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New Serum Free Light Chain Assay to Enhance and Simplify the Diagnosis of Monoclonal Gammopathies

by William T. Leeburg, MD

Traditionally it was necessary to order four tests to rule out a monoclonal gammopathy. Electrophoresis and immunofixation studies were required on both serum and a 24-hour urine collection. The urine studies were necessary because light chain disease, where the monoclonal light chains cause kidney damage and spill into the urine, may not be detectable in the serum. Bronson Laboratory now offers a Serum Monoclonal Gammopathy Panel that eliminates the need for the urine screen.

Historical Perspective

The study of immunoglobulins in the laboratory goes back to the time of Henry Bence Jones and his studies of urine, published in 1847. It was much later (1939) when serum electrophoresis demonstrated "church spires" in the serum of patients with multiple myeloma. Definitive identification of the light chains in patients dates back to 1956, with the use of the Ouchterlony technique, when Korngold and Lapiri showed that antisera raised against different groups of Bence Jones proteins (BJP) also reacted with sera from myeloma patients. The two types of BJP were designated kappa and lambda, in honor of the observations of this pair.

We now know that immunoglobulins are composed of a heavy chain (either A, D, E, G or M), which gives the specific immunoglobulin its name and the associated light chains (kappa or lambda). In patients with plasma cell disorders and other related disorders, the abnormal protein is restricted to a single light chain type, either kappa or lambda.

Abnormal proteins are detected in the lab in serum or urine by protein electrophoresis, in which a thin dark band (restriction band) or M-spike appears, most often in the gamma fraction (*see figures 1 and 2*). The abnormal protein is further characterized by serum or urine immunofixation, where the specimen is tested with specific antibodies to IgG, IgA, IgM and kappa and lambda light chains. Examples of specific abnormal proteins are IgG kappa, IgA lambda, etc. (*see figure 3*). The specific abnormal protein is important to identify as it affects the prognosis and the course of the disease.

The followup of patients with treated multiple myeloma has historically been to follow quantitative levels of serum or urine immunoglobulins or urinary BJP. Repeat immunofixation is not necessary, as the type of abnormal protein is already known.

Serum Free Light Chains

Recently it has become possible to directly measure serum free light chains (SFLC). When an immunoglobulin molecule is produced, there is a minimal excess of free light chains that then circulate in the plasma until cleared by the kidney. Under normal conditions, about twice as much kappa light chain is made compared to lambda light chain. In serum however, lambda free light chains and kappa free light chains are present in nearly equal amounts, as lambda free light chains form a dimer, and are more slowly excreted by the kidney than kappa free light chains.

Free light chain testing is abnormal when the concentration of the free light chain is greater than normal and when the ratio of the kappa free light chains to lambda free light chains (k/l light chain ratio) is abnormal. Normal ranges are given below (RF for renal failure).

Free kappa light chains	3.3-19.4 mg/l
Free lambda light chains	5.7-26.3 mg/l
k/l light chain ratio (without RF)	0.26-1.65
k/l light chain ratio (with RF)	0.37-3.1

In studies, renal failure and polyclonal gammopathy both are associated with an increase in the k/l light chain ratio, but in nearly all of these cases the ratio was <3.1. Most studies including the large study of patients with plasma cell disorders, performed at the Mayo Clinic[2] use a k/l light chain ratio of 0.26-1.65 as the normal range. Reactive states that stimulate polyclonal B cells, may cause a slight elevation in the amount of both kappa and lambda free light chains, as well as the k/l light chain ratio. It is of interest to note that nearly every low k/l light chain ratio is associated with a lambda plasma cell disorder (very few false positive results).

