

Evaluating the clinical utility of serum free light chain measurement and reference change value in monitoring monoclonal gammopathy

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ABSTRACT

Background: Serum free light chain (sFLC) measurement is highly sensitive for detecting low levels of immunoglobulins but has limited ability to distinguish between polyclonal and monoclonal increases. This study retrospectively evaluated the clinical utility of interpreting sFLC results using reference change values (RCVs) rather than conventional reference intervals (RIs) for detecting disease relapse in patients with multiple myeloma-related plasma cell disorders, where appropriate.

Methods: A total of 254 paired immunofixation electrophoresis (IFE) and sFLC results obtained from 143 patients were evaluated, including repeated measurements from 50 patients. Immunofixation electrophoresis was used as the comparative reference method. Serum kappa, lambda, and κ/λ ratio results were interpreted using population-based reference intervals, and RCVs for sFLC measurements were calculated. Sensitivity and specificity for detecting disease-related changes during follow-up were compared between RI-based and RCV-based interpretation.

Results: The calculated RCVs for increases and decreases in kappa and lambda light chains ranged from 27% to 40%. During follow-up, nine patients achieved remission, one experienced relapse, and disease status remained stable in 40 patients. Interpretation of the κ/λ ratio using reference intervals yielded a sensitivity of 50% and a specificity of 78%, whereas RCV-based evaluation of sFLC results showed a sensitivity of 80% and a specificity of 50%.

Conclusions: Given the limited sensitivity of κ/λ ratio interpretation based solely on reference intervals, its usefulness for longitudinal disease monitoring may be restricted. Application of RCV-based interpretation to serial sFLC measurements may enhance the detection of clinically meaningful disease-related changes during follow-up, particularly in patients with low-level or stable sFLC concentrations.

Keywords: free light chain, immunofixation electrophoresis, monoclonal gammopathy, multiple myeloma, reference change value

Received: 24 January 2026; Accepted: 26 March 2026; Published: 1 May 2026.

INTRODUCTION

Monoclonal gammopathies represent a heterogeneous group of plasma cell disorders in which accurate laboratory monitoring is essential for diagnosis, assessment of treatment response, and early detection of relapse. Although serum protein electrophoresis (SPE) remains the primary screening tool, its limited sensitivity restricts its usefulness in detecting low-level or evolving monoclonal components during follow-up [1].

Immunofixation electrophoresis (IFE) improves analytical sensitivity and allows immunoglobulin typing; however, its qualitative nature limits its value in longitudinal disease monitoring. These limitations are particularly relevant in conditions such as light-chain multiple myeloma, AL amyloidosis, and oligo-secretory disease,

where conventional electrophoretic methods may fail to capture clinically meaningful changes [2].

Serum free light chain (sFLC) measurement has emerged as an important complementary assay in such settings [3]. Nevertheless, interpretation of κ and λ concentrations and κ/λ ratios based on population-derived reference intervals is challenged by biological variability, analytical imprecision, and non-malignant polyclonal light chain fluctuations, potentially reducing diagnostic performance in serial measurements [4].

The κ and λ light chains are measured by turbidimetric or nephelometric methods, and the reference interval for the κ/λ ratio is 0.26–1.65 [5]. However, this approach may be insufficient to distinguish between polyclonal and monoclonal increases in serum free light chains, particularly in the presence of high gamma globulin lev-

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els [6]. These limitations are not uniform across all monoclonal gammopathies and may disproportionately affect specific disease subtypes, especially those with low tumor burden or predominant light-chain production. Consequently, there is a need for more sensitive and individualized approaches to interpret serial sFLC measurements.

The reference change value (RCV), which incorporates both intra-individual biological variation and analytical variation, offers an individualized framework for evaluating significant changes over time. RCV is calculated based on the intra-individual biological variation (CV_I), which accounts for physiological fluctuations in the analyte and the analytical variation (CV_A) of the instrument [7]. By moving beyond static reference intervals, RCV-based interpretation of serial sFLC measurements may improve sensitivity for detecting disease-related changes compared with κ/λ ratio reference-interval interpretation, supporting a patient-centered longitudinal monitoring approach [8].

In this study, we aimed to evaluate the diagnostic performance of sFLC testing in comparison with immunofixation electrophoresis and to assess the added clinical value of RCV-based interpretation for monitoring disease activity in multiple myeloma and related plasma cell disorders.

We hypothesized that RCV-based interpretation of serial sFLC measurements would improve the detection of clinically meaningful disease-related changes compared with conventional reference interval-based evaluation.

METHODS

The study was approved by the Uşak University Non-Interventional Clinical Research Ethics Committee (Approval No: 451-451-16).

Between July 2023 and August 2024, patients who underwent both IFE and sFLC analyses at Uşak Training and Research Hospital were retrospectively screened via the Laboratory Information System. CV_A were obtained from internal quality control data of the sFLC assays. CV_I values were retrieved from the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Biological Variation Database, and calculations were performed using the EFLM BV RCV calculator [9].

Patients were classified into two groups: newly admitted patients without follow-up data and patients with available follow-up measurements. Newly admitted patients were categorized as IFE-positive or IFE-negative, and the sensitivity and specificity of sFLC testing were calculated accordingly.

For patients under follow-up, disease status was determined based on IFE findings and classified as remission, relapse, or persistent positivity/negativity. RCVs calculated for sFLC measurements were applied to assess serial changes. Sensitivity and specificity were calculated by comparing sFLC-based interpretations with IFE-based classification, which served as the comparative reference method. The clinical relevance of RCV-based interpretation was evaluated by comparing its ability to detect IFE-defined remission or relapse with that of conventional reference interval-based interpretation. IFE analyses were performed using the Helena Biosciences IFE-4 system, while sFLC measurements were carried out by turbidimetry on the Bioanalysis Chemistry AutoAnalyzer using Trimer Diagnostic Free Light Chains Kappa and Lambda reagents. Reference intervals were accepted as κ : 3.3–19.4 mg/L; λ : 5.7–26.3 mg/L; and κ/λ ratio: 0.26–1.65 [10].

Statistical analyses were performed using Excel Analyse-it software. Serum free light chain results (κ , λ , κ/λ ratio) