

Serum free light chain analysis in multiple myeloma and plasma cell dyscrasias

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After the development of a reliable method to detect free light chains in serum, several investigations have been conducted to explore their importance in plasma cell dyscrasias (PCD). Detection of monoclonal proteins is very important in the diagnosis and management of PCD, which include a broad spectrum of diseases such as multiple myeloma and also benign, premalignant disorders like monoclonal gammopathy of undetermined significance. The aim of this article is to summarize the recent studies and to highlight the importance of free light chain analysis in the diagnosis of PCD, its prognostic value and role in the management of this group of diseases.

KEYWORDS: free light chain • immunoglobulin • multiple myeloma • plasma cell disorders

In the mid-19th Century, Sir Henry Bence Jones described the presence of protein aggregation in urine specimens from patients with multiple myeloma (MM). 100 years after the description of the Bence Jones protein in urine, Edelman and Gally demonstrated that light chains of immunoglobulins were identical to this protein [1]. During normal immunoglobulin synthesis, most of the immunoglobulin light chains are bound to immunoglobulin heavy chains, but there is a small excess of free light chain (FLC) production, which is rapidly cleared by the kidney. There are two types of light chains: κ and λ . Free immunoglobulin light chains are polypeptides of approximately 22 kDa. They may exist as monomers or as dimers with a molecular weight of approximately 44 kDa. The dimers may be covalently disulfide-linked or noncovalently linked [2]. κ -FLC are usually monomeric and filtered three-times faster than λ -FLC, which exist as dimers. Thus, despite the higher production of κ light chains compared with λ , the serum concentration of κ -FLC is lower than that of λ -FLC, resulting in a lower than expected κ/λ ratio. The serum concentration of FLC depends on the balance between production by plasma cells and renal clearance. Kidneys can metabolize 10–30 g of FLC per day and normal production of FLC by plasma cells is 0.5–1 g per day [3].

Since 1960, despite several efforts to measure FLC in serum, the methods used have been complicated and nonsensitive. In 2001, Bradwell

et al. developed a new assay method that used polyclonal antibodies against FLC to determine the serum concentration of free λ and κ light chains [4]. These polyclonal antibodies only react with those epitopes that are hidden when bound to heavy chains but are available when not associated with heavy chains (FIGURE 1). These antibodies have a 10,000-fold higher affinity to FLC than for light chains bound to immunoglobulin heavy chains. This nephelometric FLC assay is a commercially available method (FREELITE, The Binding Site Ltd., Birmingham, UK) that has been used successfully to provide quantitative measurement of κ - and λ -FLC in serum, and also the κ/λ -FLC ratio (FLC-R). Katzmann *et al.* studied the reference intervals and diagnostic ranges of serum FLC in healthy individuals [5]. With the 95% reference interval, serum levels were 0.33–1.94 mg/dl for κ -FLC and 0.57–2.63 mg/dl for λ -FLC. The FLC-R was 0.3–1.2 and the 100% confidence interval for this ratio was 0.26–1.65. Patients with ratios greater than 1.65 have excess κ -FLC and are presumed to be producing clonal κ -FLC. Patients with ratios less than 0.26 contain excess λ -FLC and are presumed to be producing clonal λ -FLC [5]. The urine immunoglobulin FLC assay is not recommended for practical use.

There is a correlation between age and higher absolute levels of FLC, probably due to age-dependent reduction in renal clearance.

