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Serum Free Light Chains

Quantitation and Clinical Utility
In Assessing Monoclonal Gammopathies

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Since the late 1960s, researchers have sought to develop methods to measure free light chains (FLCs) in serum. The initial experimental approaches used molecular weight differences to separate FLCs from light chains contained within intact immunoglobulins; however, these early methods did not lend themselves well to the clinical laboratory since they were technically complicated and time consuming. But the introduction of automated assays for the quantitation of immunoglobulin FLCs has now given clinical laboratories a new tool for evaluating monoclonal gammopathies. Not only have the new automated assays increased the diagnostic sensitivity for identification of light chain diseases such as light chain multiple myeloma and primary systemic amyloidosis, but they have also improved disease monitoring for light chain diseases and non-secretory myeloma. In addition, the test results have proven valuable for predicting which patients with monoclonal gammopathies of undetermined significance (MGUS) will progress to malignancy. With the availability of the FLC assay in the laboratory and the multiple advantages it can provide, the issue that many laboratories are now attempting to resolve is how to incorporate this assay into the initial evaluation of patients with suspected monoclonal gammopathies.

Monoclonal Gammopathies

Patients with monoclonal gammopathies produce monoclonal immunoglobulins by expanded, abnormal clones of plasma cells. These disorders may or may not cause symptoms. Traditionally, labs have detected the monoclonal immunoglobulins by protein

electrophoresis (PEL) and characterized the proteins by immunofixation electrophoresis (IFE). Clinically, identification of a monoclonal immunoglobulin is part of the diagnostic criteria for monoclonal gammopathies, and it serves as a marker for the abnormal plasma cell clone.

The hallmark monoclonal gammopathy is multiple myeloma (MM). Table 1 lists the distribution of the most common monoclonal gammopathies seen in the Mayo Clinic
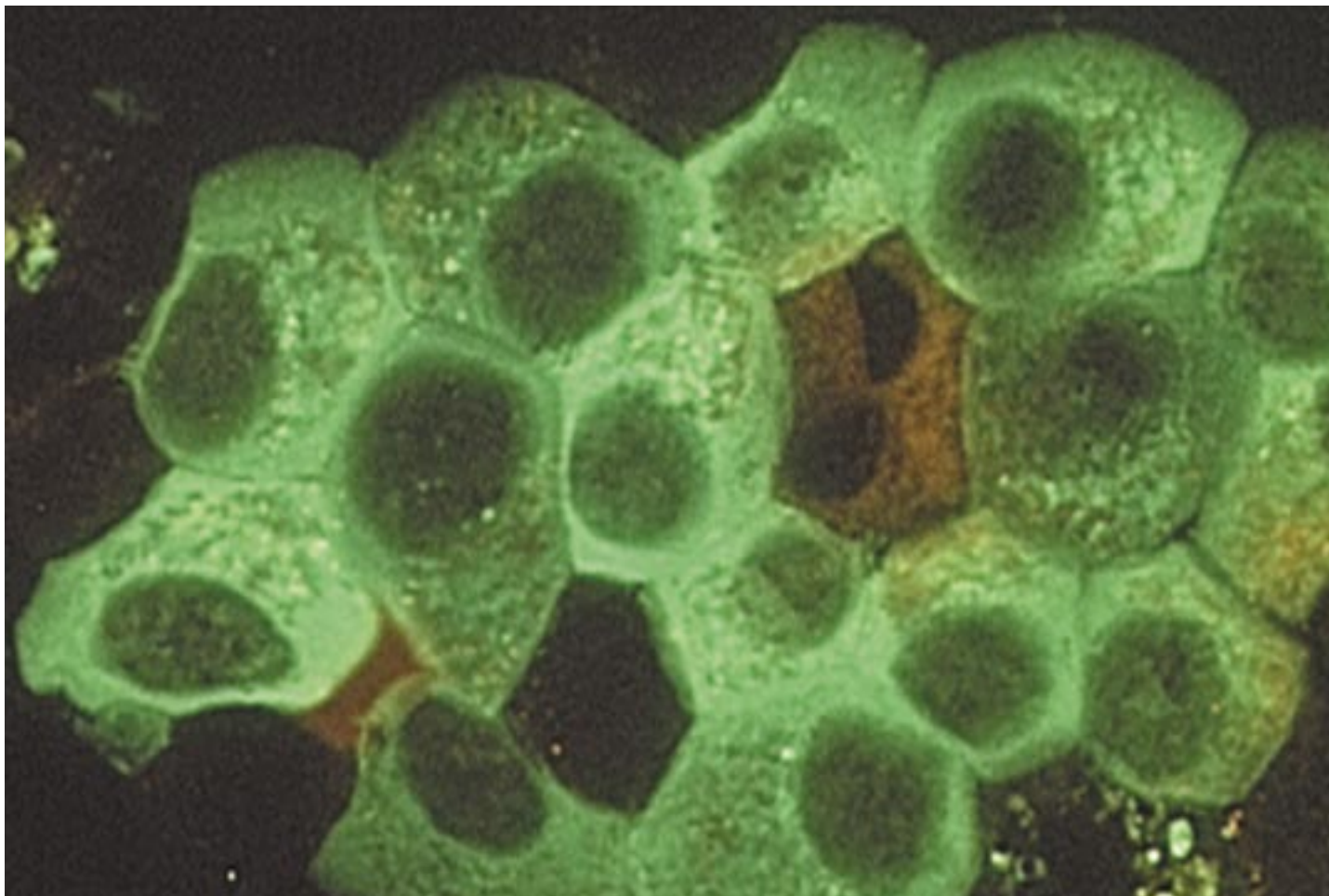
practice, including malignant disorders such as multiple myeloma, macroglobulinemia, plasmacytomas, and lymphoproliferative disorders; premalignant disorders such as MGUS and smoldering myeloma; and disorders of protein structure such as primary systemic amyloidosis.

