Serum Free Light Chain Assay and κ/λ Ratio: Performance in Patients With Monoclonal Gammopathy-High False Negative Rate for κ/λ Ratio

Gurmukh Singh

Abstract

Background: Serum free light chain assay (SFLCA) and κ/λ ratio, and protein electrophoretic methods are used in the diagnosis and monitoring of monoclonal gammopathies.

Methods: Results for serum free light chains, serum and urine protein electrophoreses and immunofixation electrophoreses in 468 patients with a diagnosis of monoclonal gammopathy were compared. The results of the two methods were graded as concordant, non-concordant or discordant with the established diagnoses to assess the relative performance of the methods. Results of κ/λ ratio in samples with monoclonal protein detectable by electrophoretic methods were also analyzed.

Results: Protein electrophoreses results were concordant with the established diagnoses significantly more often than κ/λ ratio. The false negative rate for κ/λ ratio was higher than that for electrophoretic methods. κ/λ ratio was falsely negative in about 27% of the 1,860 samples with detectable monoclonal immunoglobulin. The false negative rate was higher in lesions with lambda chains (32%) than those with kappa chains (24%). The false negative rate for κ/λ ratio was over 55% in samples with monoclonal gammopathy of undetermined significance. Even at first encounter, the false negative rates for κ/λ ratios for monoclonal gammopathy of undetermined significance, smoldering myeloma and multiple myeloma were 66.98%, 23.08%, and 30.15%, respectively, with false negative rate for lambda chain lesions being higher.

Conclusions: Electrophoretic studies of serum and urine are superior to SFLCA and κ/λ ratio. Abnormal κ/λ ratio, per se, is not diagnostic of monoclonal gammopathy. A normal κ/λ ratio does not exclude monoclonal gammopathy. False negative rates for lesions with lambda chain are higher than those for lesions with kappa chains. Electrophoretic studies of urine are underutilized. Clinical usefulness and medical necessity of SFLCA and κ/λ ratio is of questionable value in routine clinical testing.

Keywords: Monoclonal gammopathy; Serum free light chain assay; Kappa/lambda ratio; Serum protein electrophoresis; Serum protein immunofixation electrophoresis; Urine protein electrophoresis; Urine protein immunofixation electrophoresis

Introduction

The immune system makes billions of immunoglobulins, and an estimate of > 10^11 structurally different proteins is generally accepted [1]. The diverse population of immunoglobulins produces a diffuse distribution pattern, polyclonal pattern in protein electrophoresis [2]. An oligoclonal pattern may be seen in normal immune response, malignancies, and following stem cell transplants. In such circumstances, multiple low level proteins of restricted heterogeneity, i.e., clones, are noted in both protein electrophoresis and immunofixation electrophoresis. Such a pattern is often present, especially in normal immune response, in the background of polyclonal increase in immunoglobulins. An oligoclonal pattern may mature into a polyclonal pattern, although it may temporarily exhibit a monoclonal band [3-7]. Neoplastic plasma cells usually produce an immunoglobulin of only one heavy and one light chain type. Occasionally neoplastic proliferations may include a biclonal pattern [8-11].

Three major conditions with monoclonal immunoglobulins, in order of increasing severity, are: monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), and multiple myeloma or plasma cell myeloma (MM). Kyle is credited with introducing the terms MGUS and SMM to the medical lexicon [12, 13]. MM is a malignant entity. MGUS and SMM may progress to MM at a rate of 1-2% and 10-20% per year, respectively. Trials are underway to ascertain if treating SMM and asymptomatic MM would improve outcomes [14]. These entities may be associated with the secretion of intact immunoglobulin molecules, or light chains only. Even normally, light chains are produced in excess of heavy chains and a monoclonal lesion producing intact immunoglobulin also produces excess free light chains. In some cases, the immunoglobulin or light chain is not secreted or only poorly secreted, referred to as non-secretory or oligosecretory myeloma [15, 16]. Malignant lesions of plasma cells may manifest as MM, or solitary lesions of malignant plasma...
cells in bone or extra-osseous sites, designated as plasmacytomas [17-19]. Other entities with monoclonal immunoglobulins include Waldenström macroglobulinemia, B-cell lymphomas, chronic lymphocytic leukemia, amyloidosis, light chain deposition disease, heavy chain deposition disease, light and heavy chain deposition disease, polyneuropathy, and POEMS syndrome [20-26].

Electrophoretic methods, namely, serum protein electrophoresis (SPEP), serum protein immunofixation electrophoresis (SIFE), urine protein electrophoresis (UPEP), and urine protein immunofixation electrophoresis (UIFE), are classically performed to diagnose monoclonal gammopathy. If UPEP/UIFE is employed routinely, the rate of diagnosis, i.e., sensitivity, approaches 100% [2, 23, 24, 27]. Serum free light chain assay (SFLCA) and calculated \( \kappa/\lambda \) ratio are usually included in the diagnostic workup for monoclonal gammopathy. International Myeloma Workshop Consensus Panel 3 recommendation for investigative workup of monoclonal gammopathy includes testing for serum free light chain (SFLC), in addition to electrophoretic tests and bone marrow examination. It has also been pointed out that testing SFLC often does not add value, whereas others have espoused an opposite view [22-37].

There are commercially available assays for SFLCs from the Binding Site assay using polyclonal antisera, and N Latex assay from Siemens using monoclonal antibodies. Limited experience suggests that the Binding Site assay has higher sensitivity and N Latex assay has higher specificity [37, 38]. The Binding Site assay has been available for a longer time and is used more often than the N Latex assay.

Comparison of SFLCA and electrophoretic methods in diagnosis, management and prognosis of plasma cell dyscrasias has been identified as one of the research needs by the Agency for Healthcare Research and Quality [39]. In this retrospective study, the relative performances of electrophoretic methods and SFLCA and \( \kappa/\lambda \) ratio were compared in patients with monoclonal gammopathies.

Methods

This study was conducted at a 500-bed, medical school affiliated tertiary-care hospital in the Southeastern United States. The laboratory performing the tests is CLIA certified. The protocol was approved by the Institutional Review Board.

Medical records of patients who had both SPEP/SIFE and SFLCA performed between 2012 and September 2015 were reviewed. Findings of patients who did not have a diagnosis of monoclonal gammopathy were addressed in an earlier publication [36]. SPEP was performed by agarose gel electrophoresis using Helena SPIFE 3000 system with fractionation into six classic protein zones. Quantitative evaluation for the usual proteins and any M-proteins was performed by scanning the gel at 595 nm on an EDC densitometer. If the monoclonal protein overlapped a normal protein band, the concentration of the combined peak was reported. SFLCA was performed on Siemens ADVIA 2400 instrument, using Freelite kits and reagents from the Binding Site. In reporting a monoclonal spike, the approximate location of the peak was reported in descriptive terms, e.g., extreme cathodic end of SPEP, cathodal region of SPEP, mid-gamma region, anodal region, anodal end, point of application, beta region, co-migrating with C3, peak between C3 and transferrin bands, co-migrating with transferrin and uncommonly in the alpha-2 region. Analysis by SPEP, SIFE, UPEP, and UIFE is collectively referred to as electrophoretic methods.