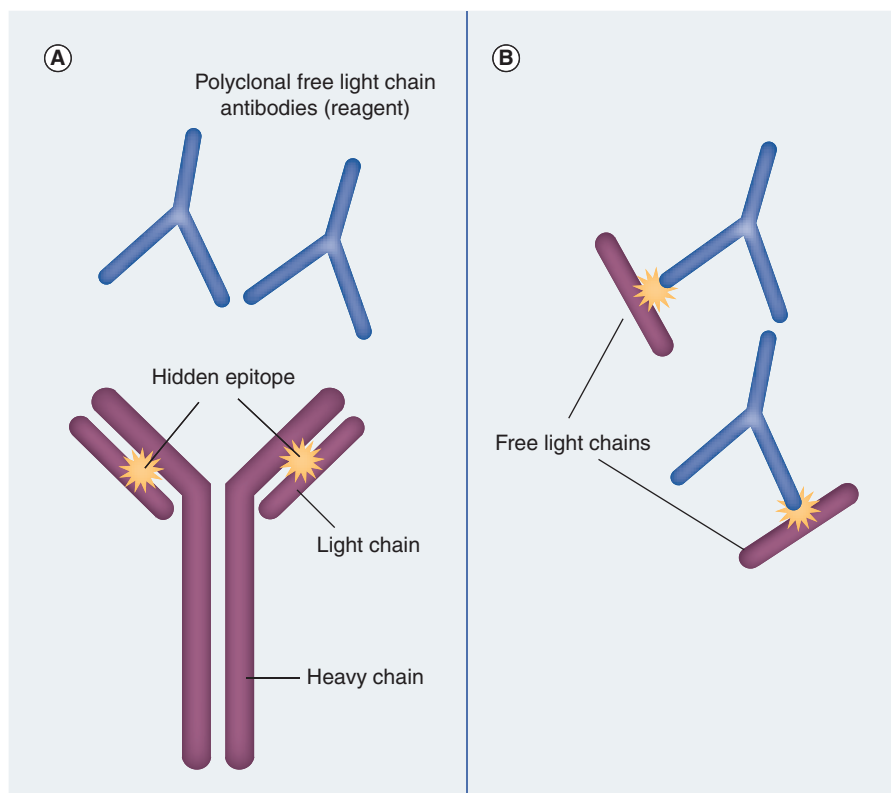


Figure 1. Principle of free light chain analysis. (A) Polyclonal immunoglobulin against hidden epitopes of the light chain cannot bind their target in intact immunoglobulin. (B) When the light chains are free in serum, the polyclonal antibodies can bind to them and liberate the epitope.

Although the levels of κ and λ -FLC increase owing to renal failure, the FLC-R changes only slightly. Based on their study of 142 patients with new dialysis-dependent renal failure, 41 of whom had MM, Hutchison *et al.* have recently proposed a new reference range for patients with renal failure: FLC-R: 0.37–3.1 [6]. The authors concluded that measurement of serum FLC concentration and calculation of serum FLC-R is a convenient, sensitive and specific method for identifying monoclonal FLC production in patients with MM and renal failure.

Polyclonal overproduction of immunoglobulins due to inflammatory or rheumatologic diseases may lead to an increase in the serum concentrations of both κ and λ FLCs, but the FLC-R usually remains within the normal range. An abnormal FLC-R indicates an excess of one light chain type versus the other. Thus, an abnormal serum FLC-R strongly suggests the presence of a monoclonal protein disorder, such as plasma cell dyscrasias (PCD). Abnormal FLC-Rs and monoclonal protein production can also be seen in B-cell lymphoproliferative disorders such as chronic lymphocytic leukemia and non-Hodgkin's lymphomas [7].

Plasma cell dyscrasias are disorders that include a broad spectrum of disease groups including: malignant diseases like MM and Waldenström's macroglobulinemia (WM), some premalignant conditions like monoclonal gammopathy of undetermined significance (MGUS), light chain MGUS and smoldering (asymptomatic) MM (SMM) and also protein conformation/deposition

disorders like immunoglobulin light chain amyloidosis (AL) and light chain deposition disease (LCDD). As the biology and presentation of PCD are heterogeneous, identification of the monoclonal immunoglobulin is very important and may be the first clue in diagnosis. Since the early 2000s, FLC analysis has moved rapidly into clinical practice. In the last decade, indications for FLC measurement have evolved. Later, the diagnostic and prognostic importance of the FLC assay and the value of serial FLC testing in PCD will be discussed in detail.