Among the patients with multiple myeloma, approximately 20% have light chain multiple myeloma (LCMM). Although these patients have large numbers of bone marrow plasma cells, they may have small or undetectable amounts of monoclonal protein in their serum. In addition to these patients with LCMM, up to 3% of myeloma patients have a form of the disease called non-secretory multiple myeloma (NSMM) in which monoclonal protein is not detectable in either serum or urine.

Like myeloma patients, individuals with primary amyloidosis (AL) have a life threatening disease that can be difficult for the lab to evaluate. Ten percent to 20% of these patients will have no detectable monoclonal protein when serum and urine are evaluated by IFE, and approximately 50% will have no M-spike—a tall, homogenous, symmetric spike resulting from the presence of monoclonal immunoglobulin protein (M protein)—when samples are analyzed by PEL.

In some cases, patients with clinically important plasma cell disorders such as LCMM, NSMM, and AL present with no apparent monoclonal gammopathy as assessed by PEL and IFE, in which case the monoclonal gammopathies are more accurately described as plasma cell proliferative disorders. The difficulty in detecting monoclonal proteins in these patients has been attributed to one of two conditions: very small amounts of immunoglobulin produced by the plasma cell clone (e.g., NSMM), or the loss of heavy chain production and the secretion of monoclonal free light chains (e.g., LCMM and AL). The body rapidly clears monoclonal FLCs from the blood and the residual low concentration may be obscured by the large amount of light chains contained in normal polyclonal immunoglobulin.

Serum FLC Assays: The Breakthrough

Given the limitations of PEL and IFE, the description by Bradwell and colleagues in 2001(*1*) of an automated nephelometric assay that was suitable for use by the clinical lab represented a major breakthrough. Now commercially available, the reagent set uses polyclonal antibodies to quantitate both κ FLC and λ FLC by immunonephelometry (FREELITE™: the Binding Site, San Diego, Calif.). The antibodies show no reactivity by immunoelectrophoresis or by Western blots to light chains contained in intact Ig or to the opposite FLC—that is, κ FLC antibodies to λ FLC and vice versa. Sensitive hemagglutination assays showed reactivity

Table 1
Distribution of Plasma Cell Proliferative Disorders

Monoclonal Gammopathy of Undetermined Significance	61%
Multiple Myeloma	18%
Primary Systemic Amyloidosis	9%
Lymphoproliferative Disease	3%
Smoldering Myeloma	3%
Solitary or Extramedullary Plasmacytoma	2%
Macroglobulinemia	2%
Other	2%

From the Mayo Clinic Dysproteinemia Data Base, 1960–2002; n=29,528.