Clinical and laboratory results were reviewed for the following: diagnoses, SPEP, SIFE, UPEP, UIFE, SFLCA, \( \kappa/\lambda \) ratio and bone marrow findings. For SPEP/SIFE, identity of the monoclonal immunoglobulin, level of the spike and other findings such as oligoclonal pattern were noted.

Bone marrow examination results were reviewed for corroborating the identity of the monoclonal immunoglobulin. In patients with non-secretory myeloma, the expression of restricted light chains in bone marrow was noted. Immunometric data collected were SFLC and \( \kappa/\lambda \) ratio. Results of \( \kappa/\lambda \) ratio were considered to be normal if the value was between 0.26 and 1.65. \( \kappa/\lambda \) ratio of \(< 0.26 \) was considered abnormally \( \lambda \) dominant, and \( \kappa/\lambda \) ratio of \( > 1.65 \) was considered to be abnormally \( \kappa \) dominant. Established diagnosis of monoclonal gammopathy was used as the reference point and the results of other assays were compared against it. The results of SPEP/SIFE and UPEP/UIFE and \( \kappa/\lambda \) ratio were classified as concordant, non-concordant or discordant using the criteria described below.

Concordant

For protein electrophoresis, the finding was classified as concordant if: 1) A monoclonal immunoglobulin, intact immunoglobulin or light chain was identified and the finding was in agreement with bone marrow examination. 2) SPEP had a visible spike that had been characterized previously and was present in the previously identified location. SIFE was not required. 3) No monoclonal immunoglobulin was recognizable on SPEP, and SIFE detected a monoclonal immunoglobulin band similar in heavy and light chain composition and location to the original finding. 4) A monoclonal light chain only was detectable on SPEP/SIFE and it was identical to the light chain identified when the diagnosis was established; it was considered concordant, even if an intact monoclonal immunoglobulin was identified at the time of the original diagnosis. 5) If an oligoclonal pattern was noted on SPEP/SIFE, the results were considered concordant if one of the clones was similar in heavy and light chain composition and location to the original finding. 6) When no monoclonal immunoglobulin was identifiable on SPEP/SIFE but urine showed a monoclonal immunoglobulin or light chain of the same type as seen at the time of initial diagnosis, the result was considered concordant. 7) In the cases of non-secretory myeloma, if a light chain was detectable on UIFE and it was identical to the light chain restriction in bone marrow, the result was considered concordant.

For \( \kappa/\lambda \) ratio, the usual range of 0.26 - 1.65 was considered normal. The \( \kappa/\lambda \) ratio was considered concordant if the dominant light chain identified by the \( \kappa/\lambda \) ratio was identical to the monoclonal light chain or the light chain component of the
intact monoclonal immunoglobulin.

Non-concordant

For protein electrophoresis, the finding was classified as non-concordant if no monoclonal immunoglobulin or light chain was detectable in SPEP, SIFE, UPEP or UIFE in a patient with an established diagnosis of monoclonal gammopathy.

For κ/λ ratio, the following was deemed to be non-concordant. The κ/λ ratio was in the normal range of 0.26 - 1.65 in a patient with diagnosis of monoclonal gammopathy.

Discordant

For protein electrophoresis, the finding was classified as discordant if: 1) A monoclonal immunoglobulin of different composition was present in SPEP, SIFE, UPEP and UIFE than that identified at initial diagnosis. 2) A monoclonal immunoglobulin and/or light chain was present in a different location than the one noted at initial diagnosis. 3) A monoclonal light chain was present in any of the electrophoretic analyses other than the light chain identified at initial diagnosis. 4) Presence of an oligoclonal pattern, especially in a patient who had undergone stem cell transplantation, was not considered discordant by itself.

For κ/λ ratio, the following were deemed to be discordant: 1) A κ/λ ratio of < 0.26 in a patient whose monoclonal immunoglobulin at initial diagnosis was kappa light chain or an intact immunoglobulin with kappa light chains. 2) A κ/λ ratio of > 1.65 in a patient whose monoclonal immunoglobulin at initial diagnosis was lambda light chain or an intact immunoglobulin with lambda light chains.

The number of discordant, non-concordant and discordant findings between electrophoretic analyses and SFLCA κ/λ ratio were compared by the Chi-square test for goodness of fit. Following the analysis described above, it was obvious that there was a high rate of false negative findings for SFLCA κ/λ ratio, therefore only the samples in which a monoclonal immunoglobulin was detectable by electrophoretic methods were analyzed to determine the false negative rate for SFLCA κ/λ ratio in such specimens.

In many instances, the results of electrophoretic methods and SFLCA κ/λ ratio were in agreement. In the specimens in which the results for the two methods were not in agreement, concordance of the results with the initial diagnosis was analyzed.

Results

Data from 468 patients were reviewed. There were 2,409 instances/observations in which results of both the electrophoretic methods and SFLCA κ/λ ratio were available. The average number of observations per patient was 5.1. However, 175 patients (37.4%) had only one observation each. The maximum number of observations in a single patient was 41.

Further breakdown of the patients and observation revealed plasma cell myeloma (patients 262, observations 1,831), SMM (patients 13, observations 66), and MGUS (patients 76, observations 295). Other patients included 11 plasmacytomas with 69 observations and a heterogeneous group of 106 patients with 148 observations. The heterogeneous group included lymphomas, including Waldenstrom and post-transplant lymphomas, neuropathy, malignancies, amyloidosis, chronic lymphocytic leukemia, osteopenia and fractures, etc.

The frequency distribution of the observations by immunoglobulin type and light chain type is given in Table 1. IgG kappa was the commonest monoclonal immunoglobulin type. Six of 262 MM patients exhibited more than one clone, and four patients had the same light chain with each clone and did

### Table 1. Frequency Distribution of Various Type of Monoclonal Immunoglobulins

| Immunoglobulin type | Number of observations | Number of patients |
|---------------------|------------------------|--------------------|
| IgG K               | 923                    | 173                |
| IgG L               | 543                    | 97                 |
| IgA K               | 305                    | 48                 |
| IgA L               | 180                    | 35                 |
| IgM K               | 88                     | 33                 |
| IgM L               | 37                     | 14                 |
| IgD K               | 10                     | 1                  |
| IgD L               | 26                     | 2                  |
| Kappa only          | 193                    | 40                 |
| Lambda only         | 88                     | 22                 |
| Non-secretory       | 16                     | 3                  |
| Total               | 2,409                  |                    |
| Total kappa (including non-secretory) | 1,535         |
| Total lambda        | 874                    |                    |
not materially alter the process for designation as concordant, non-concordant or discordant. The remaining two patients had both kappa and lambda chains and both had lambda chain clone as the dominant clone. A conservative approach was used in designation of concordance level for these two patients and benefit of doubt was given to the method being correct.

