Clinical Chemistry 49:8
1252–1257 (2003)

Minireview

Practical Considerations for the Measurement of Free Light Chains in Serum

Jillian R. Tate,[1*] Devinder Gill,[2] Ralph Cobcroft,[2] and Peter E. Hickman[1]

Background: **A new immunoassay for free light chain measurements has been reported to be useful for the diagnosis and monitoring of monoclonal light chain diseases and nonsecretory myeloma. We describe experience with and some potential pitfalls of the assay.**
Methods: **The assay was assessed for precision, sample type and stability, recovery, and harmonization of results between two analyzers on which the reagents are used. Free-light-chain concentrations were measured in healthy individuals (to determine biological variation), patients with monoclonal gammopathy of undetermined significance, myeloma patients after autologous stem cell transplants, and patients with renal disease.**
Results: **Analytical imprecision (CV) was 6–11% for κ and λ free-light-chain measurement and 16% for the calculated κ/λ ratio. Biological variation was generally insignificant compared with analytical variation. Despite the same reagent source, values were not completely harmonized between assay systems and may produce discordant free-light-chain ratios. In some patients with clinically stable myeloma, or post transplantation, or with monoclonal gammopathy of undetermined significance, free-light-chain concentration and ratio were within the population reference interval despite the presence of monoclonal intact immunoglobulin in serum. In other patients with monoclonal gammopathy of undetermined significance, values were abnormal although there was no clinical evidence of progression to multiple myeloma.**
Conclusions: **The use of free-light-chain measurements alone cannot differentiate some groups of patients with monoclonal gammopathy from healthy individuals. As with the introduction of any new test, it is essential that more scientific data about use of this assay in different subject groups are available so that results can be interpreted with clinical certainty.**
© 2003 American Association for Clinical Chemistry

Currently there is no satisfactory method to diagnose and monitor patients with light-chain myeloma, nonsecretory myeloma (NSM),[3] or primary (AL) amyloidosis. An assay for serum free light chains (FLCs) has recently become available commercially for use as a marker for monoclonal gammopathies. FLC testing is more sensitive for the detection of monoclonal light-chain diseases and NSM than previous tests. It can detect two-thirds of NSMs *(1)* that were previously missed unless more complex and expensive bone marrow investigations were performed and may permit the earlier detection of Bence Jones protein (BJP) myeloma *(2)*. The increased sensitivity of the new test over current methods may also permit increased detection of monoclonal FLCs in AL amyloidosis, with 88–98% of the monoclonal protein reported to be detected by FLC measurement compared with the 76–79% detected by traditional electrophoretic and immunofixation methods *(3, 4)*. Another potential use is earlier identification of disease recurrence in myeloma and amyloid patients after chemotherapy or transplantation *(5)* and the recurrence of myeloma for patients in apparent complete remission after autologous peripheral blood stem cell transplantation (PBSCT) *(6)*. Importantly, the quantification of serum FLCs can be used to monitor monoclonal light-chain disease, NSM, and AL amyloidosis *(3, 5)* and has the potential to reduce the number of bone marrow biopsies that are performed at regular intervals in these patients.

We have identified several practical issues for the analytical measurement of FLCs in serum. Many of these

[1] Chemical Pathology and [2] Haematology Departments, Queensland Health Pathology Service, Princess Alexandra Hospital, Woolloongabba, Queensland 4102, Australia.
*Address correspondence to this author at: Chemical Pathology Department, Queensland Health Pathology Service, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, Queensland 4102, Australia. Fax 61-7-3240-7070; e-mail jill_tate@health.qld.gov.au.
Received February 26, 2003; accepted May 23, 2003.

