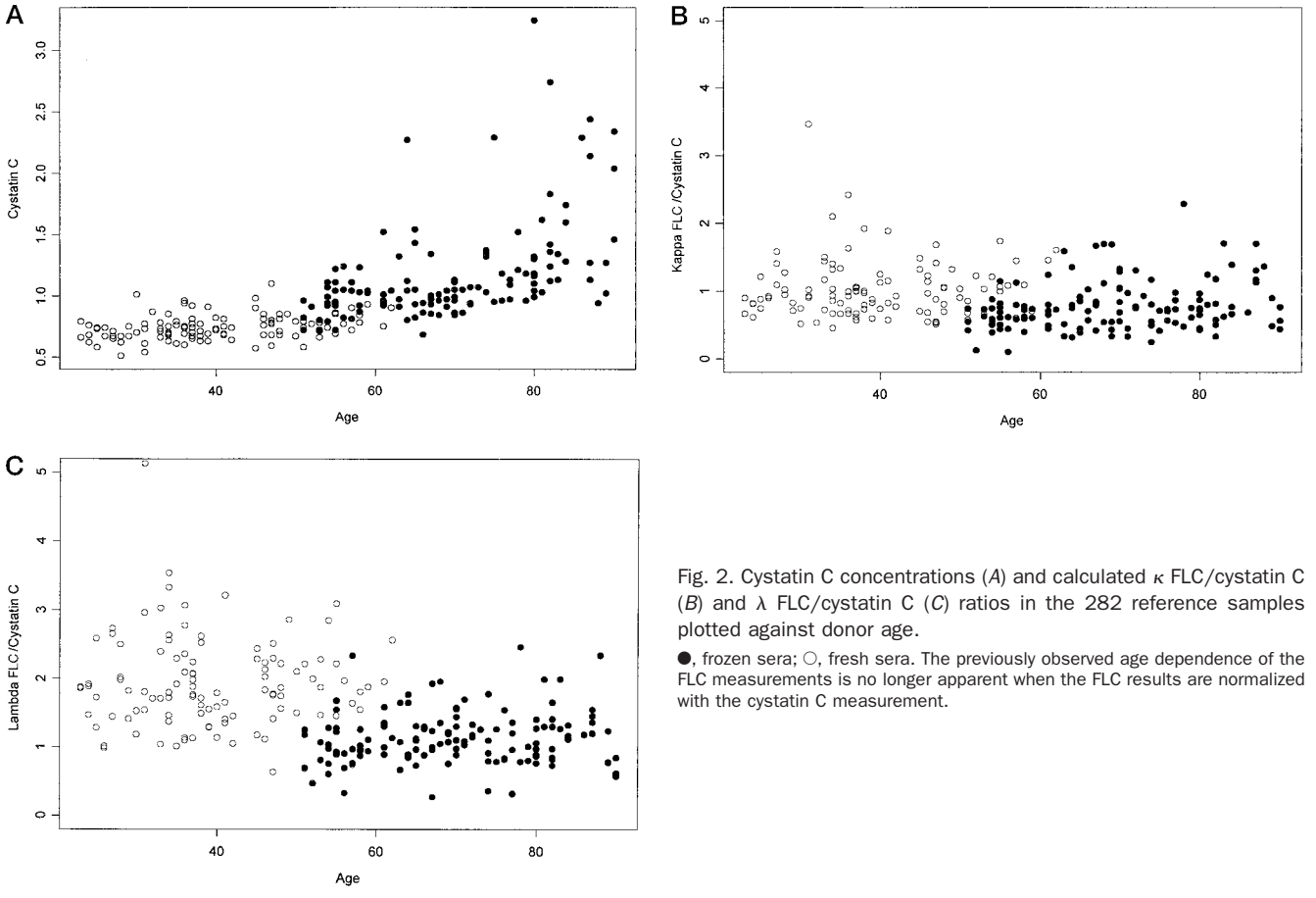

Fig. 2. Cystatin C concentrations (*A*) and calculated κ FLC/cystatin C (*B*) and λ FLC/cystatin C (*C*) ratios in the 282 reference samples plotted against donor age.

