Commentary

Quantitative Serum Free Light Chain Assay – Analytical Issues

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Abstract
Serum free light chain (FLC) assay is an important advance in the diagnosis and monitoring of monoclonal light chain diseases and a complementary test to serum protein electrophoresis and immunofixation. Immunoturbidimetric and immunonephelometric assays for serum FLC are available on routine chemistry analysers and can detect FLC down to ~1 mg/L. These assays use polyclonal anti-human FLC antisera and require acceptable imprecision, specificity, accuracy, and reproducibility between reagent batches to prevent under- or over-estimation of FLC concentration.

Assay imprecision determined between reagent lots has a variation of 8-45% for FLC concentrations and 17-32% for the calculated κ/λ FLC ratio. Dilution studies indicate some over-recovery of FLC, which may depend upon the dilution matrix. However, greater discrepancies are underestimation from nonlinear reactions and overestimation possibly from interferences or multi-reactivity to polymeric FLC. Nonlinear monoclonal FLC give concentrations which are 2- to 6-fold increased at higher sample dilution and FLC measured on different platforms may not give the same results.

Laboratory staff and clinicians should be aware of the analytical limitations of the FLC assay. Assay imprecision, especially with different lots of FLC reagent, may have a significant effect on changes in the FLC concentration and κ/λ FLC ratio. Sample dilution anomalies have the potential to confound result interpretation for patients with monoclonal light chain disease. These issues, if not adequately appreciated, have the potential to mislead clinical diagnosis and assessment of response to therapy.

Serum free light chain (FLC) assay came into routine clinical laboratories following the publication in 2001 describing the presence of monoclonal FLC in 19/28 non-secretory myeloma (NSMM) patients at diagnosis.[1] Use of the assay has grown globally since that time and other retrospective clinical studies have shown the clinical utility of FLC in serum as a complementary test to serum protein electrophoresis (SPEP) for the diagnosis and monitoring of monoclonal light chain diseases.

Monoclonal immunoglobulin free light chains are important tumour markers often present in serum and urine of patients with monoclonal gammopathies. Serum kappa (κ) and lambda (λ) FLC assays measure total polyclonal and monoclonal FLC in serum and the calculated κ/λ FLC ratio is a surrogate measure of clonality. Retrospective studies have shown serum FLC is clinically indicated for diagnosis and prognosis in plasma cell proliferative disorders, including primary amyloidosis (AL), NSMM, light chain multiple myeloma (LCMM), light chain deposition disease (LCDD) and solitary plasmacytoma; in documenting stringent complete response in multiple myeloma (MM); and for routine serial measurement to assess response in the oligosecretory diseases, NSMM, AL and LCDD.[2] Serial measurements may also be indicated in MM when serum M-protein is <10 g/L or urinary Bence Jones protein (BJP) excretion is <200 mg/24h.[3] However, there is currently no data to support its use in the monitoring of MM where disease is measurable by other methods such as SPEP. (Refer also to the review on FLC by J Katzmann in this issue).

Measurement of Serum FLC by Immunoassay
Both polyclonal and monoclonal antibody-based immunochemical methods have been developed for quantitation of FLC. Polyclonal antihuman-FLC antisera are prepared by immunising rabbits or sheep with a cocktail of BJPs, and adsorbing the product with IgG or Cohn fraction II to remove antibodies that react with bound immunoglobulin light chains.

The FLC antibodies must recognise only epitopes which are hidden in intact immunoglobulins to avoid falsely elevated FLC from cross-reaction. Ideally, polyclonal anti-human FLC antibodies target the constant domain of the light chains (C_L) which has little structural variation, and have adequate specificity and affinity to bind to individual monoclonal FLC. Non-reaction may occur if there are abnormal amino acid sequences or conformational changes of surface epitopes on C_L. Moreover, different batches of polyclonal anti-human FLC antiserum do not necessarily react in the same way.

