

## SPOTLIGHT REVIEW

# International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders

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The serum immunoglobulin-free light chain (FLC) assay measures levels of free  $\kappa$  and  $\lambda$  immunoglobulin light chains. There are three major indications for the FLC assay in the evaluation and management of multiple myeloma and related plasma cell disorders (PCD). In the context of screening, the serum FLC assay in combination with serum protein electrophoresis (PEL) and immunofixation yields high sensitivity, and negates the need for 24-h urine studies for diagnoses other than light chain amyloidosis (AL). Second, the baseline FLC measurement is of major prognostic value in virtually every PCD. Third, the FLC assay allows for quantitative monitoring of patients with oligosecretory PCD, including AL, oligosecretory myeloma and nearly two-thirds of patients who had previously been deemed to have non-secretory myeloma. In AL patients, serial FLC measurements outperform PEL and immunofixation. In oligosecretory myeloma patients, although not formally validated, serial FLC measurements reduce the need for frequent bone marrow biopsies. In contrast, there are no data to support using FLC assay in place of 24-h urine PEL for monitoring or for serial measurements in PCD with measurable disease by serum or urine PEL. This paper provides consensus guidelines for the use of this important assay, in the diagnosis and management of clonal PCD.

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## Introduction

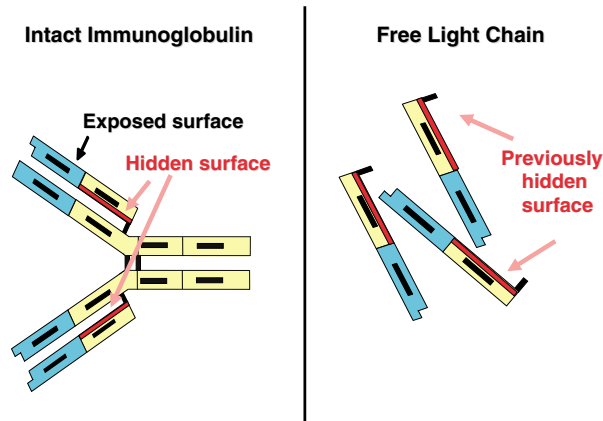
The monoclonal plasmoproliferative disorders encompass a broad spectrum of diseases ranging from the often benign monoclonal gammopathy of undetermined significance (MGUS) to the potentially curable solitary plasmacytoma to the life-threatening conditions of multiple myeloma (MM) and light chain amyloidosis (AL). For each of these diseases, measurements of circulating monoclonal immunoglobulins have been the mainstay of diagnosis, prognosis and management. Until the 1990s, the repertoire of tests to document and measure the monoclonal immunoglobulins included electrophoresis (PEL), immunoelectrophoresis, immunofixation electrophoresis (IFE) and nephelometric measurement of immunoglobulin heavy chains of serum. For most MGUS and MM patients, these measurements appeared to be sufficient; however, they were inadequate for the majority of patients with AL and more than the 3% of myeloma patients with non-secretory or oligosecretory myeloma.

In the early 2000s an assay that measured serum immunoglobulin-free light chains (FLC) was developed.<sup>1</sup> This assay differentiated itself from prior light chain reagents that were called quantitative light chain measurements in that these novel polyclonal antibodies reacted with only those epitopes that were hidden when bound to heavy chain but available when not associated with heavy chain (Figure 1). As will be discussed, this assay has moved into clinical practice based on the building evidence of its utility. The purpose of this document is to describe its potential uses and most importantly distinguish which uses have proved its utility and those which are still undergoing investigation.

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**Figure 1** Immunoglobulin-free light chain assay. (a) Shows the location of the hidden light chain determinants in the intact immunoglobulin model. (b) Shows the location of the hidden light chain determinants in the free light chain model.

### Immunoglobulin-free light chain production and measurement

Serum concentrations of FLC are dependent on the balance between production by plasma cells and renal clearance. Serum FLC are cleared rapidly through the renal glomeruli with a serum half-life of 2–4 h and are then metabolized in the proximal tubules of the nephrons. Under ordinary circumstances, little protein escapes to the urine, and serum FLC concentrations have to increase manifold before the absorption mechanisms are overwhelmed.<sup>2</sup> Approximately 10–30 g of FLC can be metabolized per day by the kidneys compared with normal plasma cell production of 0.5–1 g per day.<sup>3</sup>

Abnormal concentrations of  $\kappa$  and  $\lambda$  FLC may result from a number of clinical situations including immune suppression, immune stimulation, reduced renal clearance, or monoclonal plasma cell proliferative disorders. Sera from patients with either polyclonal hypergammaglobulinemia or renal impairment often have elevated  $\kappa$  FLC and  $\lambda$  FLC due to increased synthesis or reduced renal clearance. The  $\kappa/\lambda$  FLC ratio (rFLC), however, usually remains normal in these conditions.<sup>4</sup> A significantly abnormal  $\kappa/\lambda$  rFLC should only be due to a plasmoproliferative (or lymphoproliferative) disorder that secretes excess FLC and disturbs the normal balance between  $\kappa$  and  $\lambda$  secretion.

### Serum FLC assay

The serum FLC assay (FREELITE, The Binding Site Ltd., Birmingham, UK) is based on a commercial reagent set of polyclonal antibodies and is performed by immunonephelometry and it can be performed on a number of automated laboratory instruments.<sup>1</sup> The assay consists of two separate measurements: one to quantitate  $\kappa$  FLC and the other to quantitate  $\lambda$  FLC.