●, frozen sera; ○, fresh sera. The previously observed age dependence of the FLC measurements is no longer apparent when the FLC results are normalized with the cystatin C measurement.

Discussion

Although FLCs in serum are usually associated with monoclonal gammopathies, polyclonal FLCs have been detected at low concentrations in healthy serum *(4, 7–11)*. In monoclonal gammopathies associated with FLCs, these proteins may be present in serum in small amounts and thus be difficult to detect by IFE and often impossible to quantify by an M-spike on SPEP. Unlike IFE, the assessment of FLCs by nephelometry is a quantitative measurement, and the described sensitivity and specificity of the nephelometric FLC assay *(4)* may allow quantification and monitoring of monoclonal light chains in serum. The ability to detect abnormal amounts of FLCs and an abnormal FLC K/L is dependent, however, on accurately determined reference intervals, so that the specificity of disease detection remains high.

Table 3. Polyclonal hypergammaglobulinemia: FLC results (n = 25).[a]

	Median	Range	Abnormal, %
κ FLC, mg/L	19.6	4.3–273	52
λ FLC, mg/L	28.8	8.5–307	58
FLC K/L	0.55	0.38–1.18	0

[a] Serum samples were polyclonal hypergammaglobulinemic as determined by SPEP and IFE.

The reference intervals reported herein are close to those described in the original report of this nephelometric FLC immunoassay *(4)*. That study, however, did not include older healthy donors. The monoclonal gammopathies are more prevalent in older populations. Several epidemiologic studies have reported the incidence of monoclonal gammopathy of undetermined significance as ~1% in the population >50 years and 3% in the population >70 years of age *(12)*. The κ and λ serum FLCs showed a trend for increased values with increasing age in our study, and there was a substantial increase in these values in individuals >80 years of age. The FLC K/L ratio, however, normalized the age-dependent increases in FLC. Because total κ and λ concentrations do not show an increase with age and because the FLC K/L normalizes the increase in κ and λ FLC values, the most likely explanation for the observed increase in serum FLC concentrations is a decrease in renal clearance with advancing age. Measures of renal clearance show an age-related decrease in renal function that begins in the third decade *(13, 14)*. Cystatin C is a sensitive indicator of renal clearance, and the increase in FLC content with age is reflected by an increase in cystatin C. Dividing the FLC

Table 4. Sensitivity of IFE and FLCs.[a]