Requirements for methods using monoclonal anti-human FLC antibodies raised in mice are similar to those for polyclonal antisera - high specificity to differentiate free light chains from bound immunoglobulin light chains, high affinity to ensure assay reproducibility, and diverse reactivity with heterogeneous FLC to avoid underestimating FLC concentration.[4-7] Monoclonal antibody-based FLC methods require antibodies to be directed to the C_L domains present in Cκ allotypes and Cλ isotypes on the FLC and to have equivalent immunoreactivity for all variable region (V_L) subgroups.[7,8] ELISA-based assays provide high assay sensitivity and detect low concentrations of FLC present in serum. Moreover, automated ELISA platforms allow simultaneous analysis of multiple serum sample dilutions and should permit easy detection of antigen excess and nonlinear FLC immunoreactivity (see below); however, commercial FLC assays in this format are not currently available.

The Problem of Calibration

Standardisation and traceability of serum FLC measurement require establishment of a reference measurement system consisting of a hierarchy of reference materials and higher level measurement procedures. An essential component of standardisation is a definition of the intended analyte, i.e. the measurand 'FLC in serum'. However, the amino acid sequence variation in polyclonal and monoclonal FLC results in a heterogeneous mixture of FLC molecules in serum, essentially precluding such a definition. Use of FLC antibodies targeted to epitopes on the C_L region of FLC should minimise the effects of sequence variation. However, polymerisation of FLC can yield oligomeric complexes that may cause overestimation of FLC concentration due to multi-reactivity by these polymers.[9] Therefore, optimal anti-FLC antibody specificity requires that an antiserum has equal affinity for individual polyclonal and monoclonal κ or λ FLC from all four Vκ and five Vλ subgroups and does not exhibit multi-reactivity to polymers of FLC. In the current commercial assay (Freelite ™, The Binding Site, Birmingham, UK) depending on how well assay antibodies recognise variations in individual molecular shapes and differences in FLC polymerisation, monoclonal FLC may react differently to the manufactured polyclonal assay calibrator.[10] There is also the potential for non-reaction of FLC reported to occur in AL [11] and on change of reagent lot in LCMM.[10]

Assay Specificity

As there is only a slight difference in antigenicity between free and bound light chains associated with heavy chains, intact immunoglobulins present in g/L in serum can potentially cross-react in FLC assays. Antibody specificity in the commercial FLC assays is directed to residues on the C_L domain with a cross-reactivity of <0.01% needed to prevent the influence of intact immunoglobulin. Current data indicates that normal serum immunoglobulin levels cause overestimation of only ~10% (3–4 mg/L, representing a cross-reactivity of about 0.05%) at normal FLC concentrations.[12] Monoclonal antibody-based FLC ELISAs are reported to give even lower cross-reactivity with intact immunoglobulins.[13]

Performance of Polyclonal Antibody-based FLC Method

Immunoassay methods for anti-human FLC antisera quantitation require adequate imprecision, specificity, accuracy, and reproducibility between reagent batches. The following method performance refers to experience over several years using the Freelite™ assays.

Limit of Detection (LoD)

Immunoturbidimetric (ITA) and immunonephelometric (INA) assays for serum FLC using polyclonal anti-FLC antisera are available on a range of routine chemistry analysers and can detect FLC down to ~1 mg/L. This compares with SPEP which has an LoD of 500 -2000 mg/L, serum immunofixation electrophoresis (IFE) of 100-150 mg/L and urine IFE of 20-50 mg/L, depending on whether urine is pre-concentrated and the sensitivity of the IFE method. Ultrasensitive IFE methods may in fact be more sensitive than serum FLC assay in some patients, as has been reported for monoclonal FLC in serum and urine of AL patients.[11]

Sample Requirements

Generally serum is the preferred sample type for ITA and INA methods. However, lithium-heparin plasma and citrated plasma have been validated by the manufacturer. Specimens are stable for at least one week when stored refrigerated at 2–8 °C or frozen at -20 °C and -80 °C.[14] Lipaemic and grossly haemolysed samples should be avoided and reference made to the manufacturer's package insert for acceptable haemoglobin and triglyceride concentrations for each FLC assay and platform.