The concordance levels of electrophoretic studies and SFLCA κ/λ ratio, with established diagnoses, are shown in Tables 2 and 3. Table 2 displays the performance of the two methods evaluated for the total data. The concordance rate of the electrophoretic method was significantly greater than that for SFLCA κ/λ ratio (P << 0.00001). The performance of SPEP/SIFE was nearly flawless, with a discordance rate, at worst, of 0.58%. The discordance rate for SFLCA κ/λ ratio was 3.3%. The non-concordance rate was nearly twice as good for electrophoretic methods as compared to SFLCA κ/λ ratio findings, indicating that many of the non-concordant observations in SFLCA had detectable monoclonal immunoglobulin by electrophoretic method.

The data were further analyzed by light chain type and the results are shown in Table 3. All three patients with non-secretory myelomas had kappa chain restriction and the 16 observations were included in the kappa chain pool. The results displayed in Table 3 show that electrophoretic methods had significantly better concordance than SFLCA, and the P value for combined kappa and lambda chain specific results was < 0.00001.

Judging by numbers, percentages and the Chi-square statistic, the results for lambda light chain were far more often discordant by electrophoretic methods than was the case for SFLCA κ/λ ratio. The Chi-square statistic for kappa and lambda chains was 26.1 and 344.2, respectively. The P value for both was < 0.00001 and was not calculated further for the higher Chi-square value for lambda light chains.

The discordance rate only for kappa chains was not statistically significantly different between the two methods. The non-concordance rate for SFLCA, κ/λ ratio, for kappa chain lesions was higher than that in electrophoretic method, indicating that patients who had detectable kappa chain monoclonal immunoglobulins by electrophoretic method had normal κ/λ ratio in a significant number of observations and the SFLCA, κ/λ ratio provided a false negative result. This issue is further addressed in Table 4 and Table 5.

The samples in which a monoclonal Ig was detectable by electrophoretic methods were identified and are listed in column three of Table 4 under “Electrophoretic positive, N”. Amongst these, the samples with normal κ/λ ratio or wrong chain dominant ratio were identified and are listed in column labeled, “K/L ratio negative, N”. The last column lists the percentages of instances in which the κ/λ ratio was falsely negative. Overall, SFLCA κ/λ ratio was falsely negative in nearly 27% of the instances in which a monoclonal Ig was detectable by electrophoretic methods. The false negative rate was higher for lesions with lambda light chains than those with kappa light chains, the two rates being 31.2% and 23.7%, respectively.

The greater than 55% false negative rate for κ/λ ratio for MGUS patients is noteworthy and argues against a role of SFLCA as a screening tool for monoclonal gammapathy. This is especially important in light of the observation, reported earlier, that > 35% of samples without a monoclonal immunoglobulin have an abnormal κ/λ ratio. The high false positive rate for SFLCA κ/λ ratio in patients without monoclonal gammapathy was almost always due to falsely kappa dominant κ/λ ratio [36].

Comparative results of SFLCA κ/λ ratio and electrophoretic methods at the first encounter with a given patient are giv-

### Table 2. Performance of Electrophoretic Methods and SFLCA κ/λ Ratio With Respect to Level of Concordance With the Established Diagnosis of Monoclonal Gammapathy

| Concordance level | SFLCA, N | SFLCA, % | SPEP/SIFE, N | SPEP/SIFE, % |
|-------------------|----------|----------|--------------|--------------|
| Concordant        | 1,359    | 56.41    | 1,855        | 77           |
| Non-concordant    | 971      | 40.31    | 540          | 22.42        |
| Discordant        | 79       | 3.28     | 14           | 0.58         |

P << 0.00001

The total 2,409 observations were graded for concordance with the established diagnoses. The results of the electrophoretic methods had significantly higher concordance with the established diagnoses, P < 0.00001. The results of the electrophoretic method had significantly lower discordance rate with a P < 0.00001.

### Table 3. The Concordance Rates of the Two Methods for Light Chain Specific Results

| Concordance level | Kappa (N = 1,535) (including non-secretory) | Lambda (N = 874) |
|-------------------|---------------------------------------------|------------------|
|                   | SFLC, N | SFLC, % | SIFE, N | SIFE, % | SFLC, N | SFLC, % | SIFE, N | SIFE, % |
| Concordant        | 1010    | 66.49   | 74.85   | 82.04   |
| Non-concordant    | 521     | 34.3    | 25.81   | 150     |
| Discordant        | 4       | 0.26    | 0.39    | 75      |

P < 0.00001 (Χ² = 26.1)  
P << 0.00001 (Χ² = 344.2)

Electrophoretic methods had significantly higher concordance with the established diagnoses for both kappa and lambda light chain monoclonal gammapathies than serum free light chain κ/λ ratio assay results.
en in Table 5. Some of the MM patients had received treatment elsewhere and were not treatment naive. The false negative κ/λ ratio results are particularly noteworthy in treatment naive MGUS patients at 67%. The even higher false negative rate for MGUS lesions with lambda light chains, of nearly 90%, is in keeping with the high false negative rate for lambda light chain lesions in other settings.

The SFLCA κ/λ ratio performance was better for kappa light chain lesions than that for the total sample. However, even in kappa chain lesions the performance of SPEP/SIFE was significantly better than that for SFLCA κ/λ ratio (P < 0.00001). The discordance rate for kappa light chain was marginally, but not significantly, better for SFLCA κ/λ ratio. However, the non-concordance rates for SFLCA κ/λ ratio and SPEP/SIFE were 34.3% and 25.8%, respectively and the results for electrophoretic methods were concordant significantly more frequently.

In lesions with lambda light chains, the discordance rate was much worse for SFLCA κ/λ ratio than for SPEP/SIFE, the respective values for lambda chains being 8.6% and 0.8%.

The greater than 55% false negative rate for κ/λ ratio for MGUS patients is noteworthy and argues against a role of SFLCA as a screening tool for monoclonal gammopathy.

### Table 4.
The Samples in Which a Monoclonal Ig Was Detectable by Electrophoretic Methods Were Identified and the False Negative Rate for SFLCA κ/λ Ratio for the Grand Total, Kappa Chain Lesions, Lambda Chain Lesions and Clinical Diagnoses of MGUS, SMM and MM Are Provided

| Number of observations | Electrophoretic positive, N | K/L ratio negative, N | False negative rate for K/L, % |
|------------------------|-----------------------------|-----------------------|-------------------------------|
| Grand total            | 2,409                       | 1,860                 | 501                           | 26.94 |
| Kappa chain lesions    | 1,535                       | 1,142                 | 271                           | 23.73 |
| Lambda chain lesions   | 874                         | 722                   | 230                           | 31.86 |
| MGUS                   | 295                         | 295                   | 163                           | 55.25 |
| SMM                    | 66                          | 60                    | 23                            | 38.33 |
| MM                     | 1,831                       | 1,358                 | 467                           | 34.39 |

The table above shows the number of observations, the number of observations with a positive electrophoretic test, the number of observations with a negative K/L ratio, and the false negative rate for the K/L ratio for the grand total, kappa chain lesions, lambda chain lesions, MGUS, SMM, and MM.