Table 2
Reference Intervals and Diagnostic Ranges[1]

	95% Reference Interval	Diagnostic Range[2]
Kappa (κ) FLC	0.33–1.94 mg/dL	
Lambda (λ) FLC	0.57–2.63 mg/dL	
FLC κ/λ	0.3–1.2 mg/dL	0.26–1.65 mg/dL

1. From Katzmann et al 2002 (2).
2. The diagnostic range is a 100% range and includes all reference sera.

to the appropriate FLC at dilutions above 1:16,000 and no reactivity to light chains contained within intact Ig at dilutions of less than 1:2. The new assay reagents therefore appear to have a minimum of a 10,000-fold difference in reactivity to FLC compared to light chains contained within intact Ig. This high specificity allows κ and λ FLC to be quantitated in the presence of a large excess of serum IgG, IgA, and IgM. Furthermore, labs can perform the assays on a number of automated laboratory instruments, including the Dade Behring (Deerfield, Ill.) BN II and BN ProSpec; Beckman Coulter (Brea, Calif.) IMMAGE; Roche/Hitachi (Indianapolis, Ind.) 911, 912, 917, and module P; and the Olympus (Melville, N.Y.) AU series.

Assay Validation

In order to validate the assay for clinical use, our laboratory at Mayo Clinic performed a reference range study with sera from healthy donors aged 21–90 years (2). We screened sera by PEL and IFE to exclude samples with a monoclonal protein and quantitated κ FLC, λ FLC, and cystatin C levels, along with calculating the κ/λ FLC ratio. The results showed an apparent effect of age on FLC, but when the κ FLC was divided by the λ FLC, the κ/λ FLC ratio showed no age dependence. Cystatin C showed the same apparent age dependence. Most likely, the increase in serum FLC concentration with age is due to reduced renal clearance, and the use of the κ/λ FLC ratio normalizes the effects of reduced clearance. Table 2 presents the FLC reference intervals and diagnostic ranges.

Abnormal κ and λ FLC concentrations may be due to immune suppression, immune stimulation, reduced renal clearance, or monoclonal lymphoplasmacytic proliferative disorders. Sera from patients with either polyclonal hypergammaglobulinemia or renal impairment often have elevated κ FLC and λ FLC, but in these cases the κ/λ FLC ratio remains normal. We expect that an abnormal κ/λ FLC ratio will only result from a clonal B-lymphoid or plasma cell proliferative disorder. An abnormally high ratio suggests expansion of a κ-producing plasma cell clone whereas an abnormally low ratio suggests expansion of a λ-producing plasma cell clone. Normal ranges are usually defined by a 95% reference range. By definition, the 2.5% tails of the population distribution are abnormal. Because we don't want to define 5% of the population as having a clonal expansion, we defined a diagnostic range for the FLC K/L ratio as comprising 100% of the normal samples evaluated.

Diagnostic Sensitivity of FLC Assays: NSMM and AL

Demonstrating the clinical utility of the FLC assay, a study of 28 patients with NSMM conducted by Drayson and colleagues found that although some patients had no serum or urine monoclonal protein detectable by SPE or IFE, 19 (68%) had abnormal κ/λ FLC ratios (3). In another study of 262 AL patients, Lachmann and colleagues detected abnormal serum FLC in 98% of the AL patients, whereas the serum or urine IFE was positive in only 79% of these individuals (4).

In our initial studies using the FLC assay, we assessed a group of patients who had plasma cell proliferative disorders and a monoclonal light chain in their urine but in whom we had difficulty identifying a monoclonal serum protein by IFE. FLC assays were more sensitive than IFE in detecting serum monoclonal free light chains in this cohort of patients.

