

SERUM FREE LIGHT CHAIN ANALYSIS

Enhanced Laboratory Testing For Monoclonal Gammopathy

Monoclonal gammopathy (MG) is a laboratory diagnosis based on the detection of a monoclonal immunoglobulin in serum or urine. This finding reflects clonal plasma cell expansion, and is associated with a variety of plasma cell dyscrasias, including multiple myeloma (MM), primary amyloidosis, light chain disease, solitary plasmacytoma of bone, Waldenström's macroglobulinemia, and lymphoproliferative diseases such as leukemia or lymphoma. When a monoclonal immunoglobulin is detected in a patient without clinical symptoms of the above disorders, the condition is termed *monoclonal gammopathy of undetermined significance (MGUS)*. MGUS, a potentially pre-malignant condition that requires lifelong monitoring, has a prevalence of 3.2% among persons aged ≥ 50 years, 5.3% at ≥ 70 years and 7.5% at ≥ 85 years (Kyle *et al.* 2006). Among all patients producing a monoclonal immunoglobulin, 61% have MGUS; in comparison, 18% have MM and 9% have primary amyloidosis (Katzmann 2006).

Figure 1 demonstrates a typical case of MG, in which a monoclonal band, or M-component, is observed by screening with serum protein electrophoresis (SPE) and then characterized by immunofixation (IFE). The concept of using IFE for the laboratory evaluation of MG, now used worldwide, was originally described by FBR's Dr. Ritchie (Ritchie and Smith 1976).

The combination of SPE and IFE, performed in both serum and urine specimens, detects most cases of MG that involve intact immunoglobulin molecules. These techniques are, however, less effective in detecting conditions where only monoclonal light chains are produced (light chain disease, amyloidosis) or where there is minimal secretion of intact immunoglobulins (non-secretory or oligo-secretory MM). Recently, a sensitive nephelometric assay for kappa (κ) and lambda (λ) free light chains (FLC) has been developed; this unique assay does

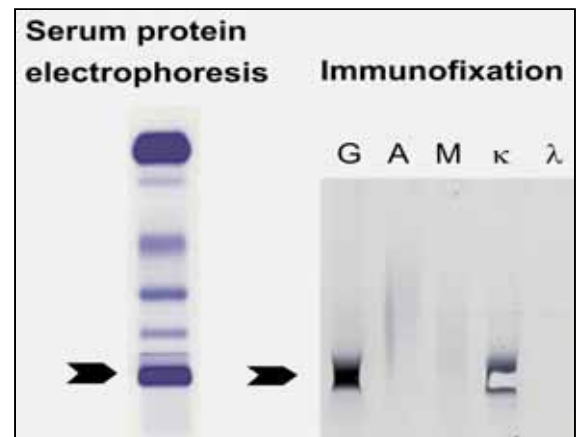


Figure 1: A monoclonal band is seen on SPE. It is identified as an IgG kappa by IFE. Note antigen excess effect in kappa lane.

not recognize light chains bound to heavy chains in intact immunoglobulins. **The addition of serum FLC testing provides significant improvements in detection rates for these conditions (see Table 1) and, moreover, provides important data for evaluating prognosis and monitoring chemotherapy of patients with plasma cell dyscrasias.**

Serum FLC analysis has recently been incorporated into international guidelines that address the detection and management of MM and related plasma cell dyscrasias (Dispenzieri *et al.* 2008, Durie *et al.* 2006). Although light chain assays have been described over the years, it is only recently that testing has become available to detect free (rather than total) light chains. Plasma cells secrete FLC (κ or λ) in addition to intact immunoglobulin molecules, and serum FLC levels are determined by their relative rates of synthesis ($\kappa > \lambda$) and renal excretion ($\kappa > \lambda$). In the presence of a monoclonal immunoglobulin, $\kappa:\lambda$ ratios may be either higher or lower than the normal range, depending on the class of the involved FLC.

SCREENING

Dispenzieri et al (2008), writing for the International Myeloma Working Group, recommend that **serum SPE, IFE and serum FLC testing be performed together** when screening for diseases associated with MG. This is particularly important for diseases where light chains are the primary clonal product, because monoclonal light chains typically occur at lower concentrations in serum than intact immunoglobulins and may not produce a visible band on SPE or IFE (Katzmann et al. 2002). The higher sensitivity of the FLC assay (1 mg/L) improves detection rates. This is also true for so-called non-

patients at risk for progression to MM. An abnormal $\kappa:\lambda$ ratio is present in 47% of patients with *solitary plasmacytoma*, and their 5-year progression rate

is 44%, compared with 26% for patients with a normal $\kappa:\lambda$ ratio (Dingli et al, 2006). Similarly for patients with *smoldering (asymptomatic) MM*, serum $\kappa:\lambda$ ratios <0.125 or >8 are associated with a 2.3-fold relative risk of progression to MM, compared with $\kappa:\lambda$ ratios of 0.125-8.0, over 15 years of follow-up (Kyle et al. 2007; Dispenzieri et al. 2008a).