### Table 5.
Comparative Results of SFLCA and Electrophoretic Methods at the First Encounter With a Given Patient Are Given Below

| Serum free light chain assay at first encounter with a given patient | Kappa chain lesions | Lambda chain lesions | False Neg, % |
|---------------------------------------------------------------------|---------------------|----------------------|--------------|
| **MGUS**                                                            |                     |                      |              |
| K/L ratio +                                                         | 32                  | 3                    |              |
| K/L ratio -                                                         | 47                  | 24                   |              |
| Total                                                               |                     |                      | 66.98        |
| Kappa chain lesions                                                 |                     |                      |              |
| Lambda chain lesions                                                |                     |                      | 88.89        |
| **SMM**                                                             |                     |                      |              |
| K/L ratio +                                                         | 8                   | 2                    |              |
| K/L ratio -                                                         | 0                   | 3                    |              |
| Total                                                               |                     |                      | 23.08        |
| Kappa chain lesions                                                 |                     |                      | 0            |
| Lambda chain lesions                                                |                     |                      | 60           |
| **MM**                                                              |                     |                      |              |
| K/L ratio +                                                         | 139                 | 44                   |              |
| K/L ratio -                                                         | 33                  | 46                   |              |
| Total                                                               |                     |                      | 30.15        |
| Kappa chain lesions                                                 |                     |                      | 19.19        |
| Lambda chain lesions                                                |                     |                      | 51.11        |

The false negative κ/λ ratio results are particularly noteworthy in treatment naive MGUS patients at a rate of 67%. The high false negative rate for lesions with lambda light chains, nearly 90%, is in keeping with the high false negative rate for lambda light chain lesions in other settings.
The non-concordance rates were similarly significantly higher (P << 0.00001) for SFLCA κ/λ ratio than that for electrophoretic methods, the rates for SFLCA κ/λ ratio and electrophoretic methods being 51.6% and 17.2%, respectively for lambda chain lesions.

The higher discordant rate for lambda chain lesions is further illustrated in Table 6 that displays the results of multiple tests in one patient. This was not the only patient with such a pattern and patients with kappa chain lesions also displayed a similar pattern.

When the rate of discordant results only was compared, the results were significantly better for the electrophoretic methods with a P < 0.00001, as shown in Tables 2, 3, 4, 5 and Table 7.

The results of comparison of the methods for the segregated MM, SMM, and MGUS patients and samples are shown in Table 7. In each of these categories, electrophoretic methods had significantly higher concordance rate than that for SFLCA, κ/λ ratio. There was only one observation in the SMM group with discordant findings by both methods and it applied to the same sample.

However, comparison of the discordant results for MGUS and MM revealed significantly better performance for the electrophoretic method. The rate of false negative SFLCA κ/λ ratio for MGUS samples was remarkably high at greater than 55%.

The newly proposed criterion for identification of SMM, in patients with involved to uninvolved light chain ratio of > 100 and light chain level of > 100 mg/L was applicable to only one observation. There was marked reduction of background immunoglobulins in the sample with IgA 16 mg/dL, and IgM 7 mg/dL.

In 1,466 of the 2,409 observations, the results of the electrophoretic method and SFLCA κ/λ ratio were in agreement, i.e., both were concordant, non-concordant or discordant. Table 8 displays the performance of electrophoretic studies vs. SFLCA κ/λ ratio results in the 943 samples in which the results

| Time of assay          | SPEP spike, g/dL | Ig type | Kappa | Lambda | κ/λ ratio |
|------------------------|------------------|--------|-------|--------|-----------|
| April 2013             | 1.69             | IgG L  | 13.24 | 17.97  | 0.74      |
| December 2012          | 1.49             | IgG L  | 17.73 | 12.77  | 1.39      |
| August 2012            | 1.44             | IgG L  | 2.59  | 4.01   | 0.65      |
| June 2012              | 1.07             | IgG L  | 3.86  | 4.17   | 0.93      |
| December 2011          | 1.1              | IgG L  | 2.24  | 3.85   | 0.58      |
| August 2011            | 1.32             | IgG L  | 2.78  | 3.59   | 0.77      |
| May 2011               | 1.07             | IgG L  | 1.67  | 3.51   | 0.48      |
| April 2011             | 0.89             | IgG L  | 2.37  | 5.68   | 0.42      |
| March 2011             | 0.95             | IgG L  | 1.56  | 3.33   | 0.47      |
| January 2011           | 0.97             | IgG L  | 1.04  | 2.71   | 0.38      |
| November 2010          | 0.82             | IgG L  | 1.59  | 2.68   | 0.59      |
| August 2010            | 0.66             | IgG L  | 1.01  | 1.38   | 0.73      |
| June 2010              | 0.74             | IgG L  | 1.6   | 2.56   | 0.63      |
| May 2010               | 1.19             | IgG L  | 1.76  | 5.11   | 0.34      |
| April 2010             | 1.2              | IgG L  | 1.82  | 3.88   | 0.47      |
| February 2010          | 1.17             | IgG L  | 1.72  | 3.89   | 0.44      |
| December 2009          | 1.34             | IgG L  | 1.98  | 2.11   | 0.94      |
| October 2009           | 1.18             | IgG L  | 1.9   | 2.21   | 0.86      |
| September 2009         | 1.38             | IgG L  | 2.27  | 2.26   | 1.00      |
| August 2009            | 1.34             | IgG L  | 1.55  | 2.59   | 0.60      |
| June 2009              | 0.77             | IgG L  | 2.03  | 2.46   | 0.83      |
| May 2009               | 0.8              | IgG L  | 2.91  | 1.5    | 1.94      |
| March 2009             | 0.9              | IgG L  | 1.95  | 1.61   | 1.21      |
| January 2009           | 0.7              | IgG L  | 2.36  | 1.64   | 1.44      |
| November 2008          | 0.7              | IgG L  | 2.33  | 1.65   | 1.41      |

The entries in this table display an example of the poor performance of SFLCA and κ/λ ratio in patients with lambda light chain monoclonal gammopathies. In this case, the SFLCA and κ/λ ratios were consistently negative and discordant despite the fact that each of the 25 samples had a readily detectable monoclonal IgG lambda monoclonal protein. The serum creatinine in this patient was 1.06 mg/dL and renal κ/λ ratio would not have been applicable.
of the two methods were not in agreement. The results of the electrophoretic methods were concordant with the initial diagnosis significantly more often than was the case for SFLCA κ/λ ratio for total as well as light chain-specific analysis. As is apparent from the numbers and percentages, the results for lambda light monoclonal gammapathies were far better for electrophoretic methods than for SFLCA κ/λ ratio. The Chi-square value for kappa chain samples was 71.0 and for lambda chain samples the Chi-square value was 695.5, hence the designation of $P << 0.00001$ for lambda chain results. For lesions with lambda light chain immunoglobulins, the electrophoretic method was in agreement with the initial diagnosis in more than 90% the instances whereas SFLCA κ/λ ratio was concordant in fewer than 10% of the instances.