Sensitive hemagglutination assays showed reactivity to cells coated with the appropriate FLC at dilutions of >1:16 000 and no reactivity to light chains contained in intact immunoglobulin at dilutions of <1:2. Although this would suggest that the reagents have at least a 10 000-fold difference in reactivity to FLC compared with light chain contained in intact immunoglobulin,<sup>1</sup> Nakano and Nagata<sup>5</sup> have shown that there is cross reactivity: 20% at an intact immunoglobulin concentration of

**Table 1** Technical limitations of the FLC assay

Limitation	Comment
Lot to lot variability of reagent	Coefficient of variability ~10–20%
Antigen excess	Actual quantity can be drastically underestimated
Unrecognizable epitopes	Uncommon
Extreme polymerization	Uncommon

12.5 mg/l for the  $\kappa$  reagent; and 0.35% at 50 mg/l for the  $\lambda$  reagent. The greater the specificity, the better is one's ability to quantitate  $\kappa$  and  $\lambda$  FLC in the presence of a large excess of serum IgG, IgA and IgM. This distinction is important, because in normal individuals and in the majority of patients with myeloma, most of the circulating light chain is bound to heavy chains—making less specific reagents a near surrogate for circulating heavy chain measurement.

Katzmann *et al.*<sup>4</sup> defined the normal range using fresh and frozen sera from 127 healthy donors 21–62 years of age and frozen sera from 155 donors 51–90 years of age from the serum bank. The 95% reference interval for  $\kappa$  FLC was 3.3–19.4 mg/l, and that for  $\lambda$  FLC was 5.7–26.3 mg/l. For the  $\kappa/\lambda$  ratio, the 95% reference interval was 0.3–1.2, but it was decided that the diagnostic range should include 100% of donors, making the normal diagnostic range for FLC  $\kappa/\lambda$  0.26–1.65. Using the 100% confidence interval increased the specificity of the test from 95 to 100%, with a drop in sensitivity from 98 to 97%. Patients with ratios greater than 1.65 contain excess  $\kappa$  FLC and are presumed to be producing clonal  $\kappa$  FLC. Patients with ratios less than 0.26 contain excess  $\lambda$  FLC and are presumed to be producing clonal  $\lambda$  FLC.

The 100% confidence interval used reduces the likelihood that polyclonal activation of B-cells will cause an abnormal ratio, but it is possible, and therefore the test must be interpreted in the context of a clinical situation. If a patient is in the midst of an infection or a flare of a rheumatologic condition, the test should be repeated at a later date.

Although the test is a major advance, it is not without its limitations.<sup>6</sup> First, there can be significant lot-to-lot variation (19–20% CV) (Table 1) between batches of polyclonal FLC antisera may result in variable immunoreactivity of individual monoclonal FLCs and inconsistent results.<sup>6</sup> Second, some monoclonal light chains (particularly  $\kappa$  FLC) do not dilute in a linear fashion and may be underestimated in the absence of additional off line dilutions.<sup>6</sup> Third, antigen excess can cause falsely low serum FLC results with nephelometric techniques, and manual dilution may be required for clinically suspicious samples.<sup>7</sup> For large multi-institutional trials, serious consideration should be had for running samples at a centralized testing facility that performs lot-to-lot comparisons. Fourth, changes in amino acid sequence of the light chain may render certain light chain epitopes unrecognizable to the FLC reagents, but apparent on immunofixation or even electrophoresis. Conversely, extreme polymerization can cause an overestimation by as much as 10-fold.

### Urine FLC assay

Most typically, the quantity of urinary light chains has been measured by 24 h urine protein electrophoresis. One can measure urinary light chains by immunonephelometry as well,<sup>1</sup> but this technique cannot be recommended routinely based on the present body of knowledge regarding their use.<sup>8</sup> Bradwell *et al.*<sup>1</sup> measured the free  $\kappa$  and  $\lambda$  concentrations in the urine of

66 normal individuals and found that the respective values are  $5.4 \pm 4.95$  and  $3.17 \pm 3.3$  mg/l with a mean  $\kappa/\lambda$  ratio of 1:0.54 (95% confidence interval, 1:2.17–1:0.25). After studying urine specimens from 20 patients analyzed by Freelite (The Binding Site) and SDS-agarose gel electrophoresis (Hydragel proteinurie, Sebia), Le Bricon *et al.* concluded that when using the  $\kappa/\lambda$  ratio the Freelite was more sensitive than electrophoresis to detect FLC, but that the concentration was overestimated in 75% of cases.<sup>8</sup> In another study of samples from patients with light chain myeloma (LCMM), correlation between concentrations of FLC in serum and urine (measured by immunoassay and corrected for urine dilution with creatinine concentrations) in the 224 patients was non-existent ( $\kappa$ ,  $r=0.29$ ,  $P=0.001$ ;  $\lambda$ ,  $r=0.13$ ,  $P=0.2$ ).<sup>3</sup> The urine immunoglobulin FLC test is NOT recommended for monitoring patients.

### Role of the serum FLC assay in diagnosis

#### Serum FLC assay in screening for plasma cell disorders

It is clear that having excess involved FLC or an abnormal rFLC is common in virtually all plasma cell disorders (Table 2). Historically, the gold standard for screening for plasma cell disorders has been PEL with immunofixation (IFE) of the serum and the urine. Important questions about the FLC assay in terms of screening are: (1) does the FLC assay add anything to IFE; and (2) if the tests are equivalent, is one test either cheaper or more convenient than the other? Neither of these questions has been answered in full, but there are pertinent data. The most important screening study was done by Katzmann *et al.*<sup>20</sup> They asked whether the serum immunoglobulin FLC assay could replace urine IFE for screening patients suspected of having a monoclonal protein-related disorder. Within the Mayo Clinic plasma cell disorder database, 428 patients who had a positive urine IFE and who had serum PEL with IFE and serum FLC assay testing as a clinical assessment were identified. Serum PEL with IFE alone would have missed the diagnosis in 28 patients (6.5%): MM ( $n=2$ ); AL ( $n=19$ ); plasmacytoma ( $n=3$ ); smoldering MM