MGUS affects the largest group of patients with MG (61%). In MGUS, the patient does not have any of the

Table 1: Sensitivity of SPE, IFE, and FLC to detect plasma cell dyscrasias

LABORATORY TEST	<i>Monoclonal immunoglobulin detected by serum testing (%)</i>			
	MM ¹	AL ²	LCMM ³	NSMM ⁴
SPE/IFE	95	70	75	0
FLC (abnormal $\kappa:\lambda$ ratio)	96	98	100	68
FLC/SPE/IFE	99	98	100	68

¹Mead et al '04; ²Lachmann et al '03; Abraham et al '03; ³Bradwell et al '03; ⁴Drayson et al '01.
MM, multiple myeloma; AL, primary amyloidosis; LCMM, light chain MM; NSMM, non-secretory MM

secretory myeloma, which has been characterized by an absence of monoclonal protein in serum and urine, as measured by SPE/IFE. In one study, two-thirds of patients with non-secretory myeloma had abnormal serum $\kappa:\lambda$ ratios (Drayson et al 2001). FLC cannot, however, replace SPE/IFE testing because the assay does not detect all cases of myeloma associated with intact monoclonal immunoglobulins (Mead et al. 2004).

Urine specimens. With the exception of suspected light chain amyloidosis, serum FLC testing can replace urine protein electrophoresis (UPE) and IFE of 24 hour urine specimens in screening for clinically important monoclonal gammopathies. Once a diagnosis of plasma cell dyscrasia has been made, however, UPE and IFE are indicated for all patients (Dispenzieri et al. 2008). FLC testing of urine itself is not recommended, as it has lower sensitivity than either serum FLC or urine IFE testing (Le Bricon et al. 2002; Herzog and Hofmann 2003; Dispenzieri et al. 2008).

clinical findings associated with MM or related disorders; monoclonal immunoglobulin concentration is < 3 g/dL, the proportion of plasma cells in bone marrow is $<10\%$, and there is no evidence of related organ or tissue impairment such as lytic bone lesions, hypercalcemia, anemia, or renal dysfunction. These patients require lifelong follow-up, because the risk of progression to MM (1%/year) does not decline over time; however, serum FLC testing helps to assess progression risk so that follow-up strategies can be modified accordingly. Whereas $\kappa:\lambda$ ratios are abnormal in the majority of patients with clinical disease, only 44% of patients with MGUS have an abnormal ratio, despite the identification of a monoclonal band by SPE/IFE (Katzmann et al. 2005). An abnormal $\kappa:\lambda$ ratio is an independent risk factor for progression in MGUS and can be used, in combination with information about other risk factors, as shown in Table 2, to identify high risk patients requiring more frequent follow-up and to reassure those at low risk (Rajkumar et al. 2005; Szarka 2005).

PROGNOSIS

Data produced by the serum FLC assay have prognostic value in most plasma cell dyscrasias (Dispenzieri et al. 2008). In both light chain amyloidosis (Dispenzieri et al. 2006) and active MM (Snozek et al. 2008), higher baseline values identify patients at increased risk of worse outcomes. In other conditions, serum FLC data can help to identify

MONITORING

Serum FLC measurements provide, for the first time, a quantitative means for monitoring patients with light chain and oligosecretory MM, and amyloidosis, who may have serum levels of monoclonal immunoglobulins below the detection limits of other assays. They are also useful for monitoring MM patients with intact monoclonal immunoglobulins.

Table 2: Progression Risk in MGUS

Risk category	Risk factors ¹	Absolute progression risk at 20 years (adjusted for death as competing risk) ²	Recommended follow-up (months) ³
	<ul style="list-style-type: none"> ● Abnormal $\kappa:\lambda$ ratio ● Non-IgG M-protein ● M-protein > 1.5 g/dL 		
LOW	0/3	2%	>12
LOW-INTERMEDIATE	1/3	10%	12
HIGH-INTERMEDIATE	2/3	18%	6-12
HIGH	3/3	27%	3-6

¹International Myeloma Working Group, 2003; ²Rajkumar *et al.* 2005; ³Unless clinical symptoms suggest progression.