### Discussion

Tremendous progress has been made in the treatment of MM, thereby increasing the importance of making an early and accurate diagnosis [22, 40]. Treatment with chemotherapy warrants monitoring, and limiting exposure to toxic drugs. Concentration of the monoclonal protein is an indicator of the tumor mass and reflects the course of disease and guides treatment. According to the International Myeloma Workshop Consensus Panel 3, initial investigation of a patient suspected with MM should include serum protein electrophoresis and immunofixation electrophoresis, 24-h urine collection for electrophoresis and immunofixation, and measurement of SFLCs. The panel recognizes serum immunofixation electrophoresis as the “gold standard” to confirm the presence of a monoclonal immunoglobulin [34]. An algorithmic approach has been recommended to avoid overuse of laboratory tests [24]. For follow-up of treatment, the International Myeloma Workshop Consensus Panel recommends quantitative measurements of immunoglobulins but prefers electrophoretic measurement of monoclonal immunoglobulin. Serial measurement of SFLCs is recommended only for patients with non-secretory or oligo-secretory myelomas [22, 34].

The panel suggests a potential use for measuring SFLC in monitoring the progress of patients with plasmacytoma and SMM. Measurements of value in predicting the development of MM in patients with a non-malignant monoclonal gammapathy include quantification of background immunoglobulins.

### Table 7. The Performance of the Two Methods, i.e., Electrophoretic Methods and SFLCA, for Plasma Cell Myeloma (MM), Smoldering Multiple Myeloma (SMM) and Monoclonal Gammopathy of Undetermined Significance (MGUS) Are Presented

| Category      | Number | Method | Concordance level for observations only | Concordant | Non-concordant | Discordant | P value (total concordance) |
|---------------|--------|--------|----------------------------------------|------------|----------------|------------|-----------------------------|
|               |        |        |                                        |            |                |            |                             |
| MM Patients   | 262    | SIFE   | 1,358                                  | 468        | 5              |            | $< 0.00001$                 |
| Observations  | 1,831  | SFLC   | 1,066                                  | 711        | 54             |            |                             |
| SMM Patients  | 13     | SIFE   | 60                                     | 5          | 1              |            |                             |
| Observations  | 66     | SFLC   | 42                                     | 23         | 1              |            | $0.00063$                   |
| MGUS Patients | 76     | SIFE   | 290                                    | 5          | 0              |            |                             |
| Observations  | 295    | SFLC   | 132                                    | 148        | 15             |            | $< 0.00001$                 |

The concordance of the electrophoretic method results with the established diagnosis was superior to the results of SFLCA in all of the three categories of patients. The level of discordance with the established diagnosis was significantly lower with electrophoretic results than for SFLCA in patients with MM and MGUS. There were insufficient observations in the SMM group for comparison of discordance levels. There was one discordant result, each, with both methods.

### Table 8. Comparison of the Electrophoretic and SFLCA Results in Samples in Which the Results of the Two Method Were Not in Agreement With Each Other

| Test method | Total | Kappa | Lambda |
|-------------|-------|-------|--------|
|             | Concordant, N | Concordant, % | Concordant, N | Concordant, % | Concordant, N | Concordant, % |
| SPEP/SIFE   | 733    | 77.73 | 303    | 63.66 | 430    | 92.08 |
| SFLCA κ/λ ratio | 210    | 22.26 | 173    | 36.34 | 37     | 7.92  |
| Total       | 943    | 476   | 467    |       |       |       |
| P value     | $< 0.00001$ | $< 0.00001$ | $P << 0.00001$ (X$^2 = 695.5$) |       |       |       |

The finding of superiority of the electrophoretic method was applicable in monoclonal gammapathies with both kappa and lambda light chains. As is apparent from the numbers and percentages, the results for lambda light monoclonal gammapathies were far better for electrophoretic methods than for SFLCA κ/λ ratio.
and measurement of involved SFLC, measurement of Hevylite chain of affected as well as unaffected immunoglobulins. At this time, it has not been established that any of these tests offers an advantage over monitoring the uninvolved immunoglobulin classes and recognizing the onset of suppression of uninvolved immunoglobulins as a risk factor for transformation of MGUS and particularly SMM into MM [40-42].

It is worth noting and emphasizing that results of the SFLCA κ/λ and κ/λ ratio, no matter how abnormal, are not a proof of monoclonal gammapathy. Any abnormal SFLCA or κ/λ ratio results must be confirmed by an electrophoretic method and/or bone marrow examination. It is conceded that a normal serum and urine electrophoretic result does not rule out a neoplastic plasma cell disorder; however, detection of a monoclonal immunoglobulin by electrophoresis is proof positive of monoclonal gammapathy. The shortcomings of SFLCA in patients with and without monoclonal gammapathy have been demonstrated by other investigators as well [32, 37, 43-53]. It could be argued that being that electrophoretic method is the gold standard, other methods are not expected to perform as well, however, the high error rates, both false positive and false negative, for SFLCA, raise the question of its usefulness, and medical necessity of this test.

Good laboratory tests are expected to have one or more of the following attributes: 1) detect disease or predisposition to disease, 2) confirm or reject a diagnosis, 3) establish prognosis, 4) guide patient management, and 5) monitor efficacy of therapy [54].

The results of SFLCA κ/λ ratio were evaluated with these performance parameters in mind. As stated earlier, SFLCA κ/λ ratio is neither diagnostic of nor exclusionary of monoclonal gammapathy, nor does it document a predisposition to disease. The high false negative rate of SFLCA κ/λ ratio, in patients with readily detectible monoclonal immunoglobulins, argues against a role in guiding management of patient or therapy. There may be a role in establishing prognosis, as has been presented for MGUS; however, given the error rate of the assay, the usefulness for prognosis would be limited at best [55]. The International Myeloma Workshop Consensus Panel recommended serial measurement of SFLCs only for patients with non-secretory or oligo-secretory myeloma are likely to be appropriate indications in patients with concordance of SFLCA data with bone marrow findings [22, 34].

If the data presented here are validated by other unbiased, non-conflicted investigators, there would be virtually no role, for SFLCA for the screening for suspected monoclonal gammapathy. If a monoclonal immunoglobulin is not detectable by SPEP and SIFE, urine should be analyzed by UPEP and UIFE. What would be gained by performing SFLCA? An abnormal SFLCA κ/λ ratio does not diagnose monoclonal gammapathy; a normal κ/λ ratio does not exclude monoclonal gammapathy.