( $n=1$ ); and MGUS ( $n=2$ ).<sup>20</sup> In contrast, serum FLC alone would have missed 14% of patients, but the combination of serum IFE and FLC identified 99.5% of patients with positive urine (Table 3). The two patients, who would have been missed had the urine IFE not been done, had low risk MGUS.<sup>20</sup> These findings are similar to those found by Beetham *et al.*,<sup>21</sup> who reported that the sensitivity and specificity of an abnormal serum rFLC as a single screening test to be 0.76 and 0.96 with negative and positive predictive values of 0.98 and 0.59, respectively. The FLC assay at diagnosis is especially relevant in patients with AL amyloidosis or any disease that has predominantly free light chains. Among 110 AL patients who had not been previously treated and who had an FLC assay performed within 120 days of diagnosis, the rFLC was positive in 91% compared with 69% for serum IFE and 83% for urine IFE. The combination of serum IFE and serum FLC assay detected an abnormal result in 99% (109 of 110) of patients with AL.<sup>14</sup>

To date, there are no data that fully address what the FLC assay adds to the serum IFE, although the Katzmann *et al.*<sup>20</sup> data come close. Its major deficiency in addressing this question is that the population tested included patients with positive urine immunofixation studies; the chosen selection criteria answered the question they posed, but increased the likelihood of a positive serum FLC assay as the median amounts of serum FLCs required to produce overflow proteinuria has been measured at 113 mg/l for  $\kappa$  (range, 7–39 500 mg/l) and 278 mg/l for  $\lambda$  (range, 6–710 mg/l).<sup>22</sup> There are several papers that show that the addition of FLC to serum PEL or capillary zone electrophoresis increases the sensitivity of these tests, which is not surprising because they only detect monoclonal proteins large enough to be seen through a normal or polyclonal background. PEL and capillary zone electrophoresis should not be considered sufficient testing when contemplating a diagnosis of plasma cell disorder. Typical sensitivity levels are 1–2 g/l for SPEP, 150–500 mg/l for IFE and intermediate in sensitivity for capillary zone electrophoresis.<sup>1</sup> Serum FLC immunoassays have a sensitivity of less than 1 mg/l.<sup>4</sup>

The conclusion drawn from these studies and others<sup>11,23–26</sup> is that for the purpose of screening for monoclonal proteins for all diagnoses except AL, the FLC can replace the 24 h urine IFE; however, once a diagnosis of monoclonal gammopathy is made, the 24 h protein IFE should be done. For AL screening, however, the urine IFE should still be done in addition to the serum tests including the serum FLC.

Not only is the screening strategy of serum IFE and FLC sensibly based on physiology, but also potential from a cost and practicality perspective. Katzmann *et al.*<sup>20</sup> noted that the 2006 Medicare reimbursement for serum FLC analysis was \$38 compared with \$71 for 24 h urine studies (total protein, PEP and IFE). Hence, the cost of adding serum FLC analysis was approximately half the cost of the comparable urine tests. In the study by Hill *et al.*<sup>23</sup> in the UK, there was an additional cost of \$9 per patient to include the FLC assay. Since in many laboratories the initial blood sample is accompanied by urine

**Table 2** Rates of abnormal FLC ratio in different plasma cell disorders

Disease	n	Abn rFLC, %
<i>Multiple myeloma (MM)</i>		
Symptomatic MM <sup>9</sup>	790	95
Symptomatic MM <sup>10</sup>	456	96
Symptomatic MM <sup>11</sup>	61	97
Symptomatic MM <sup>12</sup>	399	96
Non secretory MM <sup>13</sup>	28	68
Non secretory MM <sup>14</sup>	5	100
Light chain MM <sup>3</sup>	224	100
Light chain MM <sup>15</sup>	28	100
Smoldering MM <sup>14</sup>	72	88
Smoldering MM <sup>16</sup>	273	90
MGUS <sup>17</sup>	1148	33
MGUS <sup>14</sup>	114	44
Amyloidosis <sup>18</sup>	95	92
Amyloidosis <sup>19</sup>	262	98
Amyloidosis <sup>14</sup>	110	91
Light chain deposition disease <sup>14</sup>	28	93

Abbreviations: Abn, abnormal; FLC, immunoglobulin-free light chain; MGUS, monoclonal gammopathy of undetermined significance; rFLC, FLC ratio.

**Table 3** Four hundred and twenty-eight patients with urinary monoclonal protein detected by immunofixation electrophoresis<sup>20</sup>

Laboratory test	% abnormal
Serum immunofixation electrophoresis	93.5
Serum protein electrophoresis	80.8
Serum FLC $\kappa/\lambda$ ratio	85.7
Serum immunofixation electrophoresis or FLC ratio	99.5

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in only 40–52% of cases,<sup>21</sup> there may be cost increases. Both patients and physicians are reluctant to do 24-h urine collections because of the inconvenience posed, but depending on the indication for the original monoclonal protein study of the blood, they could be missing at least 10–17% of cases with either AL amyloidosis or LCMM by doing serum IFE alone.<sup>11,14</sup> The ease of performing the FLC measurement could rectify this deficiency and lead to earlier diagnosis of these disorders.

**Recommendations for the use of the serum FLC assay in Screening.** As shown in Table 4, the serum FLC assay in combination with serum PEL and serum IFE is sufficient to screen for pathological monoclonal plasmaproliferative disorders other than AL, which requires all the serum tests as well as the 24-h urine IFE. If a diagnosis of a plasma cell disorder is made, a 24-h urine for PEL and IFE is essential for all patients.