FLC assay as a screening test

Until the end of the 1990s, widely accepted tests to detect the M protein in PCD were: protein electrophoresis; immunofixation electrophoresis (IFE) of both serum and urine; and nephelometric measurements of immunoglobulin heavy chains of serum [8,9]. After the introduction of the reliable test for serum FLC analysis, investigators have studied the assay's utility in different PCDs (TABLE 1) [3,10–20]. Theoretically, the FLC assay should offer several advantages. First, because the sensitivity to detect monoclonal protein is 1–2 g/l for serum protein electrophoresis (SPEP), 150–500 mg/l for IFE, and less

than 1 mg/l for FLC assays, one would assume the FLC would perform better [4,5]; however, this only holds true in conditions where there is FLC excess. Second, both patients and laboratories prefer the logistics of obtaining and analyzing serum FLC over that of a 24-h urine protein electrophoresis/immunofixation. The literature shows that in routine clinical practice, the use of urine IFE analysis is very low, even when pursued as part of a clinical study (17–52%) [21,22]. Errors and failures in collecting urine and the handling of urine are the major problems, thus, serum analysis of FLC may be a more practical option. There are two important questions regarding screening for monoclonal gammopathies. First, is there benefit to adding the FLC assay to standard tests? Second, can the FLC assay replace any of the standard tests?

Katzmann *et al.* asked whether the serum immunoglobulin FLC assay could replace urine IFE for screening patients who are suspected of having a monoclonal protein-related disorder. In this retrospective study conducted on 428 patients with a monoclonal urine protein from Mayo Clinic PCDs database, abnormal FLC-R, abnormal SPEP and abnormal serum IFE ratio were 85.7, 80.8 and 93.5%, respectively [23]. They determined that had serum FLC assay been used alone, 14% of patients with positive urine IFE would have been missed; however, the combination of serum IFE and FLC identified 99.5% of patients with positive urine. The two patients who were missed had MGUS, and would not have required medical intervention [23]. Similar

Table 1. Rates of abnormal free light chain ratio in patients with newly diagnosed plasma cell dyscrasias.

Disease	Patients (n)	Abnormal FLC-R (%)	Comments/other findings	Ref.
Symptomatic MM	790	95	Worse survival in FLC-R of <1:32 or >1:32	[20]
	493	96	Faster reduction in FLC concentration in response to treatment than intact immunoglobulin	[18]
	61	97		[15]
	399	96	Baseline elevations in FLC predicted OS and PFS; faster reduction in FLC concentration in response to treatment than intact immunoglobulin	[13]
Smoldering MM (asymptomatic)	72	88		[16]
	273	90	Risk of progression to myeloma increased with FLC-R <1:8 or >1:8	[12]
Nonsecretory MM	28	68	Follow-up of six patients with elevated FLC showed that FLC concentration changed with disease activity	[14]
Light chain MM	224	100		[3]
	28	100		[10]
Amyloidosis	95	92	FLC assay was more sensitive than serum or urine IFE	[11]
	262	98	More than 50% reduction in FLC associated with survival benefit	[17]
	110	91	The combination of serum IFE and FLC assay detected abnormal results in 99% of patients	[16]
MGUS	1148	33	Abnormal FLC-R is significant predictor of progression in MGUS	[19]
	114	44		[16]
LCDD	26	92		[16]
LC-MGUS	129	100	Risk of progression to MM 0.3% per year	[32]

FLC-R: Serum immunoglobulin free light chain ratio; IFE: Immunofixation electrophoresis; LCDD: Light chain deposition disease; LC-MGUS: Light chain-MGUS; MGUS: Monoclonal gammopathy of undetermined significance; MM: Multiple myeloma; OS: Overall survival; PFS: Progression free survival.