		IFE		Serum		
Diagnosis	**Age, years**	**Serum**	**Urine**	**κ FLC, mg/L**	**λ FLC, mg/L**	**FLC K/L**
Kappa patients						
MM	64	κ	κ	2230	3.4	666
MM	52	Equivocal	κ	46	1.2	38
MM	53	κ	κ	929	0.6	1548
MM	67	κ	κ	873	6.3	139
MM	47	Equivocal	κ	630	8.8	72
MM	70	κ	κ	1620	0.6	2700
MM	84	κ	κ	109	3	36
MM	53	κ	κ	427	1.8	237
MM	60	κ	κ	802	3.7	217
MM	58	κ	κ	122	7.2	169
MM	45	κ	κ	5670	7	810
MM	67	κ	κ	2390	1.2	1992
MM	61	κ	κ	479	0.2	2395
MM	54	κ	κ	214	0.4	535
MM	68	κ	κ	60	0.2	301
AL	60	κ	κ	141	1.2	118
AL	44	Equivocal	Negative[b]	273	0.2	1365
AL	56	κ	κ	372	0.2	1860
AL	58	κ	Negative[b]	939	2	470
AL	70	κ	κ	3710	0.2	18 550
AL	64	κ	κ	899	0.2	4495
AL	52	κ	κ	1330	1.5	887
AL	47	κ	κ	218	0.4	545
LCDD	33	κ	κ	2950	6.4	466
		87% positive		100% positive		100% positive
Lambda patients						
MM	66	λ	λ	3	46	0.065
MM	45	λ	λ	3	696	0.004
MM	77	λ	λ	2	49	0.041
MM	54	λ	λ	0.9	213	0.004
MM	78	λ	λ	5	225	0.022
MM[c]	63	λ	λ	3.1	626	0.005
MM[d]	51	λ	λ	3.5	150	0.023
AL	54	Negative	λ	0.5	41	0.012
AL	73	Negative	λ	5	258	0.019
AL	59	λ	λ	5.3	652	0.008
AL	62	λ	λ	20.7	637	0.033
AL	47	λ	λ	4.5	566	0.008
AL	51	λ	Negative[f]	3.5	4660	0.001
AL	47	Negative	λ	21.2	323	0.066
AL	75	λ	NA[e,f]	13.6	716	0.019
AL	80	Negative	λ	21.3	1680	0.013
AL	55	Negative	Negative[f]	3	8.1 (negative)	0.37 (negative)
AL	52	Negative	λ	7.4	490	0.015
AL	51	λ	λ	21.9	213	0.103
AL	46	Negative	λ	5.3	212	0.025
AL	68	λ	λ	3.1	36	0.087
AL	71	Negative	λ	4.9	57	0.087
AL	72	λ	NA[f]	4.5	340	0.013
		65% positive			96% positive	96% positive

[a] Frozen serum samples from 47 patients were obtained from the Clinical Laboratory. There were 24 samples from patients with a monoclonal free κ protein and 23 from patients with a monoclonal free λ protein. These serum samples had been saved because the serum monoclonal FLC had been difficult to identify by IFE or because the serum was negative by IFE but the urine contained a monoclonal FLC detected by IFE. Two of the AL patients with a serum monoclonal λ FLC detected by IFE did not have concurrent urine samples available for testing. Four of the patients had a negative urine IFE on the concurrent sample, but these patients had a previous urine that had been positive for a monoclonal FLC by IFE. The serum IFE, urine IFE, serum FLC, and serum FLC K/L results are displayed. Patients with a history of a monoclonal κ or λ light chain had a previous urine sample that was positive for monoclonal light chain by IFE.

[b] History of urine with monoclonal κ by IFE.

[c] Indolent MM.

[d] NSMM.

[e] NA, not available.

[f] History of urine with monoclonal λ by IFE.

Table 5. LCDD: Serum FLC results.[a]

	Abnormal/Sera tested, n	
	FLC	FLC K/L
Serum, IFE κ positive	8/9	8/9
Serum, IFE λ positive	2/3	3/3
Serum, IFE negative; urine, IFE κ positive	4/4	4/4
Serum, IFE negative; urine, IFE negative; bone marrow, κ positive	1/3	2/3

[a] The κ FLC, λ FLC, and FLC K/L were determined for 19 serum samples from patients with LCDD. The fractions of patients with abnormal serum FLC and FLC K/L results are listed.

result by the cystatin C result eliminates the apparent dependence of FLC on age. The increase in FLC values is therefore likely attributable to reduced kidney function and not to age per se. Because many patients with monoclonal gammopathies also have decreased renal function and proteinuria, these could be confounding factors in interpreting FLC measurements. The FLC K/L, however, is not affected by renal function and is therefore the most straightforward representation of the data for diagnostic testing. In the group of 25 samples from patients with polyclonal hypergammaglobulinemia, the FLC values were also increased. The increment in this patient group was presumably not attributable to reduced kidney function but to increased immunoglobulin synthesis. The FLC K/L in this group of samples also normalized the κ and λ FLC increases, such that no samples had an abnormal FLC K/L.