Traditionally, serum levels of intact immunoglobulins have been used to monitor patients with plasma cell dyscrasia and to identify treatment response, remission, and relapse. Changes in serum FLC levels also reflect changes in tumor burden. As shown in Figure 2, they also detect changes far more rapidly than measurements of intact immunoglobulins, allowing for a more efficient medical response. This is due to the short half lives of FLC in serum (κ , 2-4 hours; λ , 3-6 hours) compared with those of intact immunoglobulins (21 days for IgG; 5 days for IgA, 6 days for IgM, 3 days for IgD; and, 1 day for IgE)

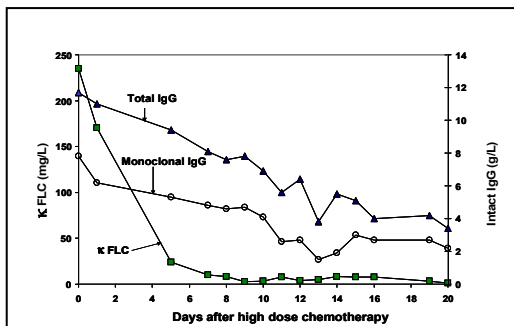


Figure 2: Response of serum FLC and intact immunoglobulin levels after chemotherapy in multiple myeloma. Modified from Bradwell AR: *Serum FLC Analysis*. 4th ed, *The Binding Site, Birmingham UK*

FALSE POSITIVE $\kappa:\lambda$ RATIOS

Renal disease

As noted above, serum FLC levels are determined in part by their relative excretion rates. In patients with renal insufficiency or failure but no evidence of MG-related disorders, both κ and λ levels can be variably elevated and $\kappa:\lambda$ ratios mildly elevated compared with a normal reference population (Hill *et al* 2006). Potential interpretive problems can be minimized by using a modified reference range for $\kappa:\lambda$ ratio in renal patients (0.37-3.1 vs 0.26-1.65). This increases the specificity of the test for MG from 93% to 99%, with no loss of sensitivity (Hutchison *et al* 2008).

Polyclonal hyperimmunoglobulinemia

In most cases, high levels of polyclonal immunoglobulins result in high levels of individual κ and λ serum FLC measurements, but a normal $\kappa:\lambda$ ratio. There are, however, exceptions and Dispenzieri *et al* (2008) recommend repeating the test at a later date if patients are experiencing infection or a rheumatic disease flare at the time of their abnormal sFLC result. Gottenberg *et al* (2008) demonstrated that serum FLC levels correlated with disease activity in rheumatoid arthritis and with extraglandular involvement in Sjögren's syndrome. Furthermore, 6% of rheumatoid arthritis patients and 8.6% of Sjögren's syndrome had abnormal $\kappa:\lambda$ ratios.

CHANGES TO FBR REPORTS

- **Reflexive testing: Block 3 and Immunoassay profile (IA),**

We have added sFLC testing to our current practice of performing reflexive IFE on samples received for Block 3 or IA testing when: a monoclonal band (or questionable band) is observed on SPE; when protein precipitates are seen at the origin on SPE; and, when hypogammaglobulinemia suggests the presence of light chain disease. Exceptions will be based on clinical laboratory judgment. We will perform only IFE (and not serum FLC) testing reflexively when SPE shows possible immune complexes. The IFE report has been revised to incorporate SPE, IFE and sFLC results.

- **NEW Block VI, Monogammopathy profile**

FBR's Block VI profile has been updated to provide efficient screening and monitoring of monogammopathy-related disorders. The profile now includes: serum quantitation of IgA, IgG and IgM; SPE; IFE; and FLC testing.

- **Interpretation of FLC results**

FBR reports will provide interpretations that integrate the findings of IFE and serum FLC testing.

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ORDER

Ordering Information for FLC testing: To order FLC as a single test check the appropriate box under Individual Studies on the FBR General Requisition form (version 02/09). To order the Block VI, Monogammopathy Profile, check the appropriate box under the profile section of the General Requisition form. IF you are using an earlier version of the Requisition, write "FLC" in the "other" box.

NOTE: If you do not want IFE or FLC testing to be performed reflexively as part of the Block 3 or Immunoassay profile, check "Omit confirmatory IFE and FLC". On earlier versions of the Requisition, check "Omit confirmatory IFE" and reflexive FLC will not be performed either.

Methodology: FBR uses particle enhanced latex nephelometry to detect free kappa and free lambda light chains (CPT code 83883 x 2 units).

Specimen: 2mL of nonhemolyzed, nonlipemic serum
Storage and shipping: Room temperature (3 days) or 4 weeks at 2-8°C

Days test set up: Testing is performed 2x per week.

Price: Refer to FBR's fee schedule.

Reference values:

Free Kappa: 3.30-19.40 mg/L

Free Lambda: 5.71-26.30 mg/L

$\kappa:\lambda$ ratio: 0.26-1.65

Note: the $\kappa:\lambda$ ratio reference range is slightly higher for patients with renal insufficiency:

$\kappa:\lambda$ ratio: 0.37-3.1

Further Information: For additional clinical information, please contact Walter C. Allan, MD; for technical information, contact Thomas B. Ledue, BA, or Wendy Y. Craig, Ph.D.