More than 35% of patients without monoclonal gammapathy have an abnormal κ/λ ratio [36]. In patients with MGUS, about 55% of the samples yield a falsely negative normal κ/λ ratio, and κ/λ ratio is normal in about 67% of MGUS patients at first encounter. If monoclonal gammapathy is suspected in patient with non-diagnostic SPEP and SIFE, 24-h urine study should be done and depending on the strength of the suspicion, a bone marrow biopsy or biopsy of the lesion ought to be considered. An argument in favor of SFLCA over urine studies has been that SFLCA is less expensive [27]. Be that as it may, SFLCA κ/λ ratio is not diagnostic of monoclonal gammapathy, nor does it exclude monoclonal gammapathy.

Polyclonal gammapathy increases the rate of erroneous κ/λ ratio finding to about 55% [36]. The results presented in Tables 2-8 demonstrating the superiority of electrophoretic methods for broad concordance as well as strictly discordant results for the two methods. The high false negative rate for SFLCA κ/λ ratio in samples with detectable monoclonal immunoglobulin makes one question the utility of this test in diagnosis of monoclonal gammapathy as well as for monitoring the course of disease.

In tracking the efficacy of treatment, it has been suggested that SFLCA may be superior to the densitometric measurement of monoclonal immunoglobulin due to the shorter half-life of light chains. While this is an apparently logical argument, empirical data argue against this notion. The half-life of lambda chains is longer than that of kappa chains [2]. If the short half-life of light chains was the determining factor, lambda dominant κ/λ ratio would be expected to be more prevalent; however, observed false negative rate of κ/λ ratio for lambda chain lesions is higher than that for kappa chain lesions, arguing against the half-life of light chains and the explanation of the high false negative rate for SFLCA, κ/λ ratio. In patients with intact immunoglobulin MM, performance of densitometric measurement of monoclonal immunoglobulin has been shown to be better than SFLCA and κ/λ ratio [56].

Observation of the high false negative results for SFLCA κ/λ ratio in general and lambda light chain lesions in particular argues against the SFLCA as monitoring tool. The false negatives rates for SFLCA are not attributable to the shorter half-life of light chains in patients with persistently negative SFLCA κ/λ ratio despite the persistent presence of monoclonal immunoglobulin in gram quantities as exemplified by the results shown in Table 6. Similarly, the results of SFLCA κ/λ ratio did not add value to the assessment of complete response to treatment for patients with MM [57, 58]. It could be argued that the high negative rate with SFLCA κ/λ ratio reflected a successful result of the treatment; however, it does not hold water in patients with persistent presence of gram quantities of monoclonal protein as illustrated in Table 6 and observed in patients with both kappa and lambda light chain monoclonal immunoglobulins. The short half-life of light chains and successful result of treatment do not explain the high false negative rate in untreated MM and SMM patients and patients with MGUS.

In more than half of the cases with light chain MM, monoclonal light chain is detectable on SPEP/SIFE; however, it may be appropriate to use SFLCA κ/λ ratio for monitoring the progress of disease as an adjunct to SPEP/SIFE. The 24-h urine excretion of the relevant light chain may be the best option; however, logistical issues are likely to preclude routine use of this test.

In the 1% or so cases of non-secretory MM, and amyloidosis, SFLCA κ/λ ratio may be appropriate if the assay result is consistent with the immunoglobulin restriction established on bone marrow examination.

In instances of light chain escape, monitoring of SFLCs

Singh  J Clin Med Res. 2017;9(1):46-57
and κ/λ ratio may offer an advantage, if the results of SFLCA κ/λ ratio are in agreement with the initial diagnosis and bone marrow finding of appropriate light chain restriction [42, 59].

Similarly, SFLCA and κ/λ ratio may offer an advantage in monitoring patients with light chain monoclonal gammopathy and patients with amyloidosis [22, 34]. However, measurements of serum beta 2 microglobulin levels have been reported to be a better marker of response to treatment [60, 61].

While the International Myeloma Working Group, in their 2014 update, recommended monitoring SMM with SFLCA to assess the risk of progression to MM and initiation of treatment, it has not been established if SFLCA is superior to other assays, e.g., quantification of background immunoglobulins, and increase in the amount of monoclonal protein [22, 34].

The predispositions for false negative and false positive results of SFLCA κ/λ ratio have been documented by others as well [32, 37, 43-53]. Factors contributing to the high error rate include hypergammaglobulinemia, hypogammaglobulinemia, and renal insufficiency in the reference range, renal failure, and propensity of light chains to aggregate or polymerize thereby potentially interfering with their measurement [55, 56, 62-65]. The reason for the higher false negative rate for lambda light chain lesions in the SFLCA κ/λ ratio is not entirely clear. A likely explanation is the greater tendency of lambda light chains to dimerize/polymerize and the polymerization possibly hiding the epitopes to which the antibodies used in the assay are directed.

Measurement of Hevylite chain has also not been shown to improve upon the performance of SPEP/SIFE in the diagnosis and monitoring of patients with intact immunoglobulin MM. Measurement of IgA Hevylite chain has been stated to provide a better estimate of the monoclonal immunoglobulin, as monoclonal IgA is often present in the beta region and may overlap the transferrin and complement bands. The clinical advantage of measuring IgA Hevylite chain over densitometric quantitation of the monoclonal band along with the underlying normal protein has yet to be demonstrated [66-69].

Alterations in the amounts of monomeric and dimeric SFLCs, in favor of dimeric light chains, have been cited as a risk factor in the development of MM and amyloidosis; however, this test has not yet made it into routine clinical testing [65].

The statement that abnormal SFLCA and κ/λ ratio is not diagnostic of monoclonal gammopathy and normal results do not exclude monoclonal gammopathy, while correct, is not meant to indicate that the test is entirely useless. However, in routine clinical practice SFLCA and κ/λ ratio should be limited to patients with non-secretory or oligo-secretory myeloma, light chain escape, and amyloidosis without detectable monoclonal immunoglobulin in serum. There may be a role for SFLCA κ/λ ratio in monitoring lympho-plasmacytic disorders, e.g., B-cell lymphoma and chronic lymphocytic leukemia [70].

Conclusions

Electrophoretic tests are superior to SFLCA κ/λ ratio in diagnosis and monitoring of monoclonal gammopathies, with the possible exception of patients with non-secretory or oligo-secreatory myeloma and light chain escape.

The κ/λ ratio has a high false positive rate in patients without monoclonal gammopathy, especially in samples with polyclonal hyper-gamma globulinemia [36].

There is a high false negative rate for the κ/λ ratio in samples with detectable monoclonal immunoglobulin, in general, and in lesions with lambda light chain monoclonal immunoglobulins, in particular. SFLCA κ/λ ratio may be useful in monitoring patients with light chain monoclonal gammopathy, non-secretory or oligo-secretory myeloma, light chain escape, and amyloidosis.

Urine protein electrophoretic studies should be used as one of the primary tools in diagnosing and monitoring monoclonal gammopathies.