[3] Nonstandard abbreviations: NSM, nonsecretory myeloma; AL amyloidosis, primary amyloidosis; FLC, free light chain; BJP, Bence Jones protein; PBSCT, peripheral blood stem cell transplantation; KFLC, κ free light chain; LFLC, λ free light chain; K/L FLC ratio, κ/λ free light chain ratio; M-protein, monoclonal protein; MM, multiple myeloma; and MGUS, monoclonal gammopathy of undetermined significance.

issues are common to all new immunoassays but are especially important when there is a single reagent source and no international standard. The assay is now available on several different platforms, including the Immage™ Protein System (Beckman Coulter), the BNII™ and BN ProSpec™ Nephelometers (Dade-Behring), the Hitachi 917 (Roche Diagnostics), and The Binding Site MiniNeph system. Despite use of the same source of commercial reagent (The Binding Site Ltd.), this does not necessarily lead to harmonization of FLC values among assay systems that use different method principles, e.g., immunonephelometric assays and immunoturbidimetric assays, or between lots of the polyclonal anti-human FLC antiserum. Variations in reagent lots and methods may affect results for patients who are monitored serially over a long period of time and may compromise the test's clinical utility.

Analytical Issues

We describe various analytical and preanalytical issues that may affect the performance and clinical interpretation of FLC measurements in serum and discuss the potential pitfalls of this new immunoassay.

METHOD HARMONIZATION

In a FLC comparison study of the Immage and ProSpec analyzers using 37 patient samples, Deming regression analysis of FLC concentrations indicated some degree of divergence in results between methods for κ FLC (KFLC) and λ FLC (LFLC) concentrations. For KFLCs in the low measuring range (3.5–19 mg/L), the regression for the ProSpec vs the Immage was: ProSpec KFLC = 1.09(Immage KFLC) − 1.6 mg/L (R^2 = 0.81; n = 24); for LFLCs in the measuring range 3.7–26 mg/L, the regression was: ProSpec LFLC = 1.28(Immage LFLC) − 2.4 mg/L (R^2= 0.87; n = 29). The mean difference between methods for KFLC concentration was −0.7 mg/L (95% confidence interval, −4.7 to 3.3 mg/L), and for LFLC concentration, the mean difference was 0.8 mg/L (−4.2 to 5.8 mg/L). For higher KFLC concentrations (20–168 mg/L), the regression was: ProSpec KFLC = 1.24(Immage KFLC) − 11.0 mg/L (R^2 = 0.95; n = 12); for LFLC in the range 29–100 mg/L, the regression was: ProSpec LFLC = 1.17(Immage LFLC) + 3.3 mg/L (R^2 = 0.96; n = 7). The mean differences between methods were 1.5 mg/L (95% confidence interval, −22.2 to 25.2 mg/L) for KFLC concentration and 11.3 mg/L (−0.5 to 23.1 mg/L) for LFLC concentration, with the difference for LFLC with the ProSpec assay being positive.

For the κ/λ (K/L) FLC ratio, the mean difference for 36 samples was −0.06 (95% confidence interval, −1.5 to 1.4). Concordance for the K/L FLC ratio calculated from KFLC and LFLC concentrations measured for 33 samples with the Immage and ProSpec assays is shown in Fig. 1 for ratios up to 3.2. K/L FLC ratios for the other four samples were in clinical agreement (Immage vs ProSpec: 4.4 vs 7.3, 8.2 vs 6.1, 17.2 vs 18.6, and 221 vs 323). For three samples,

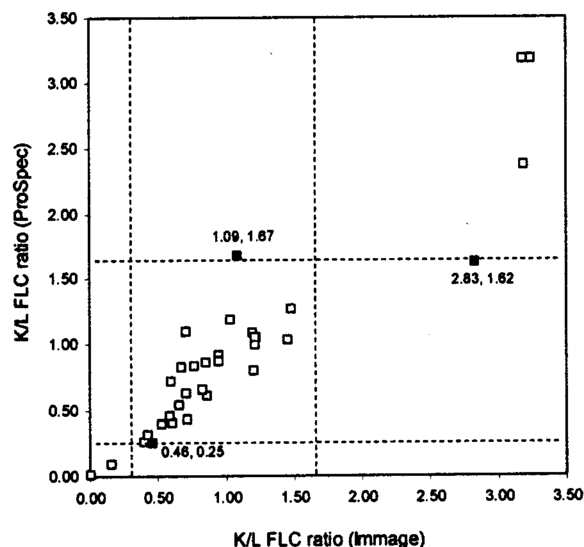

Fig. 1. Concordance plot of K/L FLC ratio calculated from KFLC and LFLC concentrations measured in 33 samples by the Immage and ProSpec assays.