findings were discovered in another study [21]. In a more recent study by the Mayo group, 1877 patients with the diagnosis of MM (n = 467), SMM (n = 191), MGUS (n = 524), plasmacytoma (n = 29), extramedullary plasmacytoma (n = 10), WM (n = 26), AL (n = 581), LCDD (n = 18) and polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin changes syndrome (POEMS; n = 31) were evaluated for single or a combination of screening tests: SPEP, serum IFE, serum FLC analysis, urine protein electrophoresis and urine IFE. A total of 26 patients had negative tests for all assays. When they excluded urine IFE from the panel, the monoclonal protein was missed in an additional 23 patients. By contrast, when the FLC test was omitted, 30 patients were missed. When serum and urine IFE were both excluded, the monoclonal protein would have been missed in 58 patients. Of note, the relative utility of the assays were disease specific. The sensitivity of SPEP plus FLC, instead of SPEP plus IFE plus FLC, differed based on the disease entity. The sensitivity of the two-test panel compared with the three-test panel was reduced by an absolute value of: 23% for POEMS; 8% for MGUS; 4% for plasmacytoma; and 1% for AL. There was no significant change for MM, SMM and WM or LCDD [24].

Vermeersch *et al.* studied the sensitivity and specificity of FLC analysis in 833 consecutive patients, who were examined for a monoclonal gammopathy [25]. A total of 28 patients were

diagnosed with PCD, 25 with B-cell non-Hodgkin's lymphoma and 156 with MGUS. They analyzed serum SPEP, serum FLC-R and IFE of serum and urine in all patients. The sensitivity of serum IFE plus serum FLC-R was comparable to the sensitivity of serum IFE plus urine IFE (93.8 vs 92.3%), but the specificity of serum IFE plus serum FLC-R was lower than that of serum IFE plus urine IFE (96.8 vs 100%); however, the majority of the cases missed were MGUS. As a single test, FLC-R was abnormal in only 28% of the MGUS patients, which is consistent with the finding of Rajkumar *et al.*, who found that only 33% of 1148 patients with the diagnosis of MGUS had abnormal FLC-R [19]. In MGUS patients, the FLC-R and concentration may be within population reference intervals despite the presence of monoclonal intact immunoglobulin [26].

In the study by Hill *et al.*, serum FLC assays were added to consecutive requests for SPEP of 923 patients. The positive predictive value for abnormal FLC-R was found to be 51% [22]. This study included a very low percentage of patients with diagnosed B-cell proliferative disorders (8%). Of patients with a polyclonal increase in immunoglobulin, 14 had an elevated FLC-R and in this group the glomerular filtration rate was lower, suggesting a polyclonal increase plus renal insufficiency may cause a false-positive abnormal FLC-R [22]. In this study, monoclonal light chains were detected by urine IFE in 15 patients, and 12 of these

patients had an abnormal serum FLC-R. Three patients with urine monoclonal proteins had a normal serum protein electrophoresis (PE) and FLC-R. Thus, the false-negative rate was 20% for serum PE plus FLC-R in this small group; however, this assay method did not miss any serious disease.

Free light chain assay for diagnosis is more important in patients with AL or LCDD. In a study, that included 110 AL patients, the FLC-R was positive in 91% of cases compared with 69% for serum IFE and 83% for urine IFE. The combination of serum IFE and FLC-R detected 99% abnormal results of patients with AL [16]. In another study of 119 patients with AL, the combination of serum IFE and the FLC assay detected the amyloidogenic light chain in 96% of patients [27]. Although the sensitivity of the two tests may appear adequate, given the gravity of the diagnosis of AL and the protean presentation, the screening approach of serum and urine IFE plus serum FLC is recommended.

Nonsecretory myeloma has been defined by the absence of monoclonal immunoglobulin by serum and urine immunofixation and includes 1–3% of myeloma patients. Of patients with nonsecretory myeloma, 68% have been shown to have abnormal FLC serum ratio and can be followed using the FLC assay [14].

Although monoclonal protein production is characteristic of PCD, it can be detected in other B-cell non-Hodgkin's lymphomas, and some patients with MGUS progress to B-cell non-Hodgkin's lymphoma. In a study including 226 patients with the diagnosis of B-cell non-Hodgkin's lymphoma, 24% had a detectable M protein and 63% of this group had an abnormal FLC-R; in 35%, the M protein was positive only by the FLC assay [7]. In another study that included 25 patients with non-Hodgkin's lymphoma, serum IFE plus abnormal serum FLC-R was positive in 13 patients, while serum IFE plus urine IFE detected monoclonal protein in 11 patients [25].