### Prognostic value of the serum FLC assay

The increased diagnostic sensitivity for the FLC diseases and the ability to eliminate urine in the diagnostic screen was somewhat predictable once the analytic sensitivity of the serum FLC assay was understood. A finding that emerged, but that was not entirely expected, was that baseline values of serum FLC can be used for prognostication (Table 4). The pathogenic rationale for this linkage is not well understood, but one possibility is that higher levels of FLC may be associated with IgH translocations<sup>27</sup> as well as increasing tumor burden.<sup>28,29</sup>

**Monoclonal gammopathy of undetermined significance**  
Approximately 1/3 of MGUS patients have an abnormal rFLC and have a higher rate of progression than those who do not (Figure 2a). Based on the size of the monoclonal protein peak, the isotype of the heavy chain, and the rFLC, a risk model for progression of MGUS to MM has been constructed.<sup>17</sup> For the purpose of prognostic modeling, a rFLC of <0.25 or >4 was selected as abnormal. In addition to abnormal rFLC, on multivariate modeling an M-spike greater than or equal to 1.5 g per 100 ml and a heavy chain isotype other than IgG were associated with risk of progression to MM or related disorders. The risk of progression at 20 years for patients with 0, 1, 2 or 3 risk factors was 5, 21, 37, or 58%, respectively (Figure 2b).

**Table 4** Uses of serum immunoglobulin free light chain assay

Screening in combination with immunofixation electrophoresis<sup>20</sup>

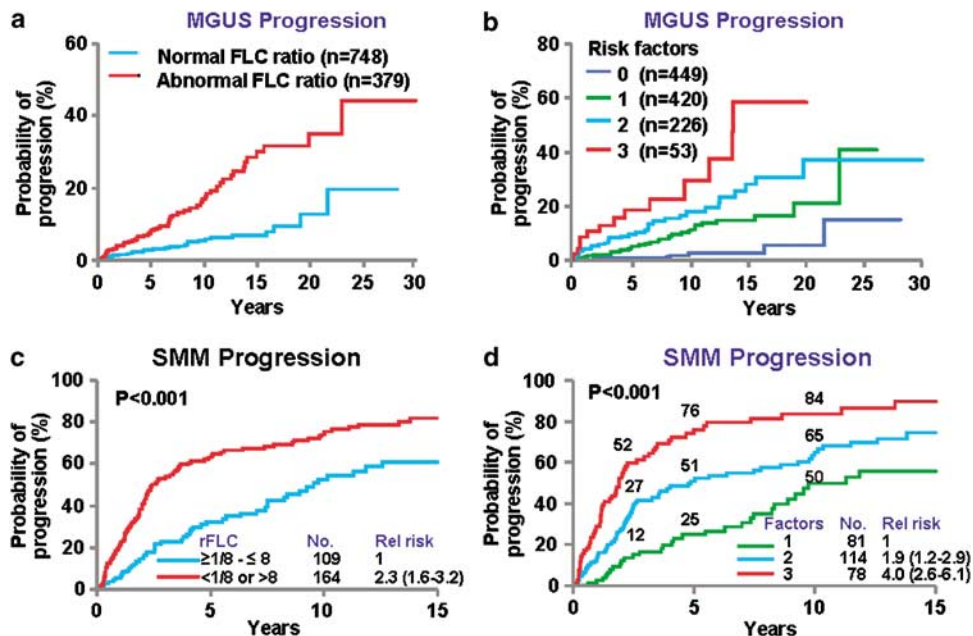
Baseline values prognostic

Monoclonal gammopathy of undetermined significance<sup>17</sup>  
Smoldering myeloma<sup>16</sup>  
Symptomatic myeloma<sup>9,12,29,32</sup>  
Plasmacytoma<sup>31</sup>  
AL amyloidosis<sup>28</sup>

Hematologic response

AL amyloidosis<sup>19,28,34–36</sup>  
'Non-secretory' myeloma<sup>a 13</sup>  
Stringent complete response in multiple myeloma<sup>a 37</sup>  
Light chain deposition disease (Personal experience of authors)

<sup>a</sup>Not yet validated.



**Figure 2** Risk of progression to symptomatic myeloma or related disorder. (a) In 1148 patients with MGUS based on abnormal rFLC (<0.26 or >1.66). (Rajkumar et al.<sup>17</sup> Serum-free light chain ratio is an independent risk factor for progression in monoclonal gammopathy of undetermined significance. *Blood*. 2005;106:812–817). (b) In 1148 patients with MGUS using a risk-stratification model that incorporates rFLC and the size and type of serum monoclonal protein. The three risk factors include: abnormal rFLC (<0.26 or >1.66); serum monoclonal protein of  $\geq 15$  g/l; and non-IgG MGUS. (Rajkumar SV et al.<sup>17</sup> Serum-free light chain ratio is an independent risk factor for progression in monoclonal gammopathy of undetermined significance. *Blood*. 2005;106:812–817). (c) In 273 patients with smoldering (asymptomatic) multiple myeloma based on rFLC <0.125 or >8 (<1:8 or >8:1) (Dispenzieri A et al.<sup>16</sup> Immunoglobulin-free light chain ratio is an independent risk factor for progression of smoldering (asymptomatic) multiple myeloma. *Blood*. 2008;111:785–789). (d) In 273 patients with smoldering (asymptomatic) multiple myeloma using a risk-stratification model that incorporates abnormal rFLC (<0.125 or >8), the size of serum monoclonal protein (greater than or equal to 30 g/l) and extent of bone marrow plasmacytosis (greater than or equal to 10%). (Dispenzieri A et al.<sup>16</sup> Immunoglobulin-free light chain ratio is an independent risk factor for progression of smoldering (asymptomatic) multiple myeloma. *Blood*. 2008;111:785–789).