K/L FLC ratios were in the range 0.01–3.2. The *dashed lines* represent the limits of the population reference interval for K/L FLC ratio (0.26–1.65). For three samples, K/L ratios were discordant as indicated on the plot (■; values are Immage ratio, ProSpec ratio) and are described in the text.

K/L ratios were discordant between methods according to the population reference interval for the K/L FLC ratio of 0.26–1.65 [The Binding Site product information *(7)*]. KFLC, LFLC, and K/L ratio values measured with the Immage analyzer for one sample were 32 mg/L, 70 mg/L, and 0.46, respectively, vs 20 mg/L, 80 mg/L, and 0.25 for the ProSpec analyzer; for the other two samples, KFLC, LFLC, and K/L ratio values were 14 mg/L, 13 mg/L, and 1.09 vs 20 mg/L, 12 mg/L, and 1.67; and 71 mg/L, 25 mg/L, and 2.83 vs 66 mg/L, 41 mg/L, and 1.62. K/L FLC ratios for all other samples were in agreement between the methods. Possible causes of discordant results among instruments include differences in analytical performance, e.g., linearity, or the effect of FLC analytical imprecision on the calculation of K/L FLC ratio, in particular at the limits of the population reference interval. These are discussed below.

OVER- OR UNDERESTIMATION OF FLC CONCENTRATION

The measurement of any monoclonal protein (M-protein), including FLCs, is subject to possible over- or underestimation on dilution in immunonephelometric and immunoturbidimetric assay immunochemical methods *(8)* and should be recognized as a potential source of error when measuring any M-protein. Intact monoclonal IgM protein is especially prone to overestimation by immunonephelometric assays because of differences in the immunoreactivity of monoclonal IgM compared with the polyclonal IgM calibrator. Such nonparallel immunoreactivity can

potentially occur for any intact monoclonal immunoglobulin or FLC on dilution *(9)* and is a property of the M-protein. The extent of this nonparallelism may vary among methods and can yield different measured M-protein concentrations *(8)*. A nonlinear assay response may also contribute to a lower recovery of FLCs on dilution of the M-protein. For example, our preliminary data showed a recovery of 62% for KFLCs for the 10-fold dilution of a monoclonal serum κ BJP measured by the ProSpec assay and 85% recovery by the Immage assay. We therefore speculate that monitoring of the FLC concentration over time in some patients is likely to be susceptible to variation depending on the selected sample dilution and the nature of the monoclonal FLC.

Serum FLC values may not correspond to the M-protein concentration quantified from electrophoretic patterns by use of densitometry and total protein or globulin concentration. If monoclonal FLCs undergo polymerization in serum, the M-protein may not be visible as a discrete band on electrophoresis but is present as a smear of different FLC polymeric forms *(10)*. It has been reported from gel filtration analysis that in sera from patients with NSM and multiple myeloma (MM), FLCs may exist in multimeric forms in addition to the usual KFLC monomers and LFLC dimers present in serum *(9, 11)*. A nephelometric overreading of FLC concentration of between 1.5- and 3.5-fold has been reported for NSM sera relative to other myeloma sera *(11)*. In a patient with an M-protein concentration of 23 g/L, determined by electrophoresis and consisting of λ light chain dimers, the LFLC concentration was measured at 344 g/L and was overestimated because of the reaction of trimolecular aggregates of the monoclonal λ FLC with the antibody *(9)*.