Acknowledgments

Drs. Roni Bollag and Wendy Bollag and Dr. Christine Litwin kindly reviewed the draft of the manuscript and the author is grateful for their corrections and suggestions. The technical assistance of Ms. Joyce Oliver and Ms. Valerie Payne is gratefully acknowledged.

References

1. Janeway CA Jr, Travers P, Walport M. The generation of diversity in immunoglobulins. Immunobiology; the immune system in health and disease. New York Garland Science. 2001.
2. Bradwell A. Serum Free Light Chain Analysis plus Hevylite (7 Ed.). Birmingham, UK: The Binding Site Group Ltd. 2015.
3. O'Connell TX, Horita TJ, Kasravi B. Understanding and interpreting serum protein electrophoresis. Am Fam Physician. 2005;71(1):105-112.
4. Rajkumar SV, Kumar S. Multiple Myeloma: Diagnosis and Treatment. Mayo Clin Proc. 2016;91(1):101-119.
5. Zeman A, McLean B, Keir G, Luxton R, Sharief M, Thompson E. The significance of serum oligoclonal bands in neurological diseases. J Neurul Neurosurg Psychiatry. 1993;56(1):32-35.
6. de Larrea CF, Cibeira MT, Elena M, Arostegui JI, Rosinol L, Rovira M, Filella X, et al. Abnormal serum free light chain ratio in patients with multiple myeloma in complete remission has strong association with the presence of oligoclonal bands: implications for stringent complete remission definition. Blood. 2009;114(24):4954-4956.
7. Pavoni E, Monteriù G, Santapaola D, Petronzelli F, Anastasi AM, Pelliccia A, D'Alessio V, et al. Tumor-infiltating B lymphocytes as an efficient source of highly specific immunoglobulins recognizing tumor cells. BMC Biotechnol. 2007;7:70.
8. Rajkumar SV, Kyle RA, Connor RF. Recognition of monoclonal proteins. UpToDate.
9. Katzmann JA, Kyle RA, Benson J, Larson DR, Snyder MR, Lust JA, Rajkumar SV, et al. Screening panels for detection of monoclonal gammopathies. Clin Chem.
26. Svahn J, Antoine JC, Camdessanche JP. Pathophysiology and biomarkers in chronic inflammatory demyelinating polyradiculoneuropathies. Rev Neurol (Paris). 2014;170(12):808-817.
27. Katzmann JA, Dispenzieri A, Kyle RA, Snyder MR, Plevak MF, Larson DR, Abraham RS, et al. Elimination of the need for urine studies in the screening algorithm for monoclonal gammopathies by using serum immunofixation and free light chain assays. Mayo Clin Proc. 2006;81(12):1575-1578.
28. Tosi P, Tomassetti S, Merli A, Polli V. Serum free light chain assay for the detection and monitoring of multiple myeloma and related conditions. Ther Adv Hematol. 2013;4(1):37-41.
29. Ludwig H, Milosavljevic D, Zojer N, Faint JM, Bradwell AR, Hubl W, Harding SJ. Immunoglobulin heavy/light chain ratios improve paraprotein detection and monitoring, identify residual disease and correlate with survival in multiple myeloma patients. Leukemia. 2013;27(1):213-219.
30. Hutchison CA, Plant T, Drayson M, Cockwell P, Kountouri M, Basnayake K, Harding S, et al. Serum free light chain measurement aids the diagnosis of myeloma in patients with severe renal failure. BMC Nephrol. 2008;9:11.
31. Bakshi NA, Gulbranson R, Garstka D, Bradwell AR, Keren DF. Serum free light chain (FLC) measurement can aid capillary zone electrophoresis in detecting subtle FLC-producing M proteins. Am J Clin Pathol. 2005;124(2):214-218.
32. Kyle RA. Sequence of testing for monoclonal gammopathies. Arch Pathol Lab Med. 1999;123(2):114-118.
33. Palladini G, Russo P, Bosoni T, Verga L, Sarais G, Lavatelli F, Nuvolone M, et al. Identification of amyloidogenic light chains requires the combination of serum-free light chain assay with immunofixation of serum and urine. Clin Chem. 2009;55(3):499-504.
34. Dimopoulos M, Kyle R, Ferrand JP, Rajkumar SV, San Miguel J, Chanan-Khan A, Ludwig H, et al. Consensus recommendations for standard investigative workup: report of the International Myeloma Workshop Consensus Panel 3. Blood. 2011;117(18):4701-4705.
35. Iwama K, Chihara D, Tsuda K, Ugai T, Sugihara H, Nishida Y, Yamakura M, et al. Normalization of free light chain kappa/lambda ratio is a robust prognostic indicator of favorable outcome in patients with multiple myeloma. Lancet Oncol. 2013;14(2):134-141.
36. Singh G. Serum Free Light Chain Assay and kappa/lambda Ratio Performance in Patients Without Monoclonal Gammopathies: High False-Positive Rate. Am J Clin Pathol. 2016;146(2):207-214.
37. Kim HS, Shin KS, Song W, Kim HJ, Park MJ. Clinical comparisons of two free light chain assays to immunofixation electrophoresis for detecting monoclonal gammapathy. Biomed Res Int. 2014;2014:647238.
38. Sasson SC, McGill K, Wienhold L, Carr A, Brown DA, Kelleher AD, Breit SN, et al. Comparison of the Freelite serum free light chain (SFLC) assay with serum and urine electrophoresis/immunofixation and the N Latex FLC assay. Pathology. 2015;47(6):564-569.
prognosis of plasma cell dyscrasias: Future research needs. AHRQ publication no 12-EHC135-EF. 2012.

40. El-Amm J, Tabbara IA. Emerging therapies in multiple myeloma. Am J Clin Oncol. 2015;38(3):315-321.

41. Holding S, Spradbery D, Hoole R, Wilmot R, Shields ML, Levoguer AM, Dore PC. Use of serum free light chain analysis and urine protein electrophoresis for detection of monoclonal gammopathies. Clin Chem Lab Med. 2011;49(1):83-88.

42. Kyle RA, Larson DR, Therneau TM, Dispensieri A, Melton LJ, 3rd, Benson JT, Kumar S, et al. Clinical course of light-chain smouldering multiple myeloma (idiopathic Bence Jones proteinuria): a retrospective cohort study. Lancet Haematol. 2014;1(1):e28-e36.

43. Pika T, Lochman P, Sandecka V, Maisnar V, Minarik J, Tichy M, Zapletalova J, et al. Immunoparesis in MGUS - Relationship of uninvolved immunoglobulin pair suppression and polyclonal immunoglobulin levels to MGUS risk categories. Neoplasma. 2015;62(5):827-832.

44. McTaggart MP, Lindsay J, Kearney EM. Replacing urine protein electrophoresis with serum free light chain analysis as a first-line test for detecting plasma cell disorders offers increased diagnostic accuracy and potential health benefit to patients. Am J Clin Pathol. 2013;140(6):890-897.