### Smoldering (asymptomatic) multiple myeloma

In addition to the use of FLC for prognosis in MGUS, baseline rFLC is useful for assessing prognosis for progression in smoldering MM.<sup>16</sup> Baseline serum samples were available in 273 patients with SMM seen from 1970 to 1995. Abnormal rFLC predicted for higher rates of progression, and the best breakpoint for rFLC was less than or equal to 0.125 or greater than or equal to eight (hazard ratio, 2.3; 95% CI, 1.6–3.2) (Figure 2c). The extent of abnormality of rFLC was independent of SMM risk categories defined by the number of bone marrow plasma cells (BMPC) and size of serum M proteins.<sup>30</sup> A risk model was constructed, incorporating the best breakpoint of rFLC, BMPC  $\geq 10\%$  and serum M protein  $\geq 3$  g per 100 ml. Patients with 1, 2, or 3 risk factors had 5-year progression rates of 25, 51 and 76% respectively (Figure 2d).

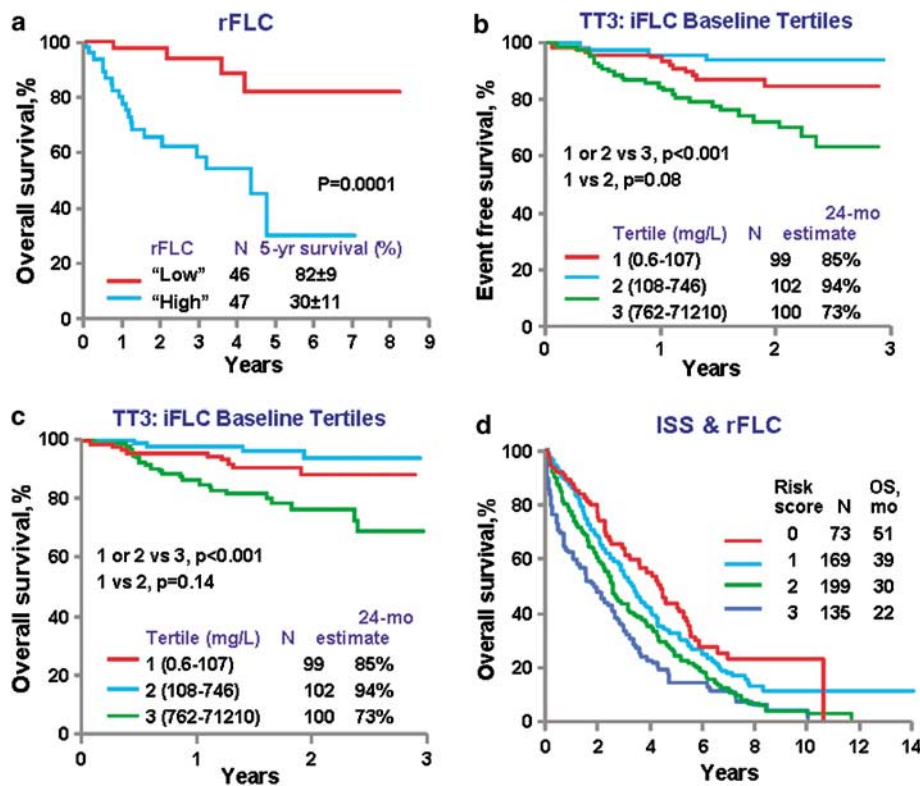
### Solitary plasmacytoma

In a cohort of 116 patients with solitary plasmacytoma the rFLC was retrospectively determined on serum collected at the time of diagnosis. An abnormal ratio was present in 47% and associated with a higher risk of progression to myeloma ( $P=0.039$ ). The risk of progression at 5 years was 44% in patients with an abnormal serum rFLC at diagnosis compared

with 26% in those with a normal rFLC. One to 2 years following diagnosis, a persistent serum M protein level of 0.5 g per 100 ml or higher was an additional risk factor for progression to MM. A risk-stratification model was constructed using the two variables of rFLC (normal or abnormal) and M protein level persistence at a level of 0.5 g per 100 ml or greater. The low risk ( $n=31$ ), intermediate risk ( $n=26$ ) and high risk ( $n=18$ ) groups had 5-year progression rates of 13, 26 and 62%, respectively ( $P<0.001$ ).<sup>31</sup>

### Multiple myeloma

Several studies have shown that baseline FLC is prognostic for survival in patients with newly diagnosed active myeloma.<sup>9,12,29,32</sup> Kyrtsolis *et al.*<sup>32</sup> found that in 94 MM patients rFLC was prognostic (Figure 3a). Median baseline rFLC was 3.6 in  $\kappa$ -MM patients and 0.02 in  $\lambda$ -MM. 'High' rFLC (worse than median) correlated with elevated serum creatinine and lactate dehydrogenase, extensive marrow infiltration and LCMM. The 5-year disease-specific survival was 82 and 30% in patients with rFLC less extreme or more extreme than median, respectively ( $P=0.0001$ ). The rFLC added to the international staging system (ISS), with ISS stage 3 patients having a 5-year disease-specific survival of 52 versus 16% depending on their rFLC.



**Figure 3** Overall survival in patients with newly diagnosed symptomatic myeloma based on baseline FLC measurement. (a) Overall survival based on rFLC thresholds in 94 patients. 'High rFLC' for patients with clonal kappa or lambda disease was 3.6 and 0.02, respectively. (Kyrtsolis MC *et al.*<sup>32</sup> Prognostic value of serum-free light chain ratio at diagnosis in multiple myeloma. *Br J Haematol.* 2007;137:240–243.) (b) Overall survival based on baseline iFLC tertiles in 301 patients undergoing Total Therapy 3. Highest tertile (greater than 750 mg/l) was associated with worse overall survival (van Rhee F *et al.*<sup>29</sup> High serum-free light chain levels and their rapid reduction in response to therapy define an aggressive multiple myeloma subtype with poor prognosis. *Blood.* 2007;110:827–832.) (c) Event-free survival based on baseline iFLC tertiles in 301 patients undergoing Total Therapy 3. Highest tertile (greater than 750 mg/l) was associated with worse overall survival (van Rhee F *et al.*<sup>29</sup> High serum-free light chain levels and their rapid reduction in response to therapy define an aggressive multiple myeloma subtype with poor prognosis. *Blood.* 2007;110:827–832.) (d) Risk stratification model using elements of the international staging system (ISS) and extreme values of rFLC adds in 790 patients diagnosed with active MM between 1995 and 1998. Patients were assigned 1 point for each of the following: abnormal rFLC ( $<0.03$  or  $>32$ ); high  $Sb2M$  ( $\geq 3.5$  g/l); or low serum albumin ( $<3.5$  g per 100 ml). (Snozek CL, Katzmann JA, Kyle RA, *et al.* Prognostic value of the serum-free light chain ratio in patients with newly diagnosed myeloma and proposed incorporation into the International Staging System Submitted).