Imprecision

Specifications for desirable FLC assay imprecision have not been determined from biological variation studies. Preliminary work using the Freelite™ FLC assay suggests intra-individual variation is low in healthy subjects (CVs <2.5% for κFLC,

λFLC, and κ/λ FLC ratio), and therefore, a lowering of the FLC assay imprecision would result in a lower measurement uncertainty of the calculated ratio.[14] The manufacturer states that FLC results are acceptable if the control values obtained are within ±20% of the specified concentration for quality control specimens matched with the FLC reagent kit. Imprecision, determined for repeat measurement of κFLC and λFLC in both normal and monoclonal samples, using several reagent lots, indicates variation of 8-45% for FLC concentrations and 17-32% for calculated κ/λ FLC ratio (Table 1).[10] Thus imprecision will have a significant effect on the calculated κ/λ FLC ratio and doubling (or halving) of the ratio can occur in the absence of a change in disease status. 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.

Interferences Causing Overestimation of FLC

Non-specific interference may result in overestimation of FLC concentration. An indication of this may be an unexpected change in the uninvolved or non-monoclonal FLC during disease monitoring but where there is no change in treatment (data not shown). Other indications are decreasing FLC concentration at higher sample dilution possibly due to interference from aggregated immunoglobulin or other protein interacting with FLC reagent to cause increased turbidity and falsely elevated concentration. Such interferences may be diluted out with lower FLC concentration observed (data not shown). It is recommended that such samples be diluted until two dilutions give comparable FLC concentration; otherwise FLC results cannot be reported with certainty.

Polymeric forms can also cause overestimation of FLC possibly by reaction at multiple antigenic sites on the FLC molecule. It has been reported from gel filtration studies that, in sera from patients with NSMM, FLC may exist in multimeric forms in addition to the usual κFLC monomers and λFLC dimers and that there is a nephelometric overestimation of FLC concentration of between 1.5- and 3.5-fold for NSMM sera relative to other myeloma sera.[9] Abraham et al. have reported an apparent λFLC concentration of 344 g/L in a patient with an M-protein concentration of 23 g/L determined by electrophoresis and consisting of light chain dimers.[15] This gross overestimation was due to the reaction of trimolecular aggregates of the monoclonal λFLC with the antibody.

Sample Dilution Anomalies

Generally FLC dilute in a linear manner. Manual off-line dilution of polyclonal and monoclonal FLC in either a normal serum or phosphate-buffered saline solution recovers 102-121% λFLC concentration at final sample dilutions of 1 in 100, 1 in 133, 1 in 200 and 1 in 400, and 102-127% κFLC concentration, when diluted in saline solution, compared with 87-100% in normal serum (Table 2). However, larger discrepancies of two- or more-fold can occur on dilution

Table 1. Variation of serum free light chain (FLC) concentration and ratio over several reagent lots.[a]

Patient Samples	CV% (mean κFLC in mg/L)	CV% (mean λFLC in mg/L)	κ/λ FLC ratio			
			Mean Ratio	Mean CV%	Range	Number of reagent lots
3 normal FLC samples	9 (42)	17 (40)	0.98	30	0.64-1.17	3
	22 (32)	20 (66)	0.50	31	0.34-0.73	7
	13 (12)	23 (15)	0.73	23	0.58-1.03	5
3 monoclonal κFLC samples	10 (168)	14 (11)	13.0	25	10.8-18.7	6
	23 (89)	45 (8.9)	11.2	17	8.4-12.6	7
	64 (30) [b]	17 (17)	1.89	75	0.76-3.49	2
3 monoclonal λFLC samples	24 (15)	21 (67)	0.27	31	0.16-0.33	5
	14 (8.4)	14 (267)	0.03	17	0.02-0.04	4
	8 (17)	24 (137)	0.13	32	0.10-0.20	5

[a] FLC concentration measured by IMMAGE (Beckman Coulter, US) over several FLC reagent lots using normal and monoclonal κFLC and λFLC samples stored in aliquots at -80 °C; [b] monoclonal κFLC showed non-reactivity for one κFLC reagent lot. [10]