45. Levinson SS. Polyclonal free light chain of Ig may interfere with interpretation of monoclonal free light chain kappa/lambda ratio. Ann Clin Lab Sci. 2010;40(4):348-353.

46. Unsworth DJ, Wallage MJ, Sarkar E, Lock RJ. Abnormalities of serum-free light chain in patients with primary antibody deficiency in the absence of B lymphocyte clonality. J Clin Pathol. 2012;65(12):1128-1131.

47. Bridoux F, Leung N, Hutchison CA, Touchard G, Sethi S, Fernand JP, Picken MM, et al. Diagnosis of monoclonal gammopathy of renal significance. Kidney Int. 2015;87(4):698-711.

48. Witzig TE, Maurer MJ, Habermann TM, Link BK, Micallef IN, Nowakowski GS, Ansell SM, et al. Elevated monoclonal and polyclonal serum immunoglobulin free light chain as prognostic factors in B- and T-cell non-Hodgkin lymphoma. Am J Pathol. 2014;89(12):1116-1120.

49. Shaheen SP, Levinson SS. Serum free light chain analysis may miss monoclonal light chains that urine immunofixation electrophoreses would detect. Clin Chim Acta. 2009;406(1-2):162-166.

50. Zamora-Ortiz G, Velazquez-Sanchez-de-Cima S, Hernandez-Reyes J, Martagon-Herrera NA, Ruiz-Arguelles A, Ruiz-Delgado GJ, Ruiz-Arguelles GJ. Poor performance of the total kappa/lambda light chain quantification in the diagnosis and follow-up of patients with multiple myeloma. Rev Invest Clin. 2014;66(4):314-318.

51. Beetham R, Wassell J, Wallage MJ, Whiteway AJ, James JA. Can serum free light chains replace urine electrophoresis in the detection of monoclonal gammopathies? Ann Clin Biochem. 2007;44(Pt 6):516-522.

52. Jaskowski TD, Litwin CM, Hill HR. Detection of kappa and lambda light chain monoclonal proteins in serum: automated immunoassay versus immunofixation electrophoresis. Clin Vaccine Immunol. 2006;13(2):277-280.

53. Wood PB, McElroy YG, Stone MJ. Comparison of serum immunofixation electrophoresis and free light chain assays in the detection of monoclonal gammopathies. Clin Lymphoma Myeloma Leuk. 2010;10(4):278-280.

54. Hilbert T, Kurec A, Lifshitz MS. General concepts and administrative issues. In: McPherson RA, Pincus MR eds. Henry's Clinical Diagnosis and Management by Laboratory Methods. 23rd edition. Elsevier. p. 2.

55. Rajkumar SV, Kyle RA, Therneau TM, Clark RJ, Bradwell AR, Melton LJ, 3rd, Larson DR, et al. Presence of monoclonal free light chains in the serum predicts risk of progression in monoclonal gammopathy of undetermined significance. Br J Haematol. 2004;127(3):308-310.

56. Uljon SN, Richardson PG, Schur PH, Anderson KC, Tanasijevic MJ, Lindeman NI. Serial serum free light chain measurements do not detect changes in disease status earlier than electrophoretic M-spike measurements in patients with intact immunoglobulin myeloma. Clin Chim Acta. 2011;412(7-8):562-568.

57. Martinez-Lopez J, Paiva B, Lopez-Andlada L, Mateos MV, Cedena T, Vidriales MB, Saez-Gomez MA, et al. Critical analysis of the stringent complete response in multiple myeloma: contribution of sFLC and bone marrow clonality. Blood. 2015;126(7):858-862.

58. Paiva B, Martinez-Lopez J, Vidriales MB, Mateos MV, Montalban MA, Fernandez-Redondo E, Alonso L, et al. Comparison of immunofixation, serum free light chain, and immunophenotyping for response evaluation and prognostication in multiple myeloma. J Clin Oncol. 2011;29(12):1627-1633.

59. Jenner E. Serum free light chains in clinical laboratory diagnostics. Clin Chim Acta. 2014;427:15-20.

60. Brioli A, Giles H, Pawlyn C, Campbell JP, Kaiser MF, Melchor L, Jackson GH, et al. Serum free immunoglobulin light chain evaluation as a marker of impact from intraclonal heterogeneity on myeloma outcome. Blood. 2014;123(22):3414-3419.

61. Pratt G, Thomas P, Marden N, Alexander D, Davis Z, Hussey D, Parry H, et al. Evaluation of serum markers in the LRF CLL4 trial: beta2-microglobulin but not serum free light chains, is an independent marker of overall survival. Leuk Lymphoma. 2016;57(10):2342-2350.

62. Liang YF, Chen WM, Wang QT, Zhai YH, Yang YJ, Liu JY, Leoguer A, et al. Establishment and validation of serum free light chain reference intervals in an ethnic Chinese population. Clin Lab. 2014;60(2):193-198.

63. Hutchison CA, Cockwell P, Stringer S, Bradwell A, Cook M, Gertz MA, Dispensieri A, et al. Early reduction of serum-free light chains associates with renal recovery in myeloma kidney. J Am Soc Nephrol. 2011;22(6):1129-1136.

64. Galvani L, Flanagan J, Sargazi M, Neithercut WD. Validation of serum free light chain reference ranges in primary care. Ann Clin Biochem. 2016;53(Pt 3):399-404.

65. Kaplan B, Golderman S, Aizenbud B, Esev K, Kukuy O, Leiba M, Livneh A, et al. Immunoglobulin-free light
chain monomer-dimer patterns help to distinguish malignant from premalignant monoclonal gammopathies: a pilot study. Am J Hematol. 2014;89(9):882-888.

66. Beaumont-Epinette MP, Moreau C, Besnard S, Latute F, Collet N, Sebillot M, Grosbois B, et al. Heavy/light chain specific immunoglobulin ratios provides no additional information than serum proteins electrophoresis and immunofixation for the diagnosis and the follow-up of intact immunoglobulin multiple myeloma patients. Pathol Biol (Paris). 2015;63(4-5):215-221.

67. Katzmann JA, Willrich MA, Kohlhagen MC, Kyle RA, Murray DL, Snyder MR, Rajkumar SV, et al. Monitoring IgA multiple myeloma: immunoglobulin heavy/light chain assays. Clin Chem. 2015;61(2):360-367.

68. Katzmann JA, Clark R, Kyle RA, Larson DR, Therneau TM, Melton LJ, 3rd, Benson JT, et al. Suppression of uninvolved immunoglobulins defined by heavy/light chain pair suppression is a risk factor for progression of MGUS. Leukemia. 2013;27(1):208-212.

69. Lorsbach RB, Hsi ED, Dogan A, Fend F. Plasma cell myeloma and related neoplasms. Am J Clin Pathol. 2011;136(2):168-182.

70. Charafeddine KM, Jabbour MN, Kadi RH, Daher RT. Extended use of serum free light chain as a biomarker in lymphoproliferative disorders: a comprehensive review. Am J Clin Pathol. 2012;137(6):890-897.