Monoclonal FLC concentrations may be similarly overestimated in urine with values higher than those quantified by electrophoresis and total urine protein concentration. Urinary FLC concentrations were reported to be 3–1609% higher by immunoassay compared with estimation by electrophoresis and the sodium dodecyl sulfate–pyrogallol red–molybdate protein assay *(12)* and up to 185% higher compared with electrophoresis and benzethonium chloride protein assay (data from our own studies). A lower reactivity of the protein reagent to monoclonal FLC in urine compared with albumin may be one reason for the discrepancy. Generally, urinary protein reagents do not show equal reactivity for albumin, γ-globulin, and tubular proteins *(13)*, with BJPs reported to be underestimated by 32–48% in a pyrogallol red–molybdate protein assay *(14)*.

Imprecision of FLC Measurements

KFLC and LFLC values in healthy individuals occur at the low end of the measuring range, with the manufacturer's reference intervals being 3–19 mg/L for KFLC and 6–26 mg/L for LFLC. Typically, in myeloma the monoclonal FLC is increased above the upper limit of the reference interval and the other, normal FLC may be within the reference interval, suppressed below the lower limit of the range, or increased if there is concurrent renal impairment. In myeloma patients who have received autologous stem cell transplants and are in complete remission, the monoclonal FLC concentration is usually within the reference interval, and the K/L FLC ratio normalizes. Performance specifications require immunoassays to show acceptable precision and linearity over the measuring range of the assay as well as suitable analytical sensitivity. The use of the latex-enhanced FLC polyclonal antibody is designed to decrease assay lower limit of detection to a FLC concentration of 2–3 mg/L. However, unless the analyzer cuvettes are washed regularly or are disposable, the polystyrene latex particles and their protein complexes can coat the cuvette surface and adversely affect assay precision. Imprecision for the Immage FLC assays was determined with the manufacturer's quality controls and a fresh-frozen serum from a healthy individual. The between-run CV was 10%, 7.4%, 7.3%, and 6.0% at KFLC concentrations of 7.1, 13.5, 27.2, and 39.7 mg/L (13–21 runs; three different reagent lots), respectively, and 11%, 11%, and 7.6% at LFLC concentrations of 10.6, 27.2, and 53.4 mg/L (13–21 runs; two different reagent lots), respectively (data from our own studies). Imprecision can increase if the instrument manufacturer's suggestions for cuvette washing are not followed. Without daily washing of the Immage cuvettes with the manufacturer's wash diluent, CVs as high as 19% and 35% were observed for KFLC and LFLC assays, respectively (our own data). To achieve maximally low imprecision for FLC assays performed on analyzers with reusable cuvettes, it is desirable to wash the cuvettes daily, in particular before FLC analyses, followed by a recalibration before proceeding with the batch analysis of patient samples. In our hands this procedure reduced imprecision, especially at the low end of the FLC measuring range.

The imprecision of either or both KFLC and LFLC measurements accentuates the error in the K/L ratio and leads to clinical uncertainty. For a K/L FLC ratio of 0.67 (7.1 mg/L KFLC and 10.6 mg/L LFLC), which is within the reference interval, total analytical imprecision (CV) was 15.5% and yielded a 95% confidence interval of 0.47–0.87 *(15)*. The three clinically discordant K/L FLC ratios shown in Fig. 1 may be partly explained by assay imprecision. Taking into account total imprecision of the ratio determined at relevant FLC concentrations with the ProSpec assay, the 95% confidence intervals for K/L ratios of 0.25, 1.67, and 1.62 were 0.19–0.31, 1.27–2.07, and 1.26–1.98, respectively. For the same samples assayed by the Immage assay, the confidence intervals for K/L ratios of 0.46, 1.09, and 2.83 were 0.37–0.55, 0.77–1.41, and 2.00–3.66, respectively, and were dissimilar from the ProSpec ratio for two of the three samples. Ratios that border on the cutoff limits of the reference interval may be misclassified as normal or abnormal, and one might expect other discordant results by simply repeating the assay on those samples with ratios close to the reference

limits. Because of the method imprecision, borderline low or high samples should be repeat tested to verify clinical classification.