(Tables 3-5). For example, samples 1, 2 and 3 from patients with monoclonal κFLC gave lower concentrations at initial sample dilution compared with 2- to 3-fold higher values on further dilution by BNII (Siemens, Germany) and IMMAGE (Beckman Coulter, US), respectively (Table 3). Nonlinear samples require several sample dilutions often 4- to 80-fold higher than the initial machine dilution to reach a final FLC concentration. For example, κFLC sample 8 (by BNII; Table 3) and λFLC samples 11 to 14 from the same patient with IgA λ myeloma (Table 4) gave 3- to 6-fold higher FLC concentration on further dilution. Depending on the antigen-antibody reactivity characteristics of the individual monoclonal FLC and the final dilution used, the concentration may be underestimated. At concentrations above the initial upper measuring range, values may be in closer agreement between methods, e.g. κFLC samples 4, 5 and 6 and λFLC sample 17 but discordant for others, e.g. λFLC samples 10 and 16. Values can be 2- to 3- fold different between IMMAGE and BNII platforms. This suggests immunoassay FLC reactivity may vary under different reaction conditions.

Gross assay nonlinearity is difficult to detect and in our experience affects monoclonal not polyclonal FLC. Due to possible differences in the titration curves for monoclonal FLC it may be useful to assay two or more sample dilutions until comparable values are found (Table 5).[10] Simply reporting the first result in the measuring range ignores the issue of nonlinear samples. For the IMMAGE and BNII methods (which have similar initial FLC measuring ranges), we recommend the following dilution scheme. If the initial FLC concentration is in the range κFLC >50 mg/L or λFLC >100 mg/L but below the assay's upper measuring range, rerun the sample at the next higher dilution. If the diluted sample does not fall in the concentration range of 10-50 mg/L for κFLC or 10-100 mg/L for λFLC, assay again at the next higher dilution. This procedure results in an additional 6-10% of dilutions required per analytical run.

Finally, FLC measurement spans the concentration range of ~1 to 100,000 mg/L. Hence, there is the possibility for antigen excess to occur with accompanying underestimation of FLC when the concentration is extremely high, e.g. samples 9 and 15 (Tables 3 and 4). Antigen excess is uncommon but may be missed if FLC results are not assessed in combination with SPEP, urine protein electrophoresis (UPEP), clinical history, bone marrow biopsy results and other chemistry results.

The advantages of the above dilution scheme are the avoidance of antigen excess with most samples and a more consistent approach to sample dilutions when monitoring patients. The dilution anomalies suggest that there is earlier antigen excess for some monoclonal FLC, well below the assay's upper measuring range due to limited antigenicity of the monoclonal molecule and selective FLC antibody consumption. Hence, similar FLC concentrations on initial sample dilution of monoclonal proteins from different individuals may not necessarily translate into the same 'true value' and FLC reaction will depend both on the antigenicity of the monoclonal FLC and total composition of the polyclonal

Table 2. Recovery of serum free light chain (FLC) concentration on dilution.[a]

Sample	Final concentration in mg/L (and % recovery) after dilution of sample in BNII Diluent or Normal Serum [b]						
	1 in 100 BNII DIL	1 in 133 BNII DIL	1 in 200 BNII DIL	1 in 400 BNII DIL	1 in 133 Normal serum	1 in 200 Normal serum	1 in 400 Normal serum
κFLC polyclonal	171 (100%)	175 (102%)	198 (116%)	197 (115%)	163 (95%)	161 (94%)	148 (87%)
λFLC polyclonal	79 (100%)	81 (102%)	88 (111%)	95 (120%)	81 (102%)	87 (110%)	96 (121%)
κFLC monoclonal	132 (100%)	151 (114%)	168 (127%)	164 (124%)	129 (97%)	131 (100%)	128 (97%)
λFLC monoclonal	278 (100%)	316 (114%)	294 (106%)	286 (103%)	314 (113%)	303 (109%)	312 (112%)

[a] FLC measured by BNII (Siemens, Germany); [b] FLC concentration of normal serum was subtracted prior to final calculation.

antibody FLC reagent. Further work is required to investigate this issue.