The amount and the ratio between the free light chains is very sensitive in detecting plasma (*continued on back*)

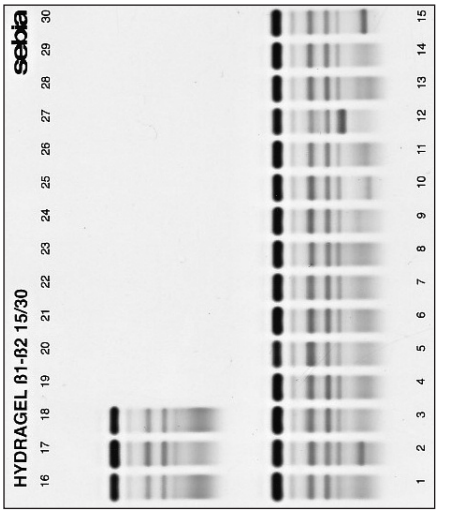

Figure 1: Serum electrophoresis gel where each lane is from a different patient. Lanes 2, 10, 12 and 15 show definitive M-spikes. Some lanes, 11 for example, may show an indistinct possible band that is best sorted out by reflexing to immunofixation.

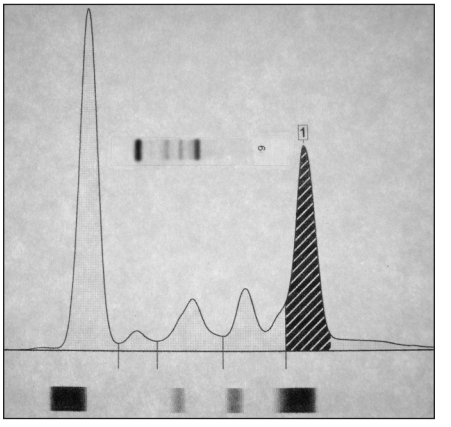

Figure 2. Serum electrophoresis and densitometry scan. The hatched area corresponding the M-spike can be measured to quantitate the monoclonal protein.

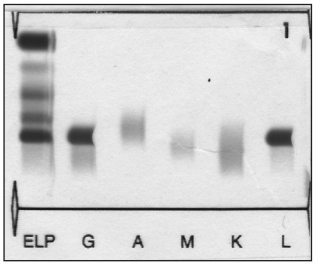

Figure 3: The abnormal band from figure 2 is characterized as an IgG lambda monoclonal protein by immunofixation.

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New Serum Free Light Chain Assay (continued)

cell disorders from serum samples including: free light chain disease (100%), light chain deposition disease (80%), AL amyloidosis (90-97%) and intact IG myeloma (96%). In addition free light chains are highly sensitive at picking up non-secretory (now oligosecretory) myeloma (82%) that are picked up by no other method, and myeloma involving heavy chains not routinely studied (IgD and IgE).

Urine testing, BJP testing versus SFLC

The sensitivity of SFLC testing is so great, that it may replace urine BJP testing. In a large study of patients with plasma cell disorders at the Mayo Clinic, 428 patients had both serum and urine for testing at presentation. All patients were tested using serum protein electrophoresis, serum immunofixation, SFLC, urine protein electrophoresis, urine immunofixation and urine free light chains (UFLC). With the serum testing alone, this study concluded that no patient requiring therapy would have been missed by using only serum samples.

UFLC are not considered as an acceptable way to follow patients with plasma cell disorders. UFLC are filtered through glomeruli, then reabsorbed and metabolized via the proximal tubules. Significant quantities of UFLC do not enter urine until reabsorption capacity of the tubules is overwhelmed and thus do not necessarily reflect either SFLC or tumor production of monoclonal free light chain. Finally as with all urine collections, there are problems with patient compliance in what is estimated at >50% of samples.

Studies have also compared urine protein electrophoresis with SFLC. There is little correlation between the two. The reasons for the lack of correlation were due to the need for the urine protein to exceed the resorption threshold to appear in the urine and that urine contained both intact IG and UFLC.

In several studies, the use of SFLC to detect AL amyloidosis at presentation has varied from 90-97% of patients. There are very rare patients with AL amyloidosis in which the SFLC are normal. If the clinical suspicion is high for AL amyloidosis, then after a negative SFLC, a 24 hour urine collection with electrophoresis and immunofixation may be required to detect a low number of additional patients with AL amyloidosis. The usefulness of UFLC has not been evaluated in this setting.

Among the most important and difficult problems with the testing of urine specimens, is the ability of the patient to collect a 24 hour specimen. All data on urine M-proteins and BJP are based on results from 24 hour collections. This is particularly important in following patients under therapy, as the 24 hour quantity of BJP is necessary to assess therapy effect. There is really no role for M-protein studies in random collections. Screening patients with suspected light chain disease utilizing random/spot urine testing is no longer appropriate, as 100% of these patients are diagnosed by SFLC alone.

Because of the high sensitivity of SFLC, it is no longer necessary to test urine for BJP as an initial step in working up a plasma cell disorders[1]. SFLC in combination with serum protein electrophoresis and serum immunofixation negates the need for 24 hour urine protein studies in nearly all cases.

Prognosis of Plasma Cell Disorders and SFLC

The baseline free light chain measurement is of major prognostic value in virtually every plasma cell disorder. Examples from several different studies are given below.

Multiple myeloma: poor prognosis when SFLC ≥750 mg/l
AL amyloidosis: shorter survival as SFLC ↑; median c/o 152 mg/l
Smoldering myeloma: ↑ risk progression c k/l lc ratio <0.125 or >8.0
Plasmacytoma: abnormal k/l lc ratio ↑ risk of progression
MGUS: abnormal k/l lc ratio ↑ risk of progression
CLL: abnormal SFLC predictive of poor survival

Role of SFLC in Assessing Response to Therapy

Free light chains are the current method to follow patients with certain types of plasma cell disorder, according to the International Myeloma Working Group (IMWG)[3]. SFLC are recommended by the IMWG, as the method to follow patients with oligosecretory myeloma. In addition the IMWG has recently published updated response criteria, which incorporate the SFLC assay (in myeloma patients without measurable serum or urine M- protein) and similar response criteria have been published for patients with AL amyloidosis.

Another study[4] showed the utility of SFLC in following patients with intact chain myeloma. SFLC were abnormal at diagnosis in 96% of 493 patients with intact chain myeloma. A small portion of the group (17) were followed with SFLC, quantitative total immunoglobulins, monoclonal immunoglobulins measured by densitometry, urine free light chains by radioimmunodiffusion, β2-microglobulin, serum creatinine, cystatin C and bone marrow plasma cell percentage estimates. They noted that SFLC fell more rapidly in response to treatment than intact immunoglobulin, had greater concordance with β2-microglobulin concentrations and bone marrow plasma cell assessments. They concluded that SFLC could be a helpful adjunct in following the therapy of patients with intact chain myeloma. However, this is not currently the recommendation of the IMWG.

Utilization of Tests to Detect Plasma Cell Disorders

As described in this article it is no longer necessary to screen both urine and serum to rule out monoclonal gammopathy. The combination of serum protein electrophoresis (SPE) and serum free light chain assay (SFLC assay) will detect virtually all cases. When the screen is positive a reflex serum immunofixation (SIFX) will be done to characterize the monoclonal protein.

To screen for a suspected Monoclonal Gammopathy:

1. SERUM MONOCLONAL GAMMOPATHY SCREEN: SPE and SFLC assay with reflex to SIFX if either is abnormal
2. SPE, SFLC and SIFX may still be ordered individually but the screen is more efficient.
3. If there is a strong suspicion of AL amyloidosis, and the serum screen is negative, then a 24 hour urine with both UPE and UIFX may be ordered.

Note: Urine studies are not necessary for screening but once a monoclonal gammopathy is diagnosed a 24 hour urine study is necessary to properly determine stage and prognosis.

For monitoring response to therapy:

1. For patients with a serum M-spike the SPE is adequate. *See Figure 2.*
2. For patients with no serum M-spike, but with a urine M-spike, urine protein electrophoresis is adequate.
3. Patients with monoclonal light chain disease but no M-spike may be followed by SFLC assay. This may also be useful for amyloidosis.
4. As described above studies are ongoing in evaluating the potential of the SFLC assay to replace measuring the M-spike.

Footnotes

1. Br J Haematol 2008:141 (May):413-422
2. Clin Chem. 2009: 55:8 1517-1522
3. Leukemia 2009: 23, 215-224
4. Br J Haematol 2004: 126, 348-354

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