PREANALYTICAL ISSUES

Together with analytical imprecision, preanalytical variables such as specimen type and stability and biological variation may contribute to the variation in FLC measurements within individuals and may add to clinical uncertainty. The effect of sample stability and specimen type was tested. In a small study, serum and lithium-heparin plasma samples were collected from six individuals with and without serum M-protein, and FLC concentrations measured with the Immage assay were compared between sample types. No statistically significant difference was observed between serum and plasma when FLC values were analyzed by the two-tailed paired *t*-test. The mean (SD) KFLC value for serum was 25.4 (13.8) mg/L, and for lithium-heparin plasma it was 25.6 (13.9) mg/L ($P < 0.65$). The mean (SD) LFLC value for serum was 85.8 (146.2) mg/L, and for lithium-heparin plasma it was 86.0 (146.6) mg/L ($P < 0.54$).

Stability of FLCs in serum was assessed after storage of serum or lithium-heparin plasma at 2–8 °C, −20 °C, and −70 °C for 1 week. FLC concentrations were determined in the stored samples collected from four individuals with and without M-protein, and values were compared with those obtained after storage of up to 24h at 2–8 °C. No statistically significant difference was observed between the FLC values for samples stored at the three temperatures for 1 week when compared with the fresh or 1-day-old samples (KFLC and LFLC in fresh samples vs samples stored at 2–8 °C for 1 week, $P < 0.38$; vs samples stored at −20 °C, $P < 0.39$; vs samples stored at −70 °C, $P < 0.34$). The variations in FLC concentrations for the 4 individuals was 2.7–8.0%, and the variation for the K/L ratio was 3.8–11.4%, with the greatest variation occurring in a κ BJP myeloma patient with increased KFLCs (measured values, 2144–2411 mg/L) and low-normal LFLC concentrations (6.0–7.0 mg/L).

BIOLOGICAL VARIATION

No data are currently available in the literature on the biological variation of serum FLCs. In a group of 10 apparently healthy individuals with normal renal function (five males, 26–50 years of age; five females, 23–50 years of age), singlicate FLC measurements of two serum samples collected up to 8 days apart from each individual were analyzed within the one run in the ProSpec assay. Subtraction of within-run FLC imprecision from the total imprecision gave intraindividual CVs <2.5% for KFLC, LFLC, and K/L FLC ratio in 8 of 10 individuals. In the two other individuals, variation of the K/L ratio was 7–8% and reflected a variation of 11–17% in KFLC concentration. Because the biological variation was generally insignificant compared with the analytical variation, a lowering of the FLC assay imprecision would be productive and reduce measurement uncertainty. This is particularly desirable within the FLC reference interval.

Clinical Issues

Information about serum FLC measurement in nonsecretory myeloma, monoclonal light chain diseases, and reference populations is reported in the literature (*1–5*, *7*), but there is limited data about FLCs in renal disease, monoclonal gammopathy of undetermined significance (MGUS), and myeloma patients after autologous stem cell transplants.

INTERCURRENT ILLNESS AND FLC CONCENTRATION

Increased FLC concentrations can occur not only when M-proteins are present but also when immunoglobulin synthesis is increased, such as in autoimmune diseases, e.g., systemic lupus erythematosus, or when kidney function is decreased and FLCs are retained for a longer time in the blood circulation. In a group of 13 patients with acute or chronic renal failure of various etiologies, including secondary amyloidosis, and end-stage renal failure on hemodialysis, FLC concentrations were increased in all patients, but the K/L FLC ratio was within the population reference interval (KFLC, 45–166 mg/L; LFLC, 32–347 mg/L; K/L ratio, 0.35–1.39). It has been shown that dialysis does not normalize increased serum concentrations of FLC in end-stage renal failure patients *(16)*. In the presence of a plasma cell dyscrasia, such as MM, NSM, or AL amyloidosis, an increased serum concentration of the abnormal, monoclonal FLC is expected *(1–4)*. In a few of these cases, FLC concentrations may be within the reference interval although the K/L ratio may be abnormal *(1)*. It is therefore important to incorporate the calculated K/L FLC ratio with FLC concentrations when interpreting clinical results.