Interpretation and Commenting

Reference Intervals

Serum FLC reference intervals have been determined using fresh and frozen sera from healthy donors using BNII and Freelite™.[16] The diagnostic range for κ/λ FLC ratio includes 100% of donors to improve assay specificity by minimising falsely abnormal ratios. Laboratories should validate FLC reference intervals by assay of at least 20 normal samples to confirm that at least 90% of sample values (18/20 samples tested) fall within the reference intervals for FLC concentration and ratio.[17]

Elevations of FLC concentration occur when monoclonal free light chains are present, when immunoglobulin synthesis is increased, when there is polyclonal FLC stimulation such as in autoimmune, liver and inflammatory diseases and with infections, and when immunoglobulin excretion is reduced due to renal impairment with prolonged retention in serum of both kappa and lambda free light chains. Ratios should remain within the diagnostic range (0.26-1.65)[16] but borderline increased κ/λ FLC ratio can occur in these conditions.[18-20] In chronic kidney disease, ratios up to 3.1 are reported,[21] hence appropriate interpretation of FLC results and commenting is required (Table 6).

Examples

Serial FLC measurement is subject to reagent lot-to-lot variation and ratios may be biased due to suppression of the uninvolved (or non-pathological) FLC on treatment of clonal plasma cell disorders.[22] Assay imprecision, especially with different lots of FLC reagent, may have a significant effect on changes in the FLC concentration and the calculated κ/λ ratio should not be reported in some instances (e.g. NSMM post treatment, shown in Table 6). In an example of a treated λ LCMM patient who went on to develop chronic kidney disease, the κ/λ FLC ratio changed to be borderline elevated although the original pathological FLC was λ not κFLC (Table 6). At the same time a trace amount of λBJP was detected on IFE of the patient's urine. Hence no FLC antiserum is able

Table 3. Recovery of serum κ free light chains (FLC) on sample dilution by two methods.

Sample ID	IMMAGE κFLC concentration (mg/L) after multiplication at final sample dilution							BNII κFLC concentration (mg/L) after multiplication at final sample dilution			
	1 in 10 [a]	1 in 50	1 in 100	1 in 200	1 in 400	1 in 1000	1 in 2000	1 in 100 [b]	1 in 400	1in 2000	1 in 8000
1) IIMM [c]	127	384	384					100	183		
2) LCMM [c]	185	352	437					162	238		
3) IIMM [c]	124	292	315					88	138		
4) IIMM	>198			574	428			>179	387		
5) IIMM	>198			798	768			>179		642	
6) IIMM	>198					4050	3440	>179			3320
7) IIMM	>162	218		206				114	164	196	
8) IIMM [d] Nonlinear	>198	>990		2500		2520		133		826	
9) No clinical notes available Antigen excess [e]	75						20,300 (1 in 5000)	N/a			

[a] IMMAGE κFLC measuring range for initial sample dilution of 1 in 10 was 6.6-198 mg/L (Freelite™ Lot 232704B) and 5.4-162 mg/L (Lot 241673D); [b] BNII κFLC measuring range for initial sample dilution of 1 in 100 was 5.6-179 mg/L (Lot 239854C); [c] Samples 1, 2 and 3 gave ~3-fold higher values by IMMAGE and ~2-fold by BNII on further sample dilution; initial FLC concentrations are within the measuring range; [d] Sample 8 was nonlinear by BNII and concentration was ~6-fold higher at 1 in 2000 final sample dilution compared with initial 1 in 100 dilution; [e] Sample 9 was underestimated at initial 1 in 10 IMMAGE dilution due to antigen excess; values were 20,300 mg/L and 17,500 mg/L at 1 in 5,000 and 1 in 10,000 final sample dilutions, respectively. N/a, not available; IIMM, intact immunoglobulin myeloma; LCMM, light chain myeloma.

