Serum free light-chain assay for the detection and monitoring of multiple myeloma and related conditions

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Abstract: Diagnosis and monitoring of multiple myeloma (MM) and related conditions are usually carried out by means of serum and urine protein electrophoresis and immunofixation. In the early 2000s, an assay aimed at evaluating serum free light chains (sFLCs) was made available and subsequently tested in different plasma cell disorders. Several reports have demonstrated the usefulness of the assay for the diagnosis and monitoring of oligosecretory MM, nonsecretory MM, Bence Jones MM, and amyloid light-chain amyloidosis. Furthermore, a prognostic role for an abnormal sFLC κ/λ ratio has been observed in the case of monoclonal gammopathy of unknown significance, smoldering MM, solitary plasmacytomas, and in newly diagnosed symptomatic MM secreting intact monoclonal immunoglobulins. In conclusion, according to present data, the sFLC assay can be considered reliable for the diagnosis, monitoring, and prognosis of different plasma cell disorders, and recently studies have been carried out to test a possible role of an sFLC evaluation in other B-cell lymphoproliferative malignancies.

Keywords: monoclonal immunoglobulins, myeloma, serum free light chains

Introduction

Monoclonal gammopathies include a wide range of hematological conditions ranging from indolent disorders such as monoclonal gammopathy of undetermined significance (MGUS) to severe, life-threatening diseases such as multiple myeloma (MM) or amyloid light-chain (AL) amyloidosis. The mainstay of the diagnosis and monitoring of these disorders is the evaluation of an abnormal monoclonal immunoglobulin, the M component, in the serum and/or urine, by means of electrophoresis and immunofixation. In several conditions, including nonsecretory or oligosecretory MM, however, no direct correspondence exists between tumor burden and the amount of M protein. Furthermore, in the case of Bence Jones MM, disease monitoring by the evaluation of light chains (LCs) in the urine can be unreliable due to errors in carrying out a correct 24-h urine collection and sampling. The sensitivity of detection of a monoclonal protein in these conditions could be increased by evaluating serum free light chains (sFLCs) [Bladè and Kyle, 1999]. For several years, attempts at the detection of sFLCs were unsuccessful as no analytical method was able to make a distinction between LCs bound to intact immunoglobulins and FLCs. In the early 2000s, however, an assay was developed aimed at evaluating an epitope 'hidden' in intact immunoglobulins and 'visible' in FLCs [Bradwell *et al.* 2001]. Subsequently, several reports have been published concerning the use of this assay in several myeloma-related conditions. A normal range for κ and λ chain concentration in the serum of healthy individuals was then set at 3.3–19.4 mg/L and 5.7–26.6 mg/L for the κ and λ chains, respectively [Katzmann *et al.* 2002]. Under normal conditions, about 500 mg/day FLCs are produced, with a κ/λ ratio of about 2/1. As λ LCs are dimeric, their renal clearance is slower compared with κ LCs [Wochner *et al.* 1967], leading to a κ/λ ratio in the serum of about 0.58 (range 0.26–1.75) [Katzmann *et al.* 2002]. FLCs have a serum half-life of 2–6 h as they are rapidly cleared by the glomeruli and metabolized in the proximal tubules. When FLCs are produced in excess, the reabsorptive capacity of the tubules can be overwhelmed, thus leading to an accumulation of

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FLCs in the serum [Bradwell *et al.* 2003]. This can occur in a number of clinical conditions including inflammation, immunological disorders, renal failure, and plasma cell neoplasms [Davids *et al.* 2010], only in this latter case, due to the overproduction of a monoclonal LC, the κ/λ ratio is abnormal, thus rendering this parameter potentially useful for the diagnosis and monitoring of these hematological disorders. A few limitations in the assay have been identified, namelya slight to substantial and inter-instrument variability [Sheldon, 2007], and the overestimation of LCs when tested in urine, so that urine FLCassay is not recommended for monitoring patients with monoclonal gammopathies [Dispenzieri *et al.* 2009].

Use of sFLC in the diagnosis and monitoring of plasma cell disorders

In over 80% of plasma cell disorders the neoplastic clone secretes a measurable amount of an intact monoclonal immunoglobulin, and although sFLC concentration is abnormal in most patients [Mead *et al.* 2004], the assay does not add any practical information to the whole diagnostic procedure, so that the gold standard for diagnosis is still represented by serum protein electrophoresis and immunofixation. Due to the short half-life of FLCs (2–6 h, compared with 21 days for intact immunoglobulin G), the assay can be useful potentially for earlier monitoring of the efficacy of a given therapeutic program [Pratt *et al.* 2006; Hajek *et al.* 2007], although the impact of these data on patients' outcome is still a matter of debate [van Rhee *et al.* 2007]. Oligosecretory MM are conditions in which the amount of monoclonal protein secreted by the neoplastic clone is much smaller than that expected by the evaluation of the tumor load; disease monitoring usually includes repeated testing of the bone marrow plasma cell infiltration and/or bone imaging [Durie *et al.* 2006]. In these cases, which usually comprise immunoglobulin D myelomas, the evaluation of the involved FLC, provided that the FLC ratio is abnormal, makes it possible to monitor disease response to therapy. For this reason the International Myeloma Working Group defined patients with a 'measurable' disease as those having either a serum M protein > 10 g/L, or a Bence Jones proteinuria > 200 mg/24 h, but also those with involved sFLCs > 100 mg/L [Durie *et al.* 2006]. According to this definition, the new International Uniform Response Criteria include a normal FLC ratio as a prerequisite for a stringently defined complete response [Durie *et al.* 2006; Dispenzieri *et al.* 2009].

