Serum free light chain analysis

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In a variety of hematologic malignancies, immunoglobulin light chains (LC) are overproduced clonally and circulate without being linked by disulphide bonds to the immunoglobulin heavy chain. The recent development of a robust assay known as κ and λ "free" LC (FLC) to quantify the levels of these unbound LC in the serum, and thereby determine their ratio, has led to an explosion of studies that demonstrate its utility in a wide range of hematologic disorders. This article summarizes laboratory testing for serum FLC, with a particular focus on clinical applications for the test. Am. J. Hematol. 85:787–790, 2010. © 2010 Wiley-Liss, Inc.

Background

Hematologic malignancies frequently result in the production of monoclonal immunoglobulin. Detection of both intact immunoglobulin and immunoglobulin free light chains (FLC) in the urine and blood has proven to be valuable in the diagnosis, prognosis, and monitoring of treatment of these diseases. One particularly useful property of serum FLC is their short half-life in the blood (κ, 2–4 hrs; λ, 3–6 hrs) in comparison to intact immunoglobulin (21 days), which provides an opportunity for real-time monitoring of disease progression and response to treatment.

For well over a century, testing for the presence of Bence Jones (BJ) protein in the urine was considered to be the gold standard to assess the clonally abnormal levels of immunoglobulin FLC. This technique can detect FLC down to the range of 10–40 mg/L; however, it can be challenging to obtain an accurate 24 hr urine collection, which is necessary for the test [1]. Moreover, due to the high resorptive capabilities of the proximal tubules of the kidney or reduced renal function, patients with low levels of FLC in the serum may not have detectable amounts in the urine [2]. Quantitative alterations of serum immunoglobulins (Igs) may result from polyclonal or monoclonal disorders. The combination of serum protein electrophoresis (SPEP), immunofixation (IFE), and densitometric analysis permits characterization and quantitation of the monoclonal immunoglobulin; however, the sensitivity of SPEP to detect FLC is poor, with a lower limit of sensitivity of 500–2,000 mg/L. Although the IFE does improve sensitivity for the detection of FLC (lower limit of sensitivity 150–500 mg/L), it is a more time-intensive assay and does not allow for quantification.

Technical Aspects, Performance, and Limitations

The development of an accurate and reproducible serum assay to determine κ and λ FLC concentration with high sensitivity has been a major challenge. A significant barrier to the development of the test is the fact that the unique epitope that is a marker of FLC is "hidden" or concealed in the conformational structure of an intact Ig. This precluded the development of a tool such as a specific antibody to detect the "hidden epitope" of FLC. This barrier was overcome in 2001 by Bradwell and coworkers [3], who dissociated the FLC from heavy chains and then raised polyclonal antibodies to detect the unique epitopes on κ and λ LC. These antibodies could then be used to develop assays that can detect only FLC in a milieu of free and bound LC. The availability of these unique antibodies ushered in an era of FLC assays, described below, for clinical studies.

Rate nephelometry is used to precisely quantify the amounts of κ or λ FLC. The level of detection in patients with abnormal κ/λ ratios is 1.5–3.0 mg/L, at least a hundred times more sensitive than that of previously available assays. The test developed by Bradwell [4] is the most specific and sensitive test for quantitation of FLC, and has a good level of precision, with percentage coefficients of variation usually less than 8%. Potential limitations in the precision of the assay are avoided by careful attention to proper laboratory technique, experienced staff, and well-maintained equipment. Care must be taken to maintain the stability of the FLC antisera, which are at risk for contamination by previously pipetted samples. The assay also requires a high level of batch-to-batch consistency, since it may be used to monitor the same patient for many years. Although antigen excess may theoretically cause the assay to underestimate high protein concentrations, clinical laboratories routinely repeat any abnormal tests at a higher dilution to identify samples with antigen excess and determine an accurate reading. A more detailed discussion of this issue and a wealth of up-to-date information about the assay and its development are available online at: www.wikilite.com [4]. In clinical practice, the assay has now been widely adopted and is generally considered to be reliable and reproducible in the real-world setting.

Normally, light chains are produced in humans at about 500 mg/day. Although approximately twice as much κ as λ is produced, renal clearance of the larger, dimeric λ light chains is slower, causing the normal κ:λ ratio to be about 0.58 (range 0.26–1.65 mg/L, median serum κ level of 7.3 mg/L, median serum λ of 12.7 mg/L) [5]. With infection or inflammatory disorders, polyclonal FLC production can increase markedly and overwhelm renal excretion. This generally results in an increase in the absolute value of both κ and λ FLC, with a relatively unchanged κ/λ ratio. With renal dysfunction, polyclonal κ and λ FLC may both accumulate in the serum, but they are eventually removed by pinocytosis by the reticuloendothelial system. Unlike renal excretion, pinocytosis is not dependent on the molecular weight of the FLC, leading to equal absorption of κ and λ. Since κ is produced in greater quantity than λ, the median κ/λ ratio in patients with advanced renal disease

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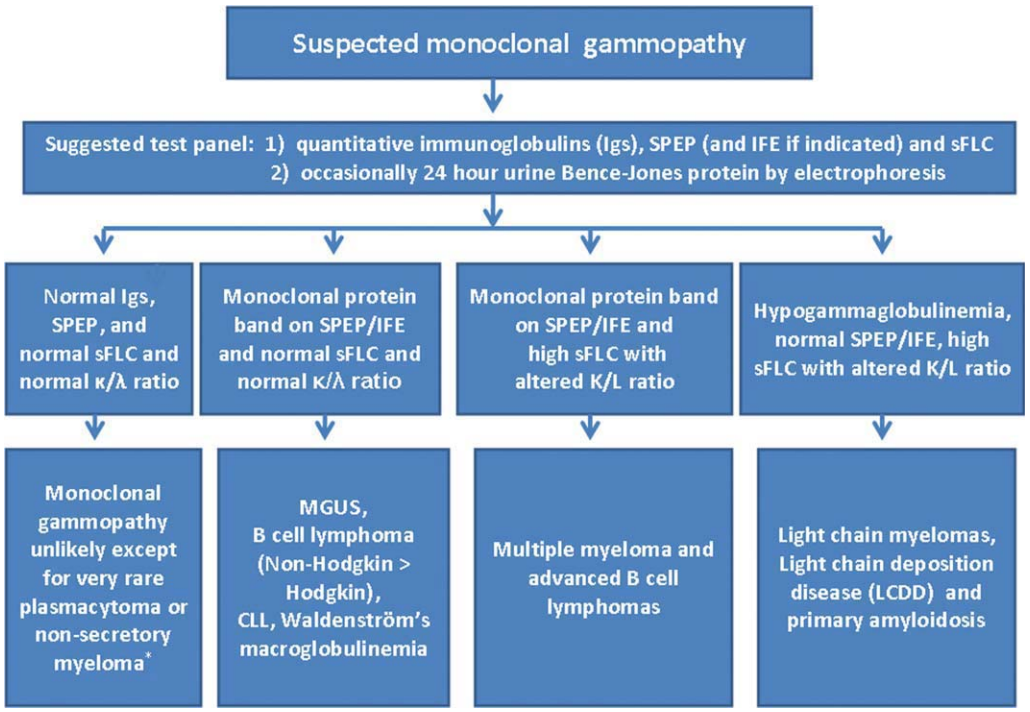

Figure 1. A diagnostic algorithm for the evaluation of a patient with a suspected monoclonal gammopathy. The screening panel includes quantitative immunoglobulins/serum protein electrophoresis (Igs/SPEP) and immunofixation electrophoresis (IFE) if necessary, along with quantitative serum free light chains (sFLC) with Kappa/Lambda ratio (κ/λ). Occasionally 24 hour urine for evidence of monoclonal FLC or Bence-Jones protein (BJP) by electrophoretic assays is required. The presence of a monoclonal band warrants an IFE and sFLC with κ/λ ratio (if not already sent), so as to characterize the monoclonal gammopathy. A normal SPEP/IFE and/or unexplained hypogammaglobulinemia warrants the performance of sFLC with κ/λ ratio so as to exclude light chain myeloma (LCM), light chain deposition disease (LCDD), or primary AL amyloidosis.
*If the work up for monoclonal gammopathy (including sFLC with κ/λ ratio and urine BJP) is negative but the clinical suspicion of AL amyloid is high, appropriate tissue biopsy with special staining for amyloid must be performed. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

increases, with a median value of 1.19 (range 0.37–3.1) [6]. In hematologic malignancies and associated conditions, monoclonal κ or λ FLC are often produced, resulting in a skewing of the κ/λ ratio. The alteration of this ratio has become an increasingly valuable way to diagnose, prognosticate, and monitor treatment of these disorders.