The International Myeloma Working Group guidelines for serum FLC analysis in MM and related disorders suggest that for screening of monoclonal proteins for all pathologic diagnoses except AL, the FLC can replace the 24-h urine IFE; however, once a diagnosis of monoclonal gammopathy is made, the 24-h urine IFE should be conducted. For AL screening, the urine IFE should still be conducted in addition to the serum test including serum FLC-R [28].

In summary, different screening approaches may be effective for different PCDs. Serum FLC analysis has several advantages over the urine IFE test. Collection and processing of urine is more complex than a simple blood test. The FLC-R is more sensitive for screening of MM and AL than urine IFE. Theoretically, the measurement of FLCs in the serum is less subject to renal dysfunction than is the measurement in the urine, where concentration is controlled by absorption in the renal tubules and not merely tumor production or serum concentration. However, the international adoption of the assay may depend on the relative costs of the assays in a given country. Thus, the FLC assay can replace the urine IFE in screening MM, WM, LCDD and SMM, and should be added to screening tests in AL and LCDD.

Prognostic importance of serum FLC

FLC as a prognostic factor in MM

In several studies, the prognostic importance of serum FLC assay for MM outcomes has been shown. Kyrtsonis *et al.* found that the 5-year disease-specific survival in those with a serum FLC-R lower than or equal to the median was 82% versus 30% in those with a serum FLC-R greater than the median ($p = 0.0001$). In this study, serum FLC-R was found to be an independent prognostic factor [29]. In another study, the serum FLC-R was incorporated into the International Staging System, which is based on serum and β 2-microglobulin levels. In this study, patients were assigned a point for any of the following risk factors: markedly abnormal FLC-R ($<1:32$ or >32); high serum β 2-microglobulin (>3.5 g/l); albumin or low serum albumin (<3.5 g/dl), for a maximum score of 3. The median survival rates depending on whether the score was 0, 1, 2 or 3 were 51, 39, 30 and 22 months, respectively [20]. van Rhee *et al.* have demonstrated the impact of the highest levels of FLC on survival. They found that patients with FLC greater than 750 mg/l had the poorest outcome (overall survival 76 vs 91%; event-free survival 73 vs 90%). The highest baseline FLC levels were significantly correlated with light chain MM, elevated creatinine, high β 2-microglobulin level, high lactic dehydrogenase levels and bone marrow plasmacytosis [30].

FLC as a prognostic factor in MGUS & SMM

Monoclonal gammopathy of undetermined significance is defined as serum M protein level less than 30 g/l, less than 10% clonal plasma cells in the bone marrow and, most importantly, the absence of myeloma-related end-organ damage (hypercalcemia, renal failure, anemia and bone lesions). MGUS was identified in 3.2% of residents of Olmsted County, MN, USA who were 50 years of age or older [31]. The prevalence increased with increasing age. The cumulative progression to other diseases (MM, WM, lymphoma amyloidosis) was 12% at 10 years, 25% at 20 years and 30% at 25 years. The risk of progression was approximately 1% per year [26]. Nearly a third of patients with MGUS have an abnormal FLC-R, and the progression rate seen in this group is higher than in those with normal FLC-R. The role of serum FLC-R as an independent risk factor for progression was demonstrated by the Mayo studies [19]. In this study, a model consisting of abnormal FLC-R (<0.25 or >1.65), M-spike greater than or equal to 1.5 g/dl and heavy chain isotype other than IgG was developed. The risk of progression at 20 years including competing risk of death for patients with 0, 1, 2 or 3 risk factors was 5, 21, 37 and 58%, respectively. More recently, Dispenzieri *et al.* demonstrated that LC-MGUS – the light chain counterpart to conventional MGUS – had a progression rate (0.3% per year) to MM and related disorders comparable to that of the lowest risk MGUS patients [32]. Results of a study by Weiss *et al.* showed that LC-MGUS was the preceding premalignant lesion for light chain MM (LCMM) [33]. Screening for LC-MGUS is not recommended but, once detected, physicians should be aware of progression of LCMM or the development of renal disease [34].