Table 4. Recovery of serum λ free light chains (FLC) on sample dilution by two methods.

Sample ID	IMMAGE λFLC concentration (mg/L) after multiplication at final sample dilution							BNII λFLC concentration (mg/L) after multiplication at final sample dilution				
	1 in 10 [a]	1 in 50	1 in 200	1 in 400	1 in 1000	1 in 10,000	1 in 20,000	1 in 100 [b]	1 in 400	1in 2000	1 in 8000	1 in 40,000
10) IIMM	>297	1055	726					>260		1710		
11) IIMM [c] Nonlinear	265	875	1350					231	379	542	684	
12) IIMM [c]	>297	1265	2580	2830				>260	631	918	1300	
13) IIMM [c]	N/a							86	161	207		
14) IIMM [c]	N/a							121	270	455		
15) LCMM [d] Antigen excess	260	>1405	>5620		>28,100	26,500	28,800	>260				42,000
16) No clinical notes available	>297	715	596					>260	1010			
17) LCMM	>297		1080		1140			>260	>1010	881	946	

[a] IMMAGE λFLC measuring range for initial sample dilution of 1 in 10 was 8.8-297 mg/L (Freelite™ Lots 232618B and 239861D); [b] BNII λFLC measuring range for initial sample dilution of 1 in 100 was 8.1-260 mg/L (Lot 239862C); [c] Samples 11 to 14 from the same patient (IgA λ myeloma) post treatment were nonlinear on dilution by both IMMAGE and BNII methods; [d] Sample 15 was underestimated at initial 1 in 10 IMMAGE dilution due to antigen excess; values were 26,500 mg/L and 28,800 mg/L at 1 in 10,000 and 1 in 20,000 final sample dilutions, respectively. N/a, not available; IIMM, intact immunoglobulin myeloma; LCMM, light chain myeloma.

to differentiate monoclonal FLC from polyclonal FLC as in this example where there was also elevated kappa and lambda FLC due to renal failure. Other cases of skewed ratio can occur in patients who have undergone autologous stem cell transplantation (ASCT) for monoclonal light chain disease where an abnormal but opposite FLC ratio post-ASCT is seen. A κ LCDD subject who, at diagnosis had a ratio of 63 went on to have a ratio of 0.06, post-ASCT (Table 6). New IgG λ bands that appeared in trace amounts at this time resulted in elevated λFLC concentration and an abnormal ratio but they were transient and the ratio rapidly normalised (also refer to paper by S. Hall et al. in this issue).

Clinical Correlations

While sample dilution anomalies may cause difficulty in result interpretation, particularly when monitoring patients with clonal plasma cell disorders, inaccuracy of FLC concentration does not preclude its use in clinical diagnosis and monitoring of disease response. INA is used for measurement of intact monoclonal immunoglobulins that overlap the beta region on SPEP to reflect disease trend. It is suggested that the same is true of FLC measurement. An example of such a trend is shown for a MM patient with predominantly λBJP being monitored for response to treatment (Table 6). Monoclonal λFLC concentration by immunoassay was 3- to 4-fold higher than by SPEP over several samples but the trend in FLC changes was similar by both methods. Overestimation by immunoassay is possibly due to multi-reactivity of monomeric, dimeric or polymeric forms of FLC.

On the other hand, FLC assays on different platforms need not always give the same clinical classification for patients with monoclonal light chain disease who are being monitored long-term using serial measurements of serum FLC. An example where clinical management might have been different is shown for a 39-year-old male with AL involving kidney, heart, lung, liver and gastrointestinal tract (Table 7). At presentation IgG κ paraprotein was 4 g/L and κBJP excretion was 24 mg/24h. BNII values were consistently higher than IMMAGE values and the diagnosis of progressive disease (and ASCT) was delayed by 6 months with the use of IMMAGE compared with BNII values.

Conclusions

Use of polyclonal FLC antibodies raises the question of the adequacy of their specificity and binding affinity to measure

Table 5. Comparison of serum free light chain (FLC) concentration by IMMAGE at starting sample dilution (1 in 10) and higher offline-sample dilutions.

Patient ID:	FLC concentration (mg/L) after multiplication at final sample dilution					
	1 in 10 [a]	1 in 20	1 in 30	1 in 50	(Other dilution)	1 in 110
κFLC: [b]						
LCMM (nonlinear)	47	90	120	141	153 (1 in 80)	154
AL (nonlinear)	63	106	113	116	-	-
AL and LCMM	143	-	245	237	-	-
LCMM	88	-	179	184	-	-
IIMM	127	236	272	289	291 (1 in 90)	-
NSMM	108	-	297	303	-	-
λFLC: [c]						
LCMM	208	420	-	411	-	391
IIMM	110	138	147	147	-	-
IIMM	212	-	327	-	277 (1 in 60)	-
LCMM	190	-	271	-	254 (1 in 60)	-
IIMM	214	-	318	286	-	-

[a] IMMAGE starting sample dilution was 1 in 10; [b] κFLC upper measuring limit: approx. 180 mg/L; [c] λFLC upper measuring limit: approx. 240 mg/L. AL, amyloidosis; IIMM, intact immunoglobulin myeloma; LCMM, light chain myeloma; NSMM, non-secretory myeloma.

monoclonal FLC in a small number of patients. Problematic samples can give FLC under- or over-estimation due to sample dilution anomalies, with difficulties in result interpretation particularly when monitoring patients with clonal plasma cell diseases. Laboratory staff and clinicians should be aware of the potential for non-reactivity of individual monoclonal FLC, the effect of dilution on FLC measurement and the impact of assay imprecision on result interpretation. Lot-to-lot variation between batches of polyclonal FLC antiserum may result in variable immunoreactivity of individual monoclonal FLC causing falsely low FLC measurement. The FLC assay is an important advance in the diagnosis and monitoring of monoclonal light chain diseases but the assay's limitations need to be understood. These issues, if not adequately appreciated, have the potential to mislead clinical diagnosis and assessment of response to therapy.

Competing Interests: The Binding Site has supplied FLC assay kits free of charge for an amyloidosis study for which Dr Mollee is the principal investigator.

Table 6. Examples of abnormal kappa/lambda free light chain (κ/λ FLC) ratios in patients tested for plasma cell disorders.

Disorder	SPEP (with IFE screening on all samples)	κFLC 3-19 (mg/L)	λFLC 6-26 (mg/L)	κ/λ FLC ratio 0.26-1.65	Reporting–Comment [a]
Renal failure	M-protein not detected. Inflammatory pattern. (eGFR 16 mL/min/1.73 m^2)	120	69	1.74	The significance of a borderline high κ/λ FLC ratio in the presence of a marked increase in γ globulins or inflammatory response is uncertain.
T-cell leukaemia	M-protein not detected. Increased polyclonal IgG (28 g/L) and IgA (18 g/L). Inflammatory pattern. (CRP 84 mg/L)	190	110	1.75	It should not be regarded as diagnostic of a monoclonal light-chain disease.
Liver disease	Small low-level bands on polyclonal γ globulins. Their clinical significance is uncertain.	370	190	1.95	Suggest follow-up FLC.
Inflammatory bowel disease	M-protein not detected. Inflammatory pattern. (CRP 32 mg/L)	340	150	2.3	Borderline κ/λ FLC ratio; add generic comment [a]
CKD	M-protein not detected. Normal pattern. (eGFR 16 mL/min/1.73 m^2)	100	48	2.1	κ/λ FLC ratios are reported to be as high as 3:1 in CKD.
NSMM	At diagnosis: M-protein not detected.	400	<4	>100	** Any calculated FLC ratio at this level of serum FLC is of uncertain significance.The κ/λ FLC ratio changes significantly with small changes in non-pathologic FLC.
	10 weeks post ASCT	7	11	0.64	
	23 weeks post ASCT	<3	13	<0.23**	
λ LCMM	At diagnosis monoclonal λBJP detected on SPEP				
	β-globulins + λBJP : 24 g/L	8	57,000	<0.01	
	18 months later λBJP not detected in serum but trace λBJP detected in urine on IFE.	280	160	1.75**	** NOTE: Original pathological FLC was λ light chain type.
κ LCDD	At diagnosis trace κBJP detected in urine on IFE				
	No monoclonal band detected in serum; 23% plasma cells (κ light chain restricted on bone marrow trephine).	1200	19	63	
	Pre ASCT: κBJP not detected; small bands on SPEP.	76	17	4.5	
	8 weeks post ASCT: κBJP not detected; trace IgG λ bands – their clinical significance is uncertain.	4	64	0.06**	** NOTE: Original pathological FLC was κ light chain type.
	10 weeks post ASCT: κBJP not detected; small bands weakly present (probably transient).	9	11	0.82	