MONOCLONAL GAMMOPATHIES

Other patient groups for which serum FLC measurement is likely to be useful and possibly an earlier marker of disease relapse or progression of MM include autologous PBSCT-treated myeloma patients in complete remission *(17)* and patients with MGUS. Serum K/L FLC ratios in a group of 10 myeloma patients who had received autologous stem cell transplant and were in complete remission (47–68 years) were 0.55–1.18 and within the manufacturer's population reference interval of 0.26–1.65, although FLC concentrations were not normalized in all patients (KFLC, 7–30 mg/L; LFLC, 13–42 mg/L). However, clinically stable PBSCT-treated myeloma patients who show no evidence of myeloma on bone marrow biopsy 6–12 weeks after transplantation but have residual M-protein can also have normalized K/L ratios. In eight patients with stable disease and intact serum immunoglobulin (2–19 g/L) or trace urine BJP, the KFLC concentration was 4–30 mg/L, the LFLC concentration was 9–40 mg/L, and the K/L ratio was 0.23–2.9 (for monoclonal κ M-protein,

the K/L ratio was 0.62–2.9; for monoclonal λ M-protein, the K/L ratio was 0.23–0.96).

In MGUS patients, the K/L FLC ratio and concentration may be within population reference intervals despite the presence of monoclonal intact immunoglobulin concentrations ≤30 g/L in serum *(18)*. The use of FLC measurements alone cannot differentiate this group from healthy individuals. Conventional serum/urine protein electrophoresis and immunofixation testing is still required in this patient group to detect M-protein. In a group of 32 MGUS patients (10 males, 40–73 years of age, 0–17 years since diagnosis; 22 females, 45–79 years of age, 0–22 years since diagnosis) with a current serum M-protein concentration of 1–22 g/L and clinically stable disease, the KFLC concentration was 7–1018 mg/L, the LFLC concentration was 9–211 mg/L, and the K/L ratio ranged from 0.10 to 48 (Table 1). In 6 of 13 patients with monoclonal κ M-protein, the K/L ratio was >1.65, and in 3 of 19 with monoclonal λ M-protein, the ratio was <0.26. Where urine samples were available for analysis, at or

Table 1. Serum KFLC, LFLC, and M-protein concentrations, urine BJP, and K/L FLC ratios for 32 MGUS patients.

Years since diagnosis	M-Protein			KFLC,[c] mg/L	LFLC,[c] mg/L	K/L FLC ratio[d]	Urine BJP
	Type[a]	Initial,[b] g/L	Current,[b] g/L				
				Reference interval: 3–19 mg/L	Reference interval: 6–26 mg/L	Population reference interval: 0.26–1.65	
4	IgML	16	22	7	67	0.10[e]	ND[f]
17	IgGL	20	18	14	73	0.19[e]	ND
2	IgGL	17	18	14	69	0.20[e]	λ BJP (IFE)[g]
9	IgML	1	3	20	69	0.29	Not detected
2	IgAL	6	7	14	48	0.29	Not detected
8	IgGL	1	15	16	53	0.30	ND
6	IgGL	9	10	10	25	0.40	λ BJP (IFE)[g]
8	IgGL	10	11	8	19	0.42	ND
4	IgAL	4	3	16	37	0.43	ND
7	IgAL	5	8	8	18	0.44	ND
4	IgGL	7	11	24	53	0.45	ND
1	IgGL	5	6	14	29	0.48	ND
2	IgGL	4	4	16	32	0.50	ND
0	IgGL	15	15	110	211	0.52	λ BJP (IFE)
22	IgGK	15	17	19	33	0.58	47 mg/day κ BJP[g]
5	IgGL	7	6	21	32	0.66	ND
5	IgGK	2	1	52	72	0.72	ND
2	IgML	10	9	19	26	0.73	ND
13	IgGK	10	9	30	41	0.73	ND
2	IgGK	6	8	38	50	0.76	ND
4	IgGK	3	5	17	17	1.00	ND
6	IgGL	4	5	27	27	1.00	Trace λ BJP[g]
0	IgGL	5	4	32	28	1.14	λ BJP (IFE)
0	IgAK	20[h]	20[h]	164	132	1.24	ND
4	IgGK	8	9	33	25	1.32	ND
1	IgMK	2	3	16	10	1.60	ND
5	IgMK	5	9	21	11	1.91[e]	20 mg/day κ BJP[g]
4	IgGK	12	11	21	11	1.91[e]	ND
1	IgAK, κ BJP (IFE)	8	9	42	15	2.8[e]	κ BJP (IFE)[g]
8	IgMK	8	6	72	9	8.0[e]	ND
7	IgGK	16	20	238	15	15.9[e]	κ BJP (IFE)[g]
1	IgGK, κ BJP (IFE)	10	10	1018	21	48[e]	104 mg/day κ BJP[g]

