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Serum Free Light Chain Assays
For Diagnosis and Monitoring of Myeloma

Introduction

Multiple myeloma and other monoclonal gammopathies represent a family of disorders that are characterized by the proliferation of a monoclonal population of plasma cells and the production of a monoclonal (M-) immunoglobulin protein (Figure 1).[1] In almost 80% of patients with myeloma, the M-protein is an intact immunoglobulin (IgG, IgA, IgE, IgD) composed of both heavy and light chains and, in 20% of patients, the M-protein is composed only of light chains, either κ or λ.[1] As much as 3% of myeloma has been classified as nonsecretory because of an inability to detect M-proteins on electrophoresis.[2] Regardless of the subtype, virtually all clonal myeloma cells secrete excess free light chains.

Testing for the presence of M-proteins is routinely performed using serum or urine protein electrophoresis, a technology that dates back more than half a century. During the last 20 years, immunofixation electrophoresis was introduced to classify the M-protein subtype and to improve diagnostic sensitivity. Nonetheless, these technologies have characteristics that reduce their sensitivity and limit their utility as tools to monitor response to treatment (Table 1).[2–7] Serum protein electrophoresis fails to detect 50% of light chain myeloma and primary systemic (AL) amyloidosis, 20% of significant plasma cell pathologies overall, and all instances of nonsecretory myeloma; therefore, better technologies are needed.[1,2,6]

Improved Technology

In 2001, testing for free κ and free λ light chains was FDA-cleared and has since become integrated into the diagnostic and monitoring guidelines for multiple myeloma that are endorsed by the International Myeloma Working Group.[3] The free light chain tests are fully automated latex-based immunoassays that are performed on high-volume nephelometric or turbidimetric platforms. The sensitivity of free light chain immunoassays is superior to other currently available methods, allowing detection within the normal range. Other assays (ie, serum protein electrophoresis and immunofixation) generally do not measure low enough to cover the normal range of FLC; therefore, they may not be reliable in differentiating normal and abnormal values.[2,8] This added sensitivity enables more accurate detection of multiple myeloma, light chain multiple myeloma, AL amyloidosis, and B-cell dyscrasia (Figure 2).[7]

The free light chain assays comprise two distinct assays: one for free κ and one for free λ. During the in vitro reaction, antisera composed of high-affinity polyclonal antibodies detect epitopes that are exposed only on free (unbound) light chains. The free light chain assays are exquisitely sensitive, measuring levels as low as 0.3 mg/L for κ and 0.4 mg/L for λ light chains.[7] Concentrations of free light chains are measurable in the serum of normal individuals and in clinical settings in which M-protein concentrations were previously undetectable using electrophoresis and immunofixation.[2,8] Importantly, virtually 100% of light chain myeloma, more than 90% of AL amyloidosis, and more than 80% of nonsecretory myeloma yield abnormal results with the serum free light chain assays.[2,9,10]

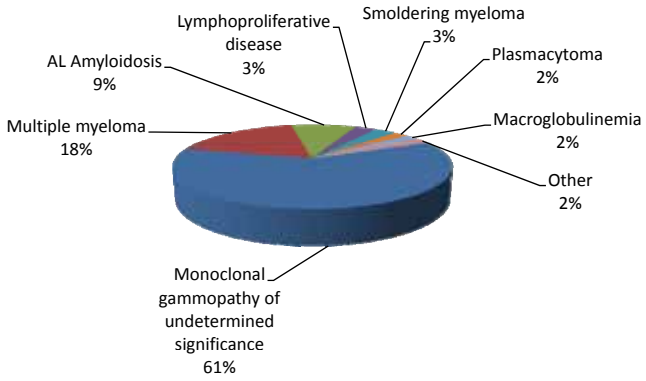

Figure 1 — Frequency of Monoclonal Gammopathies (N=29,528)[1]

Table 1 — Characteristics of Serum and Urine Protein Electrophoresis, Serum Immunofixation, and Serum Free Light Chain Assays[2-7]

	Serum Electrophoresis	Urine Electrophoresis	Serum Immunofixation	Serum Free Light Chain Assays
Interpretation	Subjective interpretation: labor intensive	Subjective interpretation: labor intensive	Subjective interpretation: labor intensive	Objective fully automated nephelometric or turbidimetric immunoassay
Sensitivity	Lower limit of light chain sensitivity: ~500 mg/L	Lower limit of light chain sensitivity: ~30 mg/L	Lower limit of light chain sensitivity: ~150 mg/L	Lower limit of light chain sensitivity: ~0.4 mg/L
Comments	• Polymerization of serum proteins leads to underestimation of M-spike • Intact immunoglobulins have long half-life	• Positive only after overflow proteinuria with results influenced by baseline renal function • Cumbersome 24-hour urine collection, labor-intensive centrifugation and concentration of specimen	• Not quantitative: only positive or negative and therefore not suitable to monitor response to treatment • Relies on the quality and specificity of antisera	• Immunochemical methods tend to overestimate light chain and intact immunoglobulin concentrations • Short half-life of free light chains enables real-time assessment of tumor kill