An algorithm for the practical implementation of FLC measurement in patients with suspected monoclonal gammopathy is outlined in Fig. 1. While some labs feel that quantitation of IgG, IgA, and IgM are not required, enumeration of these immunoglobulins has three potential advantages. First, it provides the basis for dilution of serum samples for subsequent IFE so as to maximize the immunoprecipitation and thereby enhance clarity in the detection of the monoclonal bands. Second, a normal or high IgA level with an associated decrease of IgG and IgM will necessitate exclusion of a monoclonal IgA gammopathy. Third, an unexplained decrease of IgG, IgA, and IgM often coexists with light chain gammopathies. Patients should be screened for the presence of both intact immunoglobulin by SPEP or IFE, and for FLC by the 24 hr urine collection for BJ proteins by electrophoresis, or preferably by the serum FLC assay. A normal SPEP or IFE does not exclude a monoclonal process, because a serum FLC assay may detect a clonal LC disease. If all of these tests are negative, a monoclonal gammopathy is highly unlikely, but if the clinical suspicion for AL amyloidosis is high, a tissue biopsy should be performed. If any of the above tests are positive, the gammopathy can be divided into three categories: intact Ig alone, FLC alone, or intact Ig plus FLC monoclonal proteins. Determining which of these three categories the patient falls into can help narrow the differential diagnosis and streamline the pathway to definitive diagnostic assessment.

Clinical Applications

The most widely used and best studied applications of the serum FLC assay have been in multiple myeloma. While myeloma is fundamentally a disorder of dysregulated plasma cells, a wide range of phenotypes are observed, including nonsecretory myeloma, production of FLC only (light chain disease), intact immunoglobulin, or combinations thereof.

Nonsecretory myeloma is defined by its lack of detectable intact immunoglobulin the serum or urine. It accounts for less than 3% of all myelomas, but can be challenging to manage given the inability to monitor immunoglobulin levels by SPEP. Recently, it has become clear that a majority of patients with nonsecretory myeloma actually produce detectable levels of FLC. For example, an MRC study found 19 of 28 (68%) nonsecretory myeloma patients had elevated FLC concentrations, with abnormal κ/λ ratios [7]. They also demonstrated that serum FLC can be used to monitor patients with nonsecretory myeloma by acting as a sensitive marker for progressive disease and response to therapy.

About 15% of myelomas produce only FLC, without any detectable intact immunoglobulin. In these patients, several studies have found that the serum FLC assay is more sensitive than 24-hr urine collection for BJ protein as detected by immunofixation assay, particularly in the setting of relapsed disease [8,9]. A prospective study of 82 patients with paired serum FLC and urine BJ proteins found that abnormal FLC were present in the serum in 54% of cases compared with 25% by urine BJ protein [10]. These findings have led some to conclude that when serum FLC testing is available, a 24-hr urine collection is no longer required for the measurement of light chain production [9].

The vast majority of myeloma patients (over 80%) produce intact immunoglobulin. Although the SPEP is used for

clinical management of these patients, serum FLC measurements also play a valuable role. Serum FLC at diagnosis is felt to be an independent prognostic indicator, with high levels being associated with poorer prognosis, particularly when the levels drop rapidly in response to treatment [11,12]. Serum FLC are also used to monitor for disease progression and response to treatment in nearly all myeloma patients. One especially useful property of serum FLC is their short half-life, which allows clinicians to determine response to treatment within even just a few days. One study assayed serial samples from 17 myeloma patients and found that serum FLC concentrations fell more rapidly in response to treatment than did intact immunoglobulin G, and correlated closely with the level of bone marrow plasma cells and serum β2 microglobulin concentrations [13]. There are also some early data that in patients with acute kidney injury due to myeloma, reducing serum FLC concentration by utilizing chemotherapy accompanied by a high cut-off dialyzer may lead to improved renal recovery [14]. Additionally, myeloma patients in whom the myeloma protein is undetectable by IFE as a consequence of therapy, who also normalize the κ/λ FLC ratio, have improved overall survival compared to those that do not [15]. These findings have led to the incorporation of normal FLC ratio as part of the revised definition of a stringent complete response in the Myeloma International Uniform Response Criteria [16].

Serum FLC measurement has also proven to be a valuable independent predictor of the risk of progression in premyeloma states such as monoclonal gammopathy of undetermined significance (MGUS), solitary bone plasmacytoma (SBP), and smoldering myeloma. In a large study of 1,148 MGUS patients at the Mayo Clinic, the 379 (33%) patients with an abnormal serum FLC ratio had a significantly increased risk of progression to myeloma, with a hazard ratio of 3.5 ($P < 0.001$, CI 2.3–5.5) [17]. In a separate study of 116 patients with SBP, 43 patients had progressed to myeloma by 5 years. An abnormal FLC ratio at the time of original diagnosis was associated with a higher risk of progression ($P = 0.039$), with a 5-year rate of progression of 44% compared to 26% in those with a normal FLC ratio [18]. Patients with smoldering myeloma often have an indolent course, but recent data also suggest that those with an abnormal serum FLC ratio are more likely to progress to active myeloma sooner than those with a normal ratio [19]. Of note, the utilization of the FLC assay in evaluating and monitoring monoclonal gammopathies has made the phenomenon of "free light chain escape" easier to recognize. This entity represents a shift in secretion from an intact Ig to FLC and is seen as a feature of clinical relapses in medullary and extramedullary myelomas [20].

In AL amyloidosis, the serum FLC assay has improved the ability both to make the diagnosis and to monitor patients on treatment. Although a paraprotein is detectable by serum IFE in only about 50% of AL amyloid patients, the addition of serum FLC greatly improves the sensitivity of detection, with one study showing 109 of 110 AL amyloid patients correctly identified by this combination [21]. The level of serum FLC at diagnosis also appears to correlate with the systemic burden of amyloid [22]. Importantly, a number of studies have shown that normalization or reduction of the FLC ratio with treatment correlates strongly with improved survival [23–25]. The serum FLC ratio is now a part of the international consensus guidelines for the management of AL amyloidosis [26]

The serum FLC assay has also proven to be useful in a variety of hematologic malignancies beyond myeloma. Patients with Waldenström's macroglobulinemia who present with an elevated serum FLC ratio carry a worse prognosis and require treatment sooner than those with a normal ratio [27,28]. There is also emerging evidence that serum FLC can be detected in a large fraction of patients with non-Hodgkin lymphoma. One study found that the highest incidences of monoclonal FLC excess were in mantle cell (36%) and small lymphocytic (24%) lymphoma. In a study of eight chronic lymphocytic leukemia (CLL) patients, an M-protein was detectable in six individuals only by the serum FLC assay and not by standard SPEP/IFE [29]. A prospective study found that FLC levels may be abnormal nearly 10 years before a diagnosis of CLL is made, suggesting that their production may represent an early event in the pathogenesis of the disease [30]. Other emerging data suggest that an abnormal FLC ratio confers a worse prognosis in CLL patients, and may actually identify a biologically distinct subgroup of the disease [31,32]. Elevated serum FLC levels have also recently been shown to independently confer an increased risk of development of non-Hodgkin lymphoma in HIV-infected persons [33].

Summary

The serum FLC assay has now been established as an important diagnostic, prognostic, and therapeutic marker in a wide variety of hematologic malignancies. The largest experience thus far is with multiple myeloma and its related conditions such as MGUS. The test is particularly useful in the diagnosis and management of cases of light chain disease, which may not have intact immunoglobulin detectable by SPEP or IFE, and in some cases not even by 24-hr urine collection for BJ protein. International consensus guidelines for multiple myeloma and AL amyloidosis now incorporate FLC measurement at multiple timepoints critical to classification and clinical decision making. The serum FLC assay has also demonstrated utility in Waldenström's macroglobulinemia, CLL, and NHL, and its role in these diseases will likely continue to grow as new data emerge. The advent and application of serum FLC assays has resulted in the recent recognition of an entity known as light chain monoclonal gammopathy of undetermined significance (LC-MGUS). Like the conventional MGUS, LC-MGUS seems to have the potential to evolve into light chain myeloma and related disorders [34].

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