SERUM FREE LIGHT CHAIN TESTING AND WALDENSTRÖM MACROGLOBULINEMIA

by Neal Makens, MD

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Introduction

Antibodies (immunoglobulins) are proteins produced by plasma cells (and to a limited extent by their immediate B-lymphocyte precursors) in response to antigens, which are foreign substances from microbes and other sources. A basic immunoglobulin (Ig) molecule is composed of 2 identical longer chains of amino acids called *heavy chains* and 2 identical shorter chains of amino acids called *light chains*. The resulting structure has a roughly Y-shaped configuration in which each arm of the Y is formed by an entire light chain bound to about half of a heavy chain (see diagram). Each tip of the arms of the Y constitutes an antigen binding site; the stem of the Y, consisting of the bound "lower" halves of the heavy chains, can attach to the surface of an immune cell or to an immune system protein such as complement.

The light chains can be either of two types designated by the Greek letters *kappa* (**κ**) or *lambda* (**λ**). Intact immunoglobulin (Ig) molecules can be any of five types, each determined by the type of heavy chain that it contains (**γ** = gamma in IgG, **α** = alpha in IgA, **μ** = mu in IgM, **δ** = delta in IgD, or **ε** = epsilon in IgE). A heavy chain may be paired with either of the two light chain types, but any given Ig molecule will contain only **κ** or only **λ** light chains. Likewise, the heavy chains in an individual Ig molecule will be of only one type. In the plasma, IgG is a "monomer," a single unit that

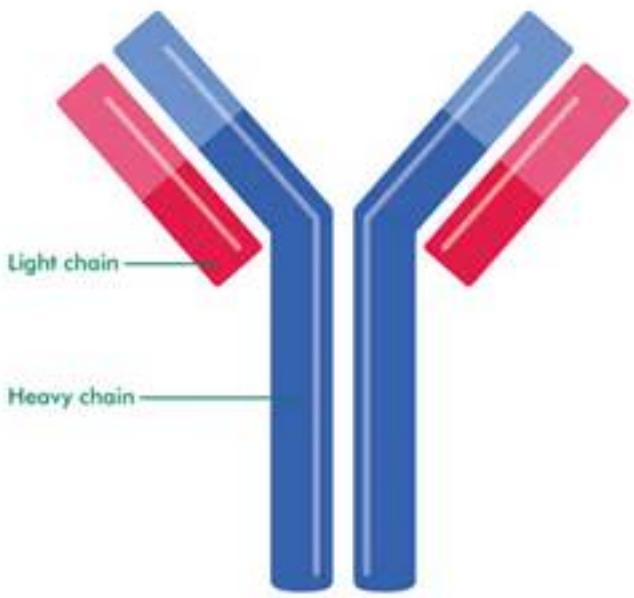

Diagram of Immunoglobulin (Antibody) Molecule

contains two identical light chains and two identical heavy chains. IgM is usually a "pentamer" that contains 5 of these units arranged radially like the spokes of a wheel, with the Y "stems" directed inward.

Free light chains

Plasma cells normally produce more light chains than heavy chains and more **κ** light chains than **λ** light chains. These excess light chains are not joined to heavy chains and they circulate in the blood as free light chains (FLCs). FLCs of **κ** type usually exist as monomers (single units), whereas **λ** free light chains are usually in pairs called dimers. Most FLCs are small enough to pass through the glomeruli, the blood filtering structures of the kidney, and are subsequently reabsorbed and metabolized in the proximal renal (kidney) tubules through which the fluid filtered by the glomeruli passes. **λ** FLC dimers are twice as large as **κ** FLC monomers, and the **λ** FLCs therefore are filtered through the glomeruli more slowly. (The half-life of **λ** FLCs in the blood stream is about 5-6 hours, which is about twice the **κ** FLC half-life.) The result is that the *serum concentration of* λ *FLCs is typically higher than that of* **κ** FLCs (**κ/λ** = 3:5).

It is important to distinguish between the measurement of *total* light chains in the blood and *free* light chains. Total light chain tests measure both free light chains and those light chains that are bound to heavy chains in intact Ig molecules without distinguishing between them. In contrast to the FLC ratio, the *total* **κ/λ** light chain ratio in serum is approximately 2:1.

Since FLC absorption and metabolism in the proximal tubules of the kidney normally far exceed the FLC synthesis rate, FLCs do not appear in the urine in more than trace amounts unless they are markedly overproduced. In multiple myeloma, however, monoclonal free light chain levels can be hundreds of times higher than normal. (In WM, the range of values is not as extreme as in myeloma.) When abnormal amounts of free light chain ("Bence Jones" protein) are present in urine, they are normally detected by urine protein electrophoresis (uPEP) and immunofixation electrophoresis (IFE). The *urine* FLC ratio by the Freelite test (see below) has been found to be less sensitive than IFE on a 24 hour urine. Consequently, *urine* FLC testing is not recommended for screening or monitoring.

The serum free light chain measurement, in contrast, is more sensitive in detecting low levels of free light chains in the blood than the traditional serum protein tests (PEP or SPEP – protein electrophoresis and IFE – immunofixation electrophoresis). The first commercially useful test for monitoring serum free light chains (sFLC) was developed by The Binding Site, Inc., Birmingham UK, and is known by the trade name of Freelite™. It uses polyclonal sheep antibodies directed against antigenic sites in the light

chain that are hidden in *intact* Ig molecules, but exposed in *free* light chains. The reference (normal) ranges for this test are given below and are those most commonly cited:

Serum Free Light Chain Reference Ranges:
κ: 3.3–19.4 mg/L
λ: 5.7–26.3 mg/L
κ/λ ratio: 0.26 –1.65

In addition to the above test, the Siemens company has developed a monoclonal antibody sFLC test that is currently available in Europe, but not in the United States. Patients who are being monitored should be followed with only one type of test, preferably performed by the same laboratory, in order to maintain the continuity of test results from one office visit to the next.

κ/λ *ratios that are increased above normal are considered as presumptive evidence of a* **κ** *monoclonal gammopathy* (a disorder in which Ig molecules are produced by a single abnormal clone of lymphoid cells), while ratios that are decreased are interpreted as evidence of a **λ** monoclonal gammopathy. However, it must be kept in mind that patients with polyclonal hypergammaglobulinemia (a broad elevation of many different Igs due to infections, autoimmune diseases, and other chronic inflammatory conditions) can have elevations of both light chain classes, and the **κ/λ** ratio can sometimes be slightly abnormal even though no monoclonal process is present. There can also be lot-to-lot variations in reagents and other technical problems that could affect the test results. In the presence of renal disease, the **κ/λ** ratio may also be altered, usually in an upward direction, due to a slower than normal rate of renal clearance of **κ** monomers. *It has been recommended that FLC test results in patients with renal impairment be evaluated using a different reference range for the* **κ/λ** *ratio: 0.37–3.1*. For these reasons, serum light chain individual **κ** and **λ** values and **κ/λ** ratio test values that are slightly outside of their reference ranges should be interpreted cautiously.

The **κ/λ** ratio should be interpreted together with the individual **κ** and **λ** values and not be considered in isolation:

- If one of the light chain measurements were elevated and the serum light chain ratio were significantly elevated, then a monoclonal gammopathy (in our case, IgM-MGUS or WM) would be likely.

- If both light chain values were elevated and the ratio were normal or borderline, then a polyclonal gammopathy due to a chronic inflammatory condition or kidney impairment would be suspected. (In rare cases, a biclonal gammopathy with one **κ** and one **λ** clone could also produce this pattern.)
- If both individual light chain values were low and the ratio were normal, bone marrow failure would be suspected.
- If all values were within the normal ranges, a monoclonal gammopathy could still be possible if the monoclonal cells were producing only an intact monoclonal Ig without a detectable increase in monoclonal free light chain. (This should be evident by PEP and IFE, however.)

Serum free light chains and Waldenström macroglobulinemia (WM)

Serum FLC (sFLC) testing is recommended as part of the initial workup for all MGUS (monoclonal gammopathy of undetermined significance) patients. The sFLC **κ/λ** ratio value is one of the 3 major risk factors used in stratifying MGUS patients into 4 groups for risk of progression to one of the plasma cell-related malignancies. sFLC testing is also recommended for *screening/diagnostic evaluation* for multiple myeloma (in its various forms), light chain (AL) amyloidosis, and a related condition known as light chain deposition disease (LCDD). It is recommended for ongoing *monitoring* of patients with oligosecretory multiple myeloma (in which the amount of secreted monoclonal protein is very low), light chain myeloma, light chain (AL) amyloidosis, and LCDD. It is also recommended for *verifying complete remission* in multiple myeloma, and may have a broader role in determining the response to treatment in myeloma.

Because FLCs have a substantially shorter half-life in the blood (up to 6 hours) than IgM (4-5 days), it has been hypothesized that sFLC testing might provide more rapid indications of changes in the course of WM that would facilitate monitoring of the disease. In 2011, Leleu and colleagues noted (in a study of sFLC monitoring of patients treated with bortezomib and rituximab) that sFLC analysis showed promising results as a sensitive marker of WM tumor measurement that correlated well with IgM and M-spike measurements, showed a more rapid change in test results in patients who responded to treatment, and also provided an earlier indication of disease progression (by about 1 month) than the results observed with traditional IgM or M-spike measurements. The article ended with a comment that the authors proposed to study sFLC measurement in future large, prospective trials to confirm whether or not early determination of sFLC would help in decision-making during the course of therapy. Kyrtsonis and colleagues in 2012 reported that the level of the involved (monoclonal) FLC and the FLC **κ/λ** ratio correlated with time to first treatment and adverse survival in WM. To date, however, there have been relatively few FLC studies in WM. As a

consequence, the status of sFLC testing in WM remains uncertain. In review articles on WM in 2015 by Drs. Gertz and Treon, sFLC testing was included in lists of tests that may be ordered as part of the *initial workup* for WM. However, at the present time, it has not been recommended for *ongoing monitoring* of patients with WM who can be followed with conventional testing.

Screening for AL amyloidosis and LCDD

It has been recommended that MGUS (monoclonal gammopathy of undetermined significance) patients whose baseline **κ/λ** ratio is abnormal and whose involved (monoclonal) FLC is elevated be monitored periodically for the development of heart or kidney damage due to amyloidosis or a related condition, light chain deposition disease. The recommended tests are serum NT-proBNP, which is a sensitive indicator of cardiac injury, and urine albumin (or microalbumin) to detect renal injury at an early stage. Such monitoring has also been recommended for selected WM patients. Signs and symptoms of amyloidosis can develop insidiously. Many of these (such as peripheral neuropathy, constipation or diarrhea, feeling lightheaded upon standing, peripheral edema, etc.) may also be seen with other diseases, so the diagnosis requires the demonstration by biopsy of one or more sites or by abdominal fat aspiration (at medical centers skilled in its detection by this technique). The need for such testing increases with additional risk factors, such as the presence of **λ** light chain as a monoclonal component of IgM gammopathy. It has been recommended that WM patients with peripheral neuropathy be considered for evaluation for amyloidosis, particularly if they have an IgMλ monoclonal protein. (While most WM patients have **κ** monoclonal light chain, the majority of patients with light chain amyloidosis have **λ** light chain. The ratio of **λ** to **κ** monoclonal light chain involvement is nearly 4:1.) For a fuller discussion of amyloidosis in WM, see Dr. Merlini's previous article in the April 2013 edition of the Torch newsletter.

Kidney injury from WM may involve infiltration by lymphoplasmacytic WM cells, deposition of intact IgM in glomerular capillaries, cryoglobulin injury, or deposition of monoclonal light chains. Light chain deposition disease always involves the kidney. The liver is the organ next most commonly involved, but numerous other organs can be involved as well. It typically presents with hypertension (high blood pressure), protein in the urine, and microscopic blood in the urine. There are microscopic and clinical differences between LCDD and light chain amyloidosis. At least half of the patients with LCDD have multiple myeloma but LCDD can occur by itself, in association with MGUS, and occasionally in WM patients. Treatment is aimed at lowering the monoclonal light chain to the maximum extent possible, which would be monitored by sFLC analysis and other tests.

Conclusion

My personal opinion is that it would be reasonable to order sFLC testing at the time of initial diagnostic evaluation to establish a baseline for future reference. At present, however, it is not considered necessary for determining response to therapy or for routine monitoring for most patients with WM. It could be useful for selected patients who are being evaluated for light chain amyloidosis or light chain deposition disease as well as those who already have either of those conditions.

For those of you who are having periodic FLC testing:
A rising involved (monoclonal) individual kappa or lambda light chain level well above the normal range associated with a rising ratio of the involved FLC/uninvolved FLC suggests that your WM is producing more monoclonal protein and may be proliferating. The reverse suggests that you are responding to treatment. These values may precede changes in your IgM by several weeks. The trend is important. They need to be correlated over time with the level of your M-spike by PEP and IgM by nephelometry, hemoglobin, platelet count, white count, kidney function tests, bone marrow findings, your general level of energy for daily tasks, the status of other illnesses that you may have, and your oncologist's impression of the status of your WM.

The future:
Over the last few years, articles have been appearing in the literature that evaluate a newer test developed by the same company that developed the sFLC test (The Binding Site). The new test is known generically as the heavy/light chain (HLC) test and is known by the trade name of Hevylite™. The HLC test uses antibodies that target junctional binding sites between the constant regions of heavy and light chains of Ig molecules, thereby measuring heavy/light chain pairs. Thus, there is simultaneous measurement both of a particular type of heavy chain (gamma, alpha, or mu) and the **κ** or **λ** light chains that are associated with that heavy chain. For example, in a case of IgMκ WM, the level of IgMκ would be increased, while the level of IgMλ might be normal or decreased. It may be that the HLC test could serve as a way to monitor the level of the M-spike. Some studies have evaluated both sFLC and HLC testing to see if the combination offers advantages over HLC alone. At the present time, only the IgG and IgA HLC tests are approved for use in the United States. In Europe, the IgM HLC test is also available. Before the HLC test is recommended for use in the diagnosis and monitoring of WM patients, additional prospective studies will be needed.

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