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Van Rhee *et al.*<sup>29</sup> have also demonstrated that among 301 patients enrolled to receive total therapy III, those with the highest levels of FLC—greater than 750 mg/l, which was the highest tercile—had the poorest outcomes (Figures 3b and c). The highest baseline FLC levels were significantly associated with LCMM, elevated creatinine (greater than or equal to 176.8  $\mu$ m or 2 mg per 100 ml),  $\beta$ -2-microglobulin (greater than or equal to 297.5 nm/l or 3.5 mg/l), lactate dehydrogenase (greater than or equal to 190 U/l) and bone marrow plasmacytosis higher than 30%.

Lastly, Snozek *et al.*<sup>9</sup> have also shown in a cohort of 790 patients diagnosed with active MM between 1995 and 1998 that baseline rFLC <0.03 or >32 ( $n=479$ ) had inferior outcomes as compared with those with an rFLC between 0.03–32 ( $n=311$ ), with median survival of 30 versus 39 months, respectively. When the abnormal rFLC was incorporated into a model using the cutoffs applied in the International Staging System,<sup>33</sup> that is, albumin <3.5 mg per 100 ml and serum  $\beta_2$ -microglobulin  $\geq$ 3.5 mg per 100 ml, it was found that rFLC was an independent risk factor. Patients with 0, 1, 2, or 3 adverse risk factors had significantly different overall survival, with median survival times of 51, 39, 30 and 22 months, respectively,  $P<0.001$  (Figure 3d).<sup>9</sup>

### Immunoglobulin light chain amyloidosis

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 FLC (hazard ratio 2.6,  $P<0.04$ ).<sup>28</sup> Baseline FLC correlated with serum cardiac troponin levels, and higher FLC levels were associated with more organs involved by amyloid, suggesting that high FLC levels may be associated with more advanced disease.

**Recommendations for the use of the serum FLC assay in prognosis.** The serum FLC assay should be measured at diagnosis for all patients with MGUS, smoldering or active multiple myeloma, solitary plasmacytoma and AL amyloidosis (Table 4).

### Role of the FLC assay in response assessment

Although FLC response can be considered in three contexts—oligosecretory diseases, light chain myeloma and measurable intact immunoglobulin disease—routine serial use of

this assay can only be recommended for the first indication. As will be discussed below, to date there have been only a few studies that have validated the usefulness of serial FLC measurements,<sup>19,28,34,35</sup> although efforts for standardizing FLC response have been proposed.<sup>36,37</sup> For serial measurements, either the involved FLC or the difference between the involved and uninvolved (dFLC) should be used.<sup>12</sup> Aside from the time of diagnosis and in the context of documenting stringent complete response, the rFLC is not useful because of the not infrequently observed treatment-related immunosuppression of the uninvolved ( $\kappa$  for monoclonal  $\lambda$  patients and  $\lambda$  for monoclonal  $\kappa$  patients) FLC during chemotherapy; the ratios generated when one of the FLC numbers is very low will be extreme, reflecting the degree of immunosuppression more than tumor burden.

### Published FLC response criteria

**Multiple myeloma.** In MM, the International Myeloma Working Group has recently published updated response criteria, which incorporate the FLC assay. The criteria are shown in Table 5 as they pertain to FLC.<sup>37</sup> There have been no formal studies performed yet to date to validate these criteria.

**AL amyloidosis.** The consensus opinion from the 10th International Symposium on Amyloid and Amyloidosis has defined FLC response in patients with AL amyloidosis as an FLC response in those individuals in involved FLC (iFLC) greater than 10 mg per 100 ml as a 50% reduction in iFLC and progression as a 50% increase in iFLC.<sup>36</sup> The definition used for amyloid patients has been partially validated based on the work of Lachmann,<sup>19</sup> Sancharawala,<sup>35</sup> and Palladini,<sup>34</sup> as described below.

### Studies evaluating FLC response in oligosecretory disease (AL amyloidosis, oligosecretory MM and light chain deposition disease)

Lachmann *et al.*<sup>19</sup> were the first to relate changes of FLC with overall survival in any disease. They demonstrated that those AL patients who achieved more than a 50% reduction in their iFLC were more likely to live longer. The majority of patients in that series were patients receiving non-myeloablative chemotherapy. Subsequently, in a group of patients undergoing hematopoietic stem cell transplant, Dispenzieri *et al.*<sup>28</sup> found that 50% reduction of iFLC was not predictive of overall survival but that this degree of reduction was associated with a trend

**Table 5** Response Criteria for FLC<sup>36,37</sup>

	Minimum to be deemed measurable	PR	CR	sCR	Progression
AL <sup>36</sup> without measurable <sup>a</sup> serum or urine M protein	iFLC $\geq$ 100 mg/l	50% reduction of iFLC	Normal rFLC and CR by IFE and bone marrow	ND	50% increase of iFLC to >100 mg/l
AL <sup>36</sup> with measurable <sup>a</sup> serum or urine M protein	ND	ND	ND	ND	ND
MM <sup>37</sup> without measurable <sup>a</sup> serum or urine M protein	iFLC $\geq$ 100 mg/l and rFLC abnormal	50% reduction of dFLC	ND	Normal rFLC & CR by IFE and bone marrow	50% increase of dFLC
MM with measurable disease <sup>12,37</sup>	Use of FLC not recommended	Use of FLC not recommended	Use of FLC not recommended	Normal rFLC & CR by IFE and bone marrow	Use of FLC not recommended

Abbreviations: dFLC, difference between iFLC and uninvolved FLC; iFLC, involved free light chain, that is,  $\kappa$  for a patient with  $\kappa$  restricted disease and  $\lambda$  for a patient with  $\lambda$  restricted disease; ND, not defined.