Serum FLC Preferred Over Urine FLC

The serum free light chain assays have supplanted urine testing when evaluating patients for monoclonal gammopathies. Light chains (also called Bence Jones proteins when detected in the urine) are freely filtered in the glomeruli, then reabsorbed and metabolized in the proximal convoluted tubules.[4] Light chains spill into the urine only after their reabsorption threshold is exceeded, and the median threshold is reached only when serum levels exceed approximately six times their normal value. Indeed, serum free light chain testing has twice the sensitivity in the detection of M-protein abnormalities among patients with myeloma compared with urine immunofixation.[4]

Serum FLC vs Urine PE Testing

In 2006, it was reported that a panel of serum free light chain testing in combination with either serum protein electrophoresis or serum immunofixation made urine testing no longer necessary in the screening guidelines for monoclonal gammopathy. More than 99% of monoclonal gammopathies were detected using a panel of serum free light chain assays and serum immunofixation.[6] In a population of veterans, 100% sensitivity and 99% specificity for plasma cell dyscrasias were reported using a panel consisting of serum protein electrophoresis and the serum free light chain assays for initial evaluation (Figure 3). In that study, the serum free light chains assays detected an additional 15 instances of multiple myeloma and one malignant lymphoma that were not detected with serum protein electrophoresis alone.[11]

Table 2 — Interpretation of Results of the Free Light Chain Assays

Kappa (κ)	Lambda (λ)	Ratio (κ : λ)	Interpretation
Normal	Normal	Normal	Normal serum
Low	Low	Normal	BM suppression without MG
		High	MG with BM suppression
		Low	
	Normal	Normal	Normal serum or BM suppression
		Low	MG with BM suppression
	High	Low	
Normal	Low	High	
		Normal	Normal serum or BM suppression
	Normal	High	MG with BM suppression
		Low	
	High	Normal	plg increase or renal impairment
		Low	MG without BM suppression
High	Low	High	MG with BM suppression
	Normal	High	MG without BM suppression
		Normal	plg increase or renal impairment
	High	Normal	
		High	MG with renal impairment
		Low	

Classification of monoclonal gammopathies according to serum FLC concentrations.
BM = bone marrow; MG = monoclonal gammopathy; plg = polyclonal immunoglobulin.

Results and Interpretation

The ratio of κ to λ normally ranges between 0.26 and 1.65. An imbalance in the ratio supports a diagnosis of monoclonal gammopathy (Table 2). The molecular mass of each light chain is approximately 22.5 kD; however, κ free light chains exist primarily as monomers, while λ free light chains circulate as covalently linked dimers with a molecular mass of approximately 45 kD. The smaller size of κ chains leads to a more rapid glomerular filtration rate and clearance, and it explains the reported median serum ratio of κ to λ free light chains of 0.6 versus the ratio of bound κ to λ of 2.0.

More than 95% of patients with intact immunoglobulin myeloma have abnormal free light chain concentrations.[12] In addition to high sensitivity for light chain myeloma, nonsecretory myeloma and AL amyloidosis, free light chain testing is also highly sensitive for the detection of light chain deposition disease.[8] In the up-front setting, an isolated abnormal free light chain ratio in the absence of clinical abnormalities and a normal serum protein electrophoresis and immunofixation likely represents free light chain monoclonal gammopathy of undetermined significance (MGUS).[13]

With a prevalence of approximately 3% in persons 50 years of age or older, MGUS is the most frequent monoclonal gammopathy.[14] Progression to myeloma or another plasma cell dyscrasia occurs in 1% of patients with MGUS per year.[15] An abnormal free light chain ratio is observed in one third of patients with MGUS. These patients have an increased risk of progression to full myeloma relative to the two-thirds of MGUS patients with a normal free light chain ratio.[16]

Free light chains have a short half-life, measurable in hours, and therefore free light chain testing provides a rapid, real-time assessment of response to treatment in patients with monoclonal gammopathy. With treatment-induced plasma cell kill, free light chain concentrations decrease rapidly and precipitously (Figure 4).[7,17] In contrast, the longer half-life of intact immunoglobulins (measurable in weeks) leads to a long lag-time in the assessment of treatment response. In a 2005 report from Memorial Sloan Kettering Cancer Center, normalization of the serum free light chain ratio after one or two cycles of chemotherapy in patients with multiple myeloma was found to be significantly associated with subsequent complete or near-complete response as assessed many cycles later using measurement of intact immunoglobulins.[18] Thus, free light chain testing may serve as a valuable tool for the rapid assessment of treatment efficacy.