Smoldering or asymptomatic MM involves the production of an M protein level of 30 g/l or more than 10% clonal plasma cells in the bone marrow, but without end-organ damage. Progression of SMM to symptomatic myeloma is approximately 10% per year. Baseline FLC-R is also useful for assessing prognosis for progression in SMM. In another Mayo Clinic study, baseline serum samples of 273 patients were examined. An abnormal FLC-R was found to be associated with higher rates of progression. The best break point for FLC-R was less than or equal to 0.125 or greater than or equal to 8 with a hazard ratio of 1.9 [12]. An abnormal FLC-R was shown to be independent of bone marrow plasmacytosis greater than 10% and serum M protein level above 30 g/l. The authors incorporated each of these risk factors to create a model for progression, with 5-year progression rates of 26, 51 and 76%.

Analysis of FLC-R as a prognostic factor was also incorporated into the 2010 International Myeloma Working Group guidelines for MGUS and SMM [35].

FLC as a prognostic factor for other PCD

In a study including 116 patients with the diagnosis of solitary plasmacytoma, 47% of patients had an abnormal FLC-R and were associated with a higher risk of progression to myeloma (44 vs 26%) [36].

In a cohort of 119 patients with AL undergoing peripheral blood stem cell transplantation, there was a significantly higher risk of death in patients with higher baseline levels of FLC. Higher FLC levels were associated with more organ involvement by amyloid [37]. High serum FLC was also found to be associated with poor prognosis in WM. The 'time to treatment' interval was shorter in the patients with elevated serum FLC levels. Serum FLC was also found to reflect tumor burden [38,39].

In summary, baseline levels of serum FLC have been used as a prognostic marker among different PCD. Higher baseline levels of serum FLC or abnormal FLC-R were associated with higher degree of disease aggressiveness or progression in almost all PCD. The pathogenic rationale for this association is not clear, but higher levels of FLC may be associated with IgH translocation, and may also reflect increasing tumor burden [30,37,40]. Moreover, high levels of light chain may be associated with increased risk of renal failure, which has a poorer prognosis [41].

FLC assay for response assessment & disease monitoring

The plasma half-life for FLC is 2–6 h, while it is 20–25 days for IgG and 6 days for IgA. The rapid half-life of λ - and κ -FLC could allow the FLC assay to provide a more rapid indication of treatment response. Although using the serum FLC assay for response is considered for all PCD, it can only be routinely recommended for oligosecretory MM, LCMM and AL [13,28]. There are a few studies investigating FLC as a predictor of response to therapy and progression [17,37,42,43]. For serial measurements, the involved FLC (rather than the FLC-R) should be the focus [13]. With highly immunosuppressive chemotherapy, the FLC-R may be markedly abnormal due to pathologically low levels of FLC, reflecting immunosuppression more than the tumor burden.

Recently, the International Myeloma Working Group published updated response criteria that incorporate the FLC assay for two purposes. The first is that it provides a means to follow those patients who would otherwise be unevaluable – that is, those patients with a serum M protein less than 10 g/l and a urine M protein less than 200 mg/24 h, but a serum immunoglobulin FLC greater than 100 mg/l.

Nonsecretory myeloma accounts for 1–3% of all myelomas and is defined by the absence of monoclonal immunoglobulins in serum and urine tests. Nearly two thirds of these patients were positive for abnormal FLC-R [14]. In this study by Drayson *et al.*, six of the patients were followed during the course of their disease and changes in concentration of FLC were in accordance with their clinical progress [14]. However, to date, there are no formal data to verify that FLC changes in patients with nonsecretory myeloma correlate with those of bone marrow plasmacytosis or overall disease status.