The increase in diagnostic sensitivity of the serum FLC assay for monoclonal light chain diseases has been a welcome, but unexpected, diagnostic advance. In a recent prospective study to further evaluate its diagnostic performance in clinical practice, we have confirmed the increased sensitivity (5). In 110 untreated AL patients, the FLC assay was more sensitive (91%) than the serum or urine IFE assay (69% and 83% sensitivity, respectively). In addition, the assays were complementary for the detection of monoclonal FLCs in AL patients. The serum and urine IFE assays showed that 95% of these patients had an abnormal result in at least one of the two assays. Similarly, for the serum IFE and FLC assays, 109 of the 110 AL patients (99%) had an abnormal result in at least one of the two tests. Furthermore, the results of the urine IFE did not add any information to the serum assays.

Monitoring Disease Activity: LCMM and AL

In addition to aiding the diagnosis of NSMM and AL, the serum FLC assay may be useful in monitoring LCMM and AL. Lachmann and colleagues detected abnormal serum FLC in 98% of AL patients tested, but only 79% of the same patients showed an abnormal IFE (4). Equally interesting was the observation that 46% of the 262 AL patients had no serum or urine M-spike which could be used to monitor treatment. For AL, therefore, the serum FLC assay provided not only a more sensitive diagnostic tool but also a quantitative assessment for monitoring disease activity. Just as a 50% reduction in M-spike values is used as a response criterion when monitoring MM, a 50% reduction in the monoclonal FLC indicated a therapeutic response and was predictive of a significant survival advantage in AL. Dispenzieri et al. have reported that rather than assessing the percent reduction of serum FLC, normalization of FLC level after stem cell transplant predicted organ response in AL patients (6).

In addition to its diagnostic sensitivity, the FLC assay may benefit patients with LCMM, since it might be used to monitor these patients in lieu of 24-hour urine studies. LCMM patients may have no M-spike, or only a minimal one, when serum is tested; however, their urine has a large M-spike of monoclonal light chain. Consequently, quantitation of the 24-hour urine M-spike can be used to monitor disease activity.

Assessing LCMM patients using the FLC assay, a 2003 study by Bradwell and colleagues (7) found that all 224 patients had an abnormal serum κ/λ FLC ratio. The correlation between the quantitation of the serum FLC and urine M-spike is not linear, however; that is, some patients with large urine M-spikes have only small elevations in serum FLC and vice versa. The correlation of the changes in serial values, however, is excellent. Abraham and colleagues (8) monitored serial changes in the serum FLC and urine M-spike values of 71 patients and found a correlation between changes in the serum and urine values of P=0.0001. Although initial baseline values for the urine M-spike are still needed, monitoring these patients may no longer require 24-hour urine collections if more clinical documentation is developed.

Determining Prognosis: Monoclonal Gammopathy of Undetermined Significance

Monoclonal gammopathy of undetermined significance (MGUS) is a pre-malignant plasma cell proliferative disorder found in approximately 3% of individuals 50 years of age and older, and it is the most common plasma cell proliferative disorder seen by the clinical laboratory. The hallmark of MGUS is the presence of a monoclonal immunoglobulin in the serum. By definition, patients with MGUS have a serum M protein < 3 g/dL, bone marrow plasma cells <10%, and no anemia, hypercalcemia, lytic bone lesions, or renal failure that would be indicative of a malignant plasma cell disorder. Because MGUS is asymptomatic, diagnosis is usually the result of a screening serum PEL performed as part of a diagnostic work-up by primary-care providers.

Although asymptomatic, individuals with MGUS progress to multiple myeloma or related malignancy at a rate of 1% per year. Therefore the risk of malignancy for a 50-year-old patient with a 25-year life span is 25%. Currently, clinicians have difficulty distinguishing individuals with stable monoclonal gammopathy from patients in whom multiple myeloma will eventually develop. Since the risk of progression does not diminish even after 25–35 years, these patients therefore require lifelong follow-up.

Given the uncertainty of the patient's prognosis and the need for long-term follow-up, clinical management of MGUS would be enhanced by identification of factors that more accurately predict progression or stability. Since myeloma has a median survival of only 3–4 years, a patient's outcome is significantly affected by the clinician's ability to delay or prevent the progression of MGUS. If patients could be risk stratified reliably, those at a high risk for progression could be considered candidates for testing preventive strategies, while low-risk patients would not be subjected to potentially harmful preventive interventions or life-long monitoring. Unfortunately, risk factors that predict the likelihood of progression have so far been difficult to identify.