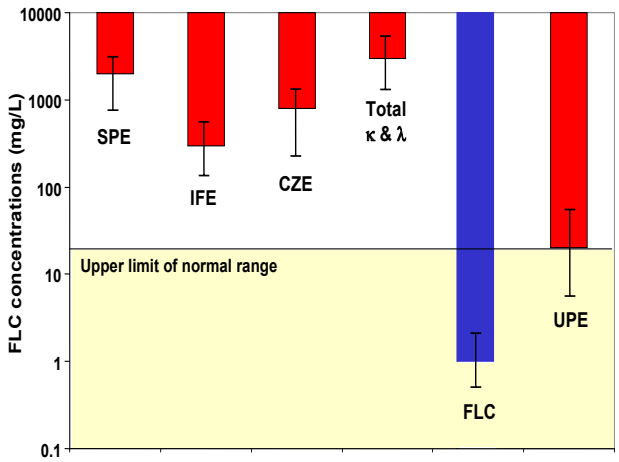

Figure 2 — Sensitivity of Free Light Chain Immunoassays Compared to Other Methods

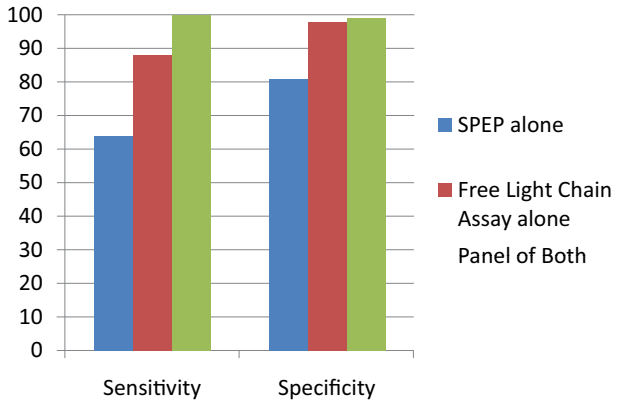

Figure 3 — Sensitivity and Specificity (in %) of Panel of Serum Protein Electrophoresis (SPEP) and Serum Free Light Chain Assays (N = 312)[11]

Current Guidelines

Guidelines for the use of serum free light chain (FLC) assays in the diagnosis and monitoring of myeloma have been established by the International Myeloma Working Group (IMWG).[19] The key elements of the guidelines include:[19]

1. Establishing a focus upon elevation of κ or λ FLC in the presence of a significantly abnormal κ/λ FLC ratio (rFLC) ie, clonally selective production of FLC.
2. Identification of serum FLC levels required to produce overflow proteinuria: medians are 113 mg/L for κ and 278 mg/L for λ. This helps correlate with the more familiar 24-hour urine measurements.
3. Serum FLC is recommended as part of screening for pathological monoclonal plasma proliferative disorders.
4. Serum FLC is recommended as a prognostic assay in MGUS, smoldering myeloma, solitary plasmacytomata, active myeloma and light chain amyloidosis. There are specific levels and cutoffs for each entity.
5. Serum FLC measurements are also recommended in the assessment of response. Oligo secretory disease is the major area for use. Rapid reduction in sFLC can be an early indicator of response. A major use of the rFLC is as the key element of stringent complete response (sCR) within the new international uniform response criteria for myeloma. Careful attention is required during ongoing therapy which can suppress the "normal" light chain levels and produce an abnormal rFLC.
6. The use of FLC measurements in many other settings is being actively studied. One potential utility is for identification of "Bence Jones escape" especially using novel therapy regimens following which "de-differentiated" relapse or extra-medullary oligo secretory progression occurs more frequently.
7. Particularly careful attention is essential for interpretation of FLC results in patients with renal insufficiency.