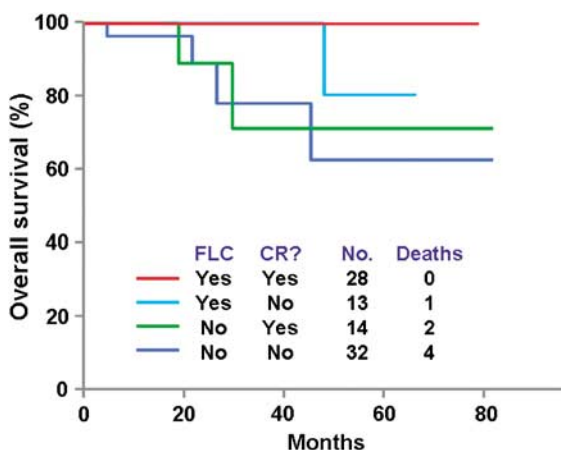
<sup>a</sup>Measurable M protein includes serum M protein of at least 1 g per 100 ml or a urine M-protein of at least 200 mg/24 h for myeloma patients (100 mg/24 h for AL patients).

toward improvement in both hematologic and organ response rate. Rather, normalization of iFLC was the most important determinant to predict for hematologic response, organ response and overall survival. In a study of 45 evaluable patients undergoing hematopoietic stem cell transplant, Cohen *et al.*<sup>38</sup> demonstrated that normalization of rFLC at 3 months predicted for both progression-free and overall survival. The discrepancy between the hematopoietic stem cell transplant and non-transplant studies may lie in the relative proportion achieving greater than a 50% reduction in iFLC. Contrary to the findings of others,<sup>28,35,38</sup> reductions in iFLC greater than 50% did not further improve prognosis in the Lachmann study.<sup>19</sup> Sanchowala and colleagues also demonstrated that the deeper the FLC response, the higher the likelihood of both organ and hematologic complete response.<sup>35</sup> Moreover, Palladini *et al.*<sup>34</sup> have shown that FLC reductions correlate with reductions of NT-proBNP, a marker of cardiac function, and predict for overall survival. In the Sanchowala series, greater than a 90% reduction in serum FLC was a better predictor of organ response than was complete hematologic response.<sup>35,39</sup> Finally, Dispenzieri *et al.*<sup>28</sup> demonstrated that immunoglobulin FLC response was a better predictor of survival in patients with AL amyloidosis than complete hematologic response (Figure 4), as defined by the Blade myeloma response criteria.<sup>39</sup>

In contrast, there are no data to date to verify that FLC changes in patients with oligosecretory myeloma correlates with those of bone marrow plasmacytosis or overall disease status, but the assumption has been made that it does based on anecdotal information.<sup>13</sup> Six of the patients studied during the course of their disease 'showed changes in concentrations of FLC that were in accordance with their clinical progress.<sup>13</sup> Finally, although there are no published data validating the use of the FLC assay in patients with light chain deposition disease, the personnel experience of the authors speaks to its utility in these cases.

#### Studies evaluating FLC response in light chain myeloma

There have been several studies that have demonstrated excellent sensitivity of the serum FLC in detecting FLC in patients with LCMM. Consistently, however, it has been shown that there is not a strong correlation between serum FLC and



**Figure 4** Free light chain response is a better predictor of overall survival than is immunofixation electrophoresis response. Dispenzieri A, Lacy MQ, Katzmann JA, *et al.* Absolute values of immunoglobulin-free light chains are prognostic in patients with primary systemic amyloidosis undergoing peripheral blood stem cell transplantation. *Blood*. 2006;107:3378–3383.

measurement or urine FLC by 24 h protein electrophoresis.<sup>3,12,15,22,40</sup> When evaluating the performance of changes of serum FLC and of urinary M-spikes over time, there is a relationship to the changes, but to date, no one has shown high correlation coefficients.

Dispenzieri *et al.*<sup>12</sup> evaluated the relationship between serum FLC and 24-h urine total protein and 24 h urine M protein using 101 patients with baseline iFLC of 5 mg per 100 ml or greater. The correlation coefficients between percentage change of iFLC and urine M protein after 2 months of chemotherapy was poor. Smaller laboratory-based studies have also been performed. Abraham *et al.*<sup>15</sup> performed serial FLC measurements in 28 LCMM patients and used a random effects model to estimate the correlation between changes in urinary M protein and serum FLC. Changes in serum FLC over a period of time correlated with changes in the amount of 24-h urinary M protein for an individual patient using a random effects model. Finally, when Bradwell *et al.*<sup>3</sup> retrospectively reviewed screening baseline serum samples of 224 patients LCMM who had enrolled onto MRC clinical trials, all were correctly identified from serum FLC samples; however, upon serial monitoring of 82 of these patients, the authors observed 'a relationship' between responses as characterized by serum FLC and 24 h urine PEL but do not provide data about correlation coefficients and time points of the serial measurements.

#### Role of FLC response in patients with measurable intact immunoglobulins

Monitoring of serum-free light chains may eventually prove to be appropriate in myeloma patients with intact immunoglobulin and those with LCMM, because approximately 95% also produce excess serum FLC;<sup>10</sup> however, outside of measuring baseline levels, there are few data to support this recommendation presently, with the exceptions noted below. One can parse the possibilities into three categories: (1) using FLC response as an earlier predictor of overall outcomes; (2) FLC response to define stringent complete response in myeloma; and (3) FLC to replace urinary measurements, in the case of light chain escape.