[a] M-Protein type was determined by immunofixation electrophoresis.

[b] M-Protein concentration was determined by densitometry after electrophoresis and protein staining of agarose gels (Beckman Coulter), or in the case of M-proteins overlapping other globulin fractions, by immunonephelometric assay with the Immage analyzer and immunoglobulin reagents (Beckman Coulter).

[c] KFLC and LFLC concentration were determined with the Immage analyzer and FLC reagents (The Binding Site Ltd.); FLC reference intervals are those quoted in the manufacturer's package insert and in Ref. *(7)*.

[d] K/L FLC ratio was calculated with use of KFLC and LFLC values; the population reference interval is that quoted in the manufacturer's package insert and in Ref. *(7)*.

[e] K/L FLC ratio outside the reference interval.

[f] ND, not determined; IFE, immunofixation electrophoresis.

[g] Urinary BJP was detected before FLC assay.

[h] M-Protein concentration was determined by immunonephelometric assay and consisted of monoclonal and polyclonal IgA.

before the time of FLC testing, BJP was detected in the urine of 10 MGUS patients, whereas immunofixation electrophoresis detected BJP in the serum of 2 of these patients (Table 1). Five of the 10 patients had an abnormal K/L FLC ratio, and 104 mg of κ BJP was excreted per day by the individual with a ratio of 48. However, BJP was also present in five other MGUS patients with ratios within the reference interval; one of these patients excreted 47 mg of κ BJP per day. Although BJP may be present in MGUS patients with FLC ratios within the reference interval, we speculate from these preliminary data that urinary BJP is likely to be present in MGUS patients with abnormal serum FLC ratios. It is possible that an abnormal FLC ratio in combination with urine BJP may predict those MGUS patients who are likely to progress to MM. Larger studies and longer follow-up times are required to confirm the significance of these findings.

Conclusions

The use of serum FLC measurements offers great promise for the investigation of monoclonal gammopathies, in particular NSM and AL amyloidosis, which are notoriously difficult to diagnose and monitor after treatment. As with the introduction of any new test, there are some reservations because the assay is not 100% sensitive or specific when used in isolation. It is essential that more scientific data about the use of this assay in different subject groups and its potential pitfalls are available before the test is used routinely in clinical laboratories. In addition, no international standard for FLC is available. At this time, only one manufacturer is producing the assay; therefore, between-method standardization is not relevant. However, as other commercial FLC assays are produced, it would be desirable that there is harmonization of FLC values among methods and that diagnostics manufacturers seek traceability of their master calibrators to The Binding Site's primary, secondary, or tertiary standards *(19)*.

We wish to acknowledge The Binding Site Ltd. (Birmingham, United Kingdom) for providing half-price FLC reagents for analysis.