In a search for risk factors, we evaluated the impact of monoclonal FLC assay results on assessing the risk of progression of MGUS to malignancy (9). Of 1,384 MGUS patients from southeastern Minnesota seen at the Mayo Clinic from 1960 to 1994, we were able to analyze 1,148 baseline serum samples obtained within 30 days of diagnosis. Malignant progression had occurred in 87 patients (7.6%). An abnormal FLC ratio was detected in 379 patients (33%). The risk of progression in patients with an abnormal FLC ratio was significantly higher compared to patients with a normal ratio and was independent of the size and isotype of the serum monoclonal protein.

MGUS patients with an abnormal serum κ/λ FLC ratio, IgA or IgM isotype, and a serum M-protein level ≥1.5 gm/dL had a risk of progression at 20 years of 58% (high-risk MGUS), versus 37% with any two of these risk factors (high-intermediate-risk),

Table 3

Risk-Stratification Model to Predict Progression of MGUS to Myeloma[1]

Risk Group	Absolute Risk of Progression at 20 Years	Absolute Risk of Progression at 20 Years Accounting for Death as a Competing Risk
Low Risk Serum M protein <1.5 gm/dL, IgG isotype, normal FLC ratio	5%	2%
Low-Intermediate Risk Any one factor abnormal	21%	10%
High-Intermediate Risk Any two factors abnormal	37%	18%
High-Risk All three factors abnormal	58%	27%

1. From Rajkumar et al. 2005 (9).

21% with one risk factor (low-intermediate risk), and 5% when none of the risk factors were present (low-risk) (See Table 3). In the group of low risk patients, comprising 39% of the MGUS population, the serum M protein levels may need to be rechecked only if symptoms of myeloma or a related disorder become apparent. On the other hand, the smaller group of high-risk patients, comprising only 24% of the MGUS population, needs to be monitored more closely before pathologic fractures, hypercalcemia, renal failure, paraplegia from extramedullary plasmacytoma, primary amyloidosis, overwhelming infection, or other serious complications develop. This is a finding of significant importance for the management of MGUS.

Finding a Role for FLC Assays

Given its increased diagnostic sensitivity, improved monitoring capabilities, and potential use for risk stratifying patients, the serum free light chain assay could provide the laboratory with an important complementary methodology to PEL and IFE. For the evaluation of monoclonal gammopathies, the FLC assay clearly adds diagnostic sensitivity to the traditional serum PEL and IFE assays for patients with NSMM and AL. In addition, the FLC assay provides a quantitative parameter to monitor patients with no serum M-spike on PEL. Whether the FLC assay will replace urine PEL and IFE for monitoring LCMM patients is not yet clear, but studies are underway to evaluate the assay as

a potential surrogate for 24-hour urine studies. Lastly, the FLC assay provides a prognostic marker for risk stratification for progression of MGUS to malignant disease.

Currently, the standard approach for evaluating possible monoclonal gammopathies is to perform serum PEL and IFE. The PEL identifies and quantitates the M-spike, and the IFE characterizes the monoclonal protein. In addition, because the IFE is more sensitive than the PEL, it will identify some monoclonal proteins that are missed by PEL. Now, some laboratories may want to begin including the FLC assay as part of the initial panel of tests for monoclonal gammopathies. As discussed above, the FLC assay will increase the diagnostic sensitivity for the difficult to diagnose light chain diseases, will provide a quantitative value for monitoring patients who do not have an M-spike on PEL, and will provide prognostic information for MGUS patients. For MGUS patients, whose samples represent the majority of the abnormal results that we detect, the combined use of PEL, IFE, and FLC assays could prove particularly valuable as it would allow risk stratification for these patients; low risk MGUS patients would not only be given peace of mind, but also would not have to be routinely monitored for progression. These patients and their primary care physicians can be reassured that they need to pay attention to the "usual suspects" of diet, exercise, and smoking, but that it is unlikely that they need be concerned about their monoclonal gammopathy. **CLN**

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