It has been noted that measurements of serum FLC may be more sensitive for early response and early relapse than are standard measurements of the involved heavy chain. With regards to detecting early response or lack thereof, the rationale is logical. FLC half-life is 2–4 h, whereas that for a typical IgG is approximately 8–21 days. Graphs have been presented demonstrating this effect in patients.<sup>10</sup> However, no one has shown that early detection of lack of response predicts for ultimate treatment failure, or that the 3–4 week time delay that may occur when using measurements of heavy chains actually affects the ultimate outcome of the patient. Serial measurement of serum FLC may also detect relapse sooner than do the protein electrophoresis studies. Once again, the difficulty lies in the absence of data to support the fact that knowledge of disease reactivation or drug failure a few months early has any impact on overall patient outcome. Although the interesting argument has been made that earlier prediction of drug failure could provide economic benefit in an era when novel agents are extremely expensive,<sup>41</sup> there are not sufficient data to recommend abandonment of a treatment regimen based on the FLC alone in patients with non-oligosecretory disease.

Van Rhee *et al.*<sup>29</sup> reported that patients treated with VDT-PACE who had the deepest FLC reductions after 2–3 cycles of therapy did worse than those who did not, but their analysis was confounded by the fact that patients with lower levels of FLC were incapable of having very high percentage reduction of

their free light chains. For example a patient with a baseline FLC of 5 mg per 100 mg cannot achieve a 96% reduction, which would be dropping the level to 0.2 mg per 100 ml—a pathologically low value. The authors provide no information about whether very high percentages of reduction were independent of baseline FLC. In contrast, Dispenzieri *et al.*<sup>12</sup> demonstrated that although FLC response at 2 months into alkylator-based therapy predicted for ultimate PEL response, it did not predict for overall or progression-free survival. The major limitation of this study, however, is that the induction chemotherapy employed did not contain novel chemotherapeutic agents.

Although normalization of rFLC has been incorporated into the definition of stringent complete response in the International Myeloma Working Group Uniform Response Criteria,<sup>37</sup> there are no data as of yet to document that complete response with or without the rFLC criteria is prognostic for progression-free survival or overall survival. There is one published study in which patients were treated with doxorubicin and dexamethasone for 2 or 3 months followed by thalidomide and dexamethasone for 2 months.<sup>42</sup> The authors found that normalization of the rFLC after one or two cycles of treatment, which occurred in 8 of 37 patients, was significantly associated with the achievement of CR or nCR ( $P < 0.003$ ). The significance of this finding is uncertain because no information about PFS or OS as related to rFLC normalization is provided.

For those patients with intact immunoglobulin myeloma without significant Bence Jones proteinuria, 24 h protein electrophoresis is typically done infrequently. However, patients with advanced disease can develop light chain escape with or without extramedullary disease. For unclear reasons, a subclone of malignant plasma cells expand, which is incapable of producing significant amounts of immunoglobulin heavy chain, but retains the ability to make light chains. Without doing periodic urinary evaluations or serum FLC measurements, this phenomenon can be missed.<sup>43</sup>

**Recommendations for the use of the serum FLC assay in response assessment.** Serial FLC ascertainment should be routinely performed in patients with AL amyloidosis and multiple myeloma patients with oligosecretory disease. It should also be done in all patients who have achieved a CR to determine whether they have attained a stringent CR.

### Using the FLC assay in the setting of renal insufficiency

There is limited information about the use of this assay in the context of renal insufficiency, but several generalizations are possible. Although renal failure will increase the levels of both  $\kappa$  and  $\lambda$  FLC in a given individual, it will not cause an abnormal rFLC. Interpreting serial measurements of iFLC in patients with oligosecretory myeloma, LCDD or amyloidosis who are on dialysis or who have markedly abnormal renal function is very challenging, and response assessment has not been validated. However, following the dFLC or the iFLC, while noting the uninvolved FLC and as an additional indicator of renal status, can provide information in these very complicated patients.

### Conclusion

In summary, there are four major indications for the FLC assay in the evaluation and management of MM and related clonal plasma cell disorders. In the context of screening for the

presence of myeloma or related disorders, the serum FLC assay in combination with serum protein electrophoresis and immunofixation yields high sensitivity, and negates the need for 24-h urine studies when screening for multiple myeloma; once diagnosis of a plasma cell disorder is made, 24-h urine studies are required for all patients. Second, the FLC assay is of major prognostic value in virtually every plasma cell disorder, including monoclonal gammopathy of undetermined significance, smoldering myeloma, active myeloma, immunoglobulin light chain amyloidosis and solitary plasmacytoma. Third, the FLC assay allows for quantitative monitoring of patients with oligosecretory plasma cell disorders, including patients with AL, oligosecretory myeloma and nearly two-thirds of patients who had previously been deemed to have non-secretory myeloma. In AL patients and patients with oligosecretory myeloma, measurement of FLC is essential. The FLC assay cannot replace the 24-h urine protein electrophoresis for monitoring myeloma patients with measurable urinary M proteins. Fourth, the rFLC a requirement for documenting stringent complete response according to the International Response Criteria.

Although the serum FLC is a valuable assay in patients with plasma cell disorders, there are technical limitations of the assay which make its use as a serial measurement potentially problematic including: lot-to-lot variation; assay imprecision; and instances in which they do not dilute in a linear fashion. The most important area for future investigation includes defining the clinical relevance of early FLC 'response' or 'relapse' in patients with measurable intact serum immunoglobulins or measurable urinary M proteins. Apart from initial diagnosis and documentation of stringent complete response, its use is not advocated in these patients.

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## Appendix

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