Using the reference intervals and diagnostic ranges developed in this study, we assessed the relative sensitivity of the FLC assay for detecting monoclonal κ and λ FLC concentrations in sera of a cohort of patients who had a variety of monoclonal gammopathies. In this small, selected group of samples, the FLC measurements had a higher sensitivity than did IFE for detecting small concentrations of monoclonal light chains in serum. Interestingly, those serum samples that were negative by IFE had FLC concentrations that were similar to those in samples with positive IFE results. The lower sensitivity of the serum IFE assay in this group may be attributable to polyclonal immunoglobulins that obscure the small, monoclonal FLC band. The binding of the FLC assay is reported to be >10 000-fold higher for FLCs in comparison with the light chains bound to heavy chains in intact immunoglobulin *(4)*. This binding preference may allow detection of a small increase in FLC concentration when polyclonal immunoglobulins are present. Alternatively, the serum IFE assay may not detect FLCs in some of these samples because of polymerization of monoclonal light chains. The polymerization of some light chains yields complexes that electrophorese in very broad patterns that are not recognized as monoclonal light chains *(5)*. The increased sensitivity of the FLC K/L compared with serum IFE makes the FLC method a useful diagnostic assay. Primary systemic amyloidosis and LCDD are often difficult to diagnose, and the presence of a monoclonal FLC is an important differential diagnostic clue. The sensitivity of the IFE method in serum or urine is ~70% for AL and ~90% when both serum and urine assays are performed. The enhanced diagnostic sensitivity may be useful for disease detection and may be an additional laboratory assessment for patients suspected of having a light-chain disease.

Table 6. FLC K/L: Comparison of reference intervals and diagnostic ranges.[a]

	Reference interval (0.3–1.2)		Diagnostic range (0.26–1.65)	
	Estimate	95% CI[b]	Estimate	95% CI
Sensitivity, %	98	91–100	97	89–100
Specificity, %	95	92–98	100	98–100
PPV, %	78	65–89	100	91–100
NPV, %	100	98–100	99	97–100
Accuracy, %	96	93–98	99	98–100

[a] Sera from the 282 reference individuals and 25 polyclonal hypergammaglobulimia patients as well as the 66 sera from the AL, LCDD, and MM patients were used to calculate the sensitivity, specificity, PPV, NPV, and accuracy of the FLC K/L ratio. The PPV and NPV assumed a prevalence of 15% in the test population. Both the 95% central reference interval and the diagnostic range were evaluated.

[b] CI, confidence interval.

The FLC data for the 19 LCDD serum samples demonstrate the differences between the FLC and IFE assays. Seven of these samples were negative for a serum monoclonal light chain by IFE, and 6 of these had an abnormal FLC K/L. Surprisingly, the FLC K/L was not increased in one of nine sera with a monoclonal κ light chain detected by IFE. This serum sample was retested, and the IFE and FLC results were reproducible. The κ FLC value in this sample was at the low end of the usual reference interval and was much lower than the other eight samples in this group. The assay selectivity for cryptic light-chain sites that are "hidden" when bound to heavy-chain proteins most likely depends on reactivity with very few sites on the light chain. We speculate that the lack of reactivity with the monoclonal light chain from this patient may have been attributable to truncation of the light chains and the consequent loss of antigenic sites. The increased sensitivity of the FLC assay compared with the serum IFE assay, coupled with the inability of the FLC assay to detect certain light chains, suggests that these two assays be used as complementary diagnostic tests.

In addition to the increased sensitivity and diagnostic potential of the FLC assays, the ability to quantify monoclonal FLCs may be useful to monitor the disease process in light-chain diseases. The disease course of AL can be difficult to assess and is currently monitored by evaluating organ function (e.g., kidney function by urine protein measurements). Changes in organ function, however, may take a long time to manifest, and therefore, direct

measurements of the serum or urine M-spike provide a real-time marker for monitoring AL. Fewer than one-half of AL patients have a measurable M-spike, and the quantification of FLC may provide a more universal and timely assessment of disease activity. Studies correlating disease activity and FLC quantification remain to be done.

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