References

1. Drayson M, Tang LX, Drew R, Mead GP, Carr-Smith H, Bradwell AR. Serum free light-chain measurements for identifying and monitoring patients with nonsecretory multiple myeloma. Blood 2001;97: 2900–2.
2. Abraham RS, Clark RJ, Bryant SC, Lymp JF, Larson T, Kyle RA, et al. Correlation of serum immunoglobulin free light chain quantification with urinary Bence Jones protein in light chain myeloma. Clin Chem 2002;48:655–7.
3. Abraham RS, Katzmann JA, Clark RJ, Bradwell AR, Kyle RA, Gertz MA. Quantitative analysis of serum free light chains. A new marker for the diagnostic evaluation of primary systemic amyloidosis. Am J Clin Pathol 2003;119:274–8.
4. Lachmann HJ, Gallimore R, Gillmore JD, Smith L, Bradwell AR, Hawkins PN. Detection of monoclonal free light chains by nephelometry in 262 patients with systemic AL amyloidosis [Abstract]. Clin Chem 2002;48(Suppl 6):A164.
5. Lachmann HJ, Gallimore R, Gillmore JD, Smith L, Bradwell AR, Hawkins PN. Correlation of changes in nephelometric quantification of serum monoclonal free light chains following chemotherapy and outcome in 137 patients with systemic AL amyloidosis [Abstract]. Clin Chem 2002;48(Suppl 6):A165.
6. Tate JR, Grimmett K, Mead GP, Cobcroft R, Gill D. Free light chain ratios in the serum of myeloma patients in complete remission following autologous peripheral blood stem cell transplantation [Abstract]. Clin Chem 2002;48(Suppl 6):A166.
7. Katzmann JA, Clark RJ, Abraham RS, Bryant S, Lymp JF, Bradwell AR, et al. Serum reference intervals and diagnostic ranges for free κ and free λ immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. Clin Chem 2002;48:1437–44.
8. Whicher JT, Warren C, Chambers RE. Immunochemical assays for immunoglobulins. Ann Clin Biochem 1984;21:78–91.
9. Abraham RS, Charlesworth MC, Owen BAL, Benson LM, Katzmann JA, Reeder CB, et al. Trimolecular complexes of λ light chain dimers in serum of a patient with multiple myeloma. Clin Chem 2002;48:1805–11.
10. Sölling K. Polymeric forms of free light chains in serum from normal individuals and from patients with renal diseases. Scand J Clin Lab Invest 1976;36:447–52.
11. Mead GP, Stubbs PS, Carr-Smith HD, Drew RL, Bradwell AR. Nephelometric measurement of serum free light chains in nonsecretory myeloma [Abstract]. Clin Chem 2002;48 (Suppl 6):A23.
12. Le Bricon T, Bengoufa D, Benlakehal M, Bousquet B, Erlich D. Urinary free light chain analysis by the Freelite® immunoassay: a preliminary study in multiple myeloma. Clin Biochem 2002;35: 565–7.
13. McElderry LA, Tarbit IF, Cassells-Smith AJ. Six methods for urinary protein compared. Clin Chem 1982;28:356–60.
14. Watanabe N, Kamei S, Ohkubo A, Yamanaka M, Ohsawa S, Makino K, et al. Urinary protein as measured with a pyrogallol red-molybdate complex, manually and in a Hitachi 726 automated analyzer. Clin Chem 1986;32:1551–4.
15. Badrick T, Hickman PE. Confidence intervals and free prostate-specific antigen. Clin Chem 1998;44:1789.
16. Cohen G, Rudnicki M, Schmaldienst S, Hörl WH. Effect of dialysis on serum/plasma levels of free immunoglobulin light chains in end-stage renal disease patients. Nephrol Dial Transplant 2002; 17:879–83.
17. Badros A, Barlogie B, Siegel E, Roberts J, Langmaid C, Zangari M, et al. Results of autologous stem cell transplant in multiple myeloma patients with renal failure. Br J Haematol 2001;114: 822–9.
18. Kyle RA, Therneau TM, Rajkumar SV, Offord JR, Larson DR, Plevak MF, et al. A long-term study of prognosis in monoclonal gammopathy of undetermined significance. N Engl J Med 2002;346:564–9.
19. Bradwell AR, Carr-Smith HD, Mead GP, Tang LX, Showell PJ, Drayson MT, et al. Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. Clin Chem 2001;47:673–80.