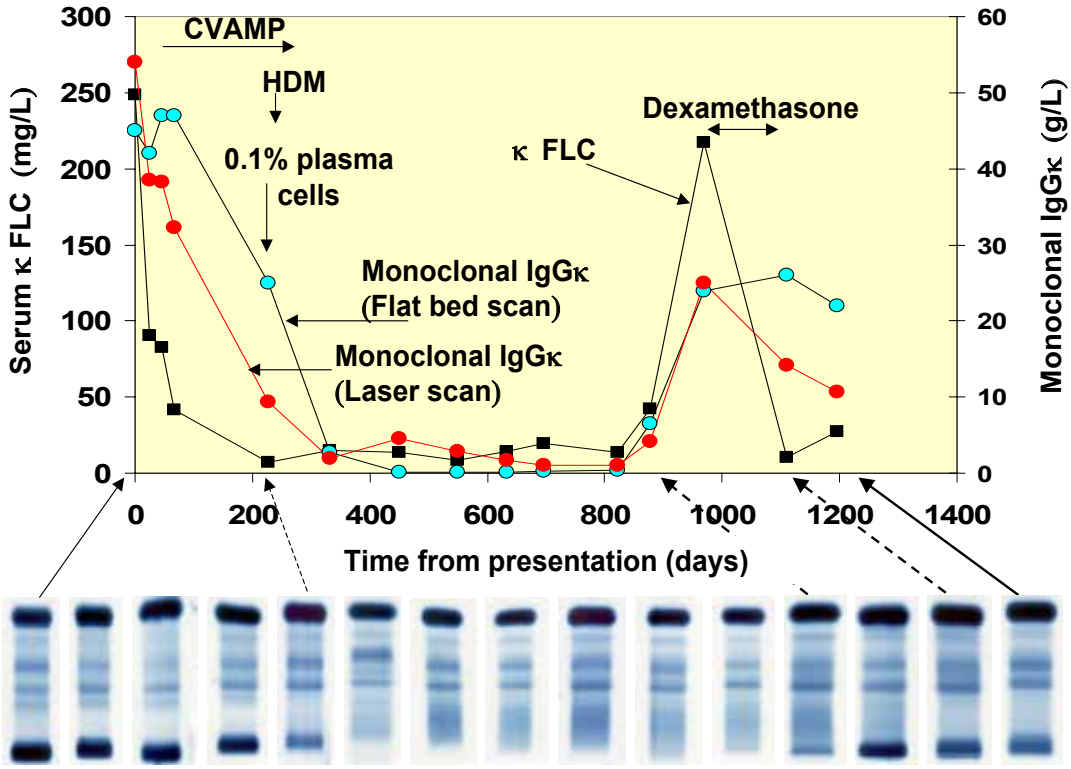

Figure 4 — Changes in Kappa Free Light Chains (κFLC) and IgGκ During Treatment With Chemotherapy (CVAMP) Followed by High Dose Melphalan (HDM) and Stem Cell Transplantation, Subsequent Relapse, and Retreatment in a Patient With Kappa-producing Myeloma (Courtesy of A. R. Bradwell)[7]

Summary and Updated Guidelines

After reviewing numerous clinical studies, the International Myeloma Working Group has recently published guidelines for serum-free light chain analysis in multiple myeloma and related disorders.[20] These guidelines make the following statements:

- In the context of screening, the serum free-light chain assay in combination with serum protein electrophoresis and immunofixation yields high sensitivity, and negates the need for 24-hour urine studies for diagnoses other than light chain amyloidosis.
- When taken at baseline, serum free light chain analysis yields prognostic information in virtually every plasma cell dyscrasia.
- The free light chain assay can be used to quantitatively monitor patients with oligosecretory plasma cell dyscrasias, including AL, oligosecretory myeloma and nearly two-thirds of patients who had previously been deemed to have non-secretory myeloma.
- In AL amyloidosis patients, serial free light chain measurements outperform serum protein electrophoresis and immunofixation.

Ordering Options

Serum is the preferred specimen for analysis of free light chains. Testing for free κ and free λ may also be performed in urine but is not necessary if a serum sample is available.[6] Furthermore, increased levels of free light chains will be observed earlier in serum than in urine due to their reabsorption and catabolism in renal tubular cells.[4]

Relevant Assays*

Test Name	Test Number
Free κ and λ Light Chains Plus Ratio, Quantitative, Serum	121137
Free κ and λ Light Chains Plus Ratio, Quantitative, Urine	121228
Free κ and λ Light Chains Plus Ratio, Quantitative, Serum (Serial Monitor)	121155
Immunofixation (IFE), Serum, Protein Electrophoresis (PE), Serum, and Quantitative Free κ and λ Light Chains (FLC) Plus Ratio, Serum	120256
Protein Electrophoresis (PE), Serum and Quantitative Free κ and λ Light Chains (FLC) Plus Ratio, Serum	121210
Free κ and λ Light Chains Plus Ratio, Quantitative, Urine (Serial Monitor)	121243

*For the most current information regarding test options, including specimen requirements and CPT codes, please consult the online Test Menu at www.LabCorp.com.

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