Nonsecretory MM represent about 3% of all MM. The addition of sFLC evaluation to the diagnostic work up has demonstrated that an average 70% of cases produce a monoclonal LC [Drayson *et al.* 2001], thus rendering the sFLC assay useful in monitoring disease response and relapse.

The major application of sFLC assays in the diagnosis and monitoring of clonal plasma cell disorders is in MM secreting only LCs (Bence Jones). Several studies have demonstrated that sFLC measurement has a greater correspondence to tumor load compared with Bence Jones proteinuria, especially when small amounts to be evaluated as in residual disease need [Bradwell *et al.* 2003; Katzmann *et al.* 2002]. This could make LC quantification in 24-h urine samples no longer necessary to monitor Bence Jones MM, even though an estimation of proteinuria should be performed at diagnosis to better characterize the disease [Singhal *et al.* 2007]. In the case of AL amyloidosis, a monoclonal protein is detected in about 50% of patients, but usually in small amounts. A recent report showed that adding sFLC assay to the diagnostic work up allows a correct identification of monoclonal protein in most patients [Katzmann *et al.* 2005]. Another recent report demonstrated that a reduction in sFLC level correlates to a response to therapy [Kumar *et al.* 2010], so, although 24-h proteinuria with electrophoresis must be evaluated at diagnosis in patients with AL amyloidosis, disease monitoring should be accomplished by evaluation of FLCs only [Dispenzieri *et al.* 2009].

Prognostic role of the sFLC assay

As soon as the sFLC assay became available and widely tested, a number of studies reported a possible prognostic role of sFLC or the sFLC ratio in most MM-related disorders. It has been demonstrated that an abnormal FLC ratio in MGUS predicts for a higher probability of progression to MM [Rajkumar *et al.* 2005]. A risk model including the sFCL ratio, size of monoclonal protein peak, and isotype of the heavy chain was thus designed, and patients showed a risk of progression at 20 years ranging from 5% to 58%, depending on the number of risk factors they displayed [Rajkumar *et al.* 2005]. Similarly, in patients with smoldering MM, an abnormal sFLC ratio predicts a shorter time to

progression, and a risk model was constructed including the sFLC ratio, percentage of bone marrow plasma cell infiltration, and the size of the M protein [Dispenzieri *et al.* 2008].

Several reports indicated a possible prognostic role for the sFLC assay even in MM secreting intact immunoglobulins. Baseline sFLC level predicts for survival in patients with newly diagnosed symptomatic MM [Kyrtsonis *et al.* 2007]: patients showing a κ or λ LC level above the median level had a significantly shorter survival (30% at 5 years) than patients showing an sFLC level within the median range (80% at 5 years). Also the sFLC ratio has a prognostic significance in symptomatic MM, as a 9-month survival advantage was observed in patients with a normal ratio compared with those with an abnormal one. An abnormal sFLC ratio was also incorporated in the cut-offs included in the International Staging System, and it showed an independent prognostic significance [Snozek *et al.* 2008].

In the case of solitary plasmacytomas, an abnormal sFLC ratio at diagnosis was reported to predict for a higher risk of progression to overt MM [Dingli *et al.* 2006]. This was incorporated into a risk model also including the persistence of a detectable M protein (higher than 0.5g/dl), and patients having both risk factors had a risk of progression at 5 years of 62%, compared with 13% of patients with no risk factors.

In AL amyloidosis a higher level of sFLCs at diagnosis predicts for a higher risk of death [Kumar *et al.* 2010]. A reduction of sFLCs on treatment appears to be more strongly related to an improvement in overall survival than intact immunoglobulin decrease after therapy [Kumar *et al.* 2011]. Another parameter that has been recently taken into consideration in this field is the difference between involved and uninvolved sFLCs (FLC-diff), which has been incorporated into a prognostic model that also includes troponin T and N-terminal pro-B-type natriuretic peptide; this allows stratification of patients into four risk categories showing a significantly different survival [Kumar *et al.* 2012].

sFLCs in MM-related renal failure

In patients with renal failure, removal of sFLC occurs primarily by reticuloendothelial pinocitosis; this leads to an increase in sFLC half-life and serum levels 20–30 times normal [Bradwell *et al.* 2003]. As the differential ability to clear κ and λ LCs by the kidney is lost, the sFLC ratio can also change, and this renders the assay potentially unreliable. It has been suggested for this reason that, for this patient population, normal range should be increased to 0.37–3.1, thus leading to an increased sensitivity of the assay [Hutchison *et al.* 2008]. The management of kidney myeloma relies on the rapid removal of nephrotoxic LCs from the serum; for this reason plasma exchange or, more recently, high cut-off hemodialysis, has been proposed as an adjunct to dexamethasone ± bortezomib-based regimens. Evaluation of sFLCs could also be useful in this setting to monitor the efficacy of these dialytic procedures aimed at the selective elimination of sFLCs [Hutchison *et al.* 2007].

Further applications

sFLC assay has definitely been demonstrated to be useful in the diagnostic work up and monitoring of several MM-related conditions, and to possess a prognostic role in a majority of them. More recently, the role of sFLC assay has been investigated in other B-cell lymphoproliferative disorders, such as Waldenström macroglobulinemia, non-Hodgkin lymphoma (NHL) and B-cell chronic lymphocytic leukemia (B-CLL) [Charafeddine *et al.* 2012]: sFLC level or an abnormal sFLC ratio predicts a shorter survival in B-CLL, while in diffuse large B-cell NHL, an increased sFLC level represents an independent adverse prognostic factor for overall and event-free survival. AIDS could represent another future application of FLC assay as a marker of B-cell dysfunction. It has been reported that, in HIV-infected patients, polyclonal sFLCs above the normal range is correlated to advanced age, higher viral load, lower CD4 counts, and a higher predisposition to AIDS-related events such as opportunistic infections [Shiels *et al.* 2012].

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Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

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