MM (mainly λBJP with monoclonal IgA λ present in trace amounts in serum)	DATE	SPEP λBJP (mg/L)	UPEP λBJP (mg/24h)				
	24.10.08 (presentation)	8,000	9,400	42	21,000**	0.002	** λFLC concentration by immunoassay was 3-to 4-fold higher than by SPEP over several samples.
	28.11.08	8,000	-	12	31,000**	<0.001	
	Post treatment:						λBJP quantitated from SPEP was similar at presentation and 5 weeks later whereas λFLC concentration increased by ~ 50%.
	9.12.08	3,000	7,300	2	15,000	<0.001	
	26.12.08	1,000	-	21	4,300	0.005	
	12.1.09	Trace on IFE	-	14	580	0.02	Quantitation of low-level monoclonal bands (1-2 g/L) by scanning densitometry is prone to large imprecision. However, trends in λFLC change were similar by both immunoassay and SPEP when monitoring this patient.
	3.2.09	Trace on IFE	216	36	350	0.10	

[a] Generic comment is 'Increased serum FLC concentrations can occur not only when monoclonal light chains are present but also when immunoglobulin synthesis is elevated (e.g. autoimmune, liver and inflammatory diseases, infection), or immunoglobulin excretion is reduced (e.g. renal impairment).' ASCT, autologous stem cell transplantation; BJP, Bence Jones protein; CKD, chronic kidney disease; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; IFE, immunofixation electrophoresis; LCDD, light chain deposition disease; LCMM, light chain myeloma; M-protein, monoclonal protein; NSMM, non-secretory myeloma; SPEP, serum protein electrophoresis; UPEP, urine protein electrophoresis.

Table 7. Monitoring AL amyloidosis in 39-year-old male with amyloidogenic κ free light chains (FLC) by FLC immunoassay, and serum and urine protein electrophoresis.

Date of sample collection	κFLC IMMAGE [a](mg/L)	κFLC BNII [b](mg/L)	SPEP IgG κ (g/L)	UPEP κBJP (mg/24h)
12/8/04 (At time of diagnosis)	560	654	4	24
30/08/04 (Post 7 days treated)	118 (54)	143	-	-
20/09/04	239	352	4	34
22/10/04	146 (62)	199	3	30
7/02/05	81 (38)	85	3	27
16/05/05 *	110 (54)	185 *	4	27
15/08/05	100 (-)	-	-	-
14/11/05 **	210 (109) **	-	-	-
24/11/05 (Date of ASCT)	-	-	-	-
1/12/05	57 (28)	42	5	33
29/05/06	62 (39)	51	3	15
28/08/06	70 (33)	62	3	Trace by IFE

[a] κFLC measured over several reagent lots by IMMAGE; concentrations shown in brackets are at initial 1 in 10 dilution; final concentrations are at 1 in 50 or higher dilution; [b] κFLC measured retrospectively by BNII using one reagent lot; samples with κFLC >160 mg/L went on to the next higher sample dilution. * FLC progress detected by BNII; ** FLC progress detected by IMMAGE; ASCT; autologous stem cell transplantation; BJP, Bence Jones protein; IFE, immunofixation electrophoresis; SPEP, serum protein electrophoresis; UPEP, urine protein electrophoresis.

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