The second use of the FLC condoned by the International Myeloma Working Group is for the response category of stringent complete response. This response category not only includes absence of a monoclonal protein on immunofixation of the serum and urine, and the absence of clonal plasma cells in the bone marrow, but also considers normalization of the FLC-R. Stringent complete remission (sCR) was proposed by the International Uniform Response Criteria for Multiple Myeloma group [44]. The definition of sCR is satisfied by that of complete remission (CR) plus the absence of clonal plasma cells in bone marrow and normalization of FLC in the serum. CR is defined as negative IFE in serum and urine, disappearance of any soft tissue plasmacytoma, and are 5% or less plasma cells in bone marrow. However, there is little data indicating that a stringent complete response with a normal FLC-R is prognostic for progression-free survival or overall survival. In another study, 52 patients diagnosed with MM were monitored for FLC, using the value of FLC assay to determine sCR [45]. Owing to the short half-life of FLC, patients were only included if the CR remained stable for at least 3 months. The result of this study revealed that the FLC-R is, due to its shorter half-life, a useful marker for faster detection of remission or progression in MM. No additional value of FLC-R to determine the depth of remission in IFE-negative patients was found. In a different study, which included 203 myeloma patients who underwent autologous plus allogeneic stem-cell transplantation, achievement of CR or sCR had no significant impact on event-free and overall survival [46]. In another recent study, the prevalence of abnormal FLC-R was studied in 34 patients with MM in complete response after hematopoietic stem-cell transplantation. Abnormal FLC-R was found in 73% of patients with oligoclonal band group versus 26% without oligoclonal band and 41% in total [47]. An oligoclonal immune response is known to be associated with good outcome, and because they found higher abnormal FLC-R in the oligoclonal band-producing subgroup, they highlighted this group and suggest further investigation.

The role of measuring a response in LCMM and intact immunoglobulin MM remains controversial. Approximately 96% of new cases of MM with intact immunoglobulin have abnormal

serum FLC concentrations [18]. Consistently, however, there is a poor correlation between the concentrations of the tumor FLC and intact immunoglobulin [10,13,18]. Owing to the shorter plasma half-life of FLC, the FLC assay may be valuable to assess early treatment response. Poorly responding patients could be identified earlier, however, there are no data to show that early detection of a lack of response predicts treatment failure. In the case of LCMM, serum FLC concentrations were reported as being more specific for complete response than immunofixation of the urine because a third as many achieved CR as judged by normal serum concentrations of FLC compared with FLCs in the urine. This led the authors to suggest that serum FLC may be able to replace the urine studies [3]. Unfortunately, this study is insufficient to change practice since both the methodology of studying the urine and the criteria used for remission were vague. When changes in FLC were evaluated in another clinical trial setting, the correlation between the 24-h urine M protein and serum FLC was poor in patients with LCMM and in those with intact immunoglobulin myeloma with FLCs in their urine; in addition FLC response at 2 months did not predict overall or progression-free survival [13]. In a small study, serum FLC was monitored in patients with MM who achieved negative immune fixation after allogeneic stem-cell transplantation [48]. Normalization of FLC occurred approximately 3 months earlier, probably due to the shorter half-life of FLC.

Although the use of FLC measurement is appealing in lieu of 24-h urine protein electrophoresis, no data have yet been produced to show that when there is discordance between the two assays, the FLC performs better. The inconvenient reality is that because the FLC is the newer test, the burden of proof lies on demonstrating its superiority.

'Free light chain escape', is a shift from intact immunoglobulin secretion to FLC secretion during relapse [49,50]. Kuhnemund *et al.* reported that this process was more common in IgG patients than IgA patients (8:2) and in males than females (9:1) [50]. It was shown that patients with intact immunoglobulin myeloma also have some tumor cells producing only FLC, and there was no impact of treatment modality on this phenomenon [51]. The diagnosis of this process is often delayed because clinicians often do not perform 24-h urine proteins electrophoresis. The FLC assay may facilitate earlier diagnosis of this entity given its ease of performance on routine blood samples.

In the 10th International Symposium on Amyloid and Amyloidosis, consensus opinion has been defined for FLC response in patients with AL. This recommendation is based on several studies [17,42,43,52]. In a study including AL patients undergoing hematopoietic stem-cell transplant, 50% reduction in their intact FLC was not predictive of overall survival, but this degree of reduction was associated with a trend towards improvement in both hematologic and organ response rate [37]. In this study, immunoglobulin FLC response was a better predictor of survival than complete hematologic response. In a very recent Mayo study, 125 AL patients with high serum FLC were evaluated for FLC response. A total of 90% reduction in FLC was found to be correlated with prolonged survival [53].

Restrictions

As is true for most tests, the FLC assay is not 100% sensitive or specific for diagnosing plasma cell malignancy when used in isolation. As expected with any assay, there are several technical limitations of the FLC assay. Lot-to-lot variation in assay reagents can be an issue. Both polyclonal and monoclonal serum FLC have often been found to dilute in a nonlinear fashion, leading to underestimation in the absence of off-line dilution [54]. Falsely high results may occur due to polymerization of light chains [10,55]. In the case of antigen excess, false low serum FLC results may be obtained [56,57]. Patients' clinical picture, renal function status, coexisting infections and concomitant treatments should be taken into account.

Expert commentary & five-year view

In summary, the FLC assay in combination with serum PE and IFE yields high sensitivity and negates the need for 24-h urine analysis when screening for PCD other than amyloidosis – the one condition in which urine IFE is also still recommended. Once the diagnosis of PCDs has been made, a 24-h urine protein electrophoresis plus or minus a urine IFE is required for all patients. Serum FLC levels and ratio at diagnosis is an independent prognostic factor for MGUS, SMM and solitary plasmacytoma, active myeloma and light chain AL. In addition, the FLC assay is useful for monitoring patients with LCMM, AL and nonsecretory myeloma. The FLC assay may eventually replace urine for monitoring light chains in LCMM, but this requires more study before it can be accepted as a practice guideline. It is also needed to document SCR in the myeloma uniform response criteria.

It is not only important to know when to use the test, but also how to use it [58]. In general, for screening, the FLC-R is of major importance. The ratio abnormality should be due to elevation of at least one of the FLCs and these results should be considered in the context of the renal range in cases of renal failure [6]. For prognosis, various extremes of the FLC-R are most commonly used (MGUS, SMM, plasmacytoma and MM), but in AL the absolute value of the involved FLC is prognostic and some authors have also used the absolute value for prediction in MM. Finally, for the instances in which the FLC assay is used for serial monitoring of the clone, the absolute value of the involved FLC is the preferred parameter (instead of the ratio). In this context, the uninvolved FLC and the ratio can provide additional insight into subtle renal dysfunction, severe immunoparesis and polyclonal activation. However, these same factors can be confounding – especially immunoparesis – and this is the reason why the FLC-R cannot be recommended as the serial measure of tumor burden.

The role of FLC in other B-cell neoplasms and polyclonal autoimmune disorders is in its infancy. Interestingly, in some autoimmune diseases such as rheumatoid arthritis and primary Sjögren's syndrome, abnormal FLC-R without monoclonal gammopathy and monoclonal B-cell proliferation is described [59]. Whether these abnormalities will prove to be prognostic or point to the need for a less restrictive FLC-R range is yet to be

determined. Whether there will be prognostic value for serum FLC in non-Hodgkin's lymphomas and rheumatologic diseases will be elucidated in the future.

Finally, the novel immunoassay panels for immunoglobulin heavy/light chain pairs have been designed and launched. These polyclonal antibodies recognize junctional epitopes between heavy chain and light chain constant regions of intact immunoglobulins [60]. This new analysis may provide additional valuable information in combination with the FLC assay in the future.

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Key issues

- Monoclonal protein production is characteristic of plasma cell proliferative disorders.
- In the last decade, a sensitive and specific method to measure free light chains in the serum has been defined.
- In the context of screening plasma cell proliferative disorders, serum free light chain assay with serum protein electrophoresis and immune fixation electrophoresis yields high sensitivity and specificity.
- Baseline free light chain measurement provides an important prognostic value for plasma cell proliferative disorders.
- Free light chain analysis is very helpful for monitoring oligosecretory multiple myeloma and immunoglobulin light chain amyloidosis. Eventually, it may be validated to replace the 24-h urine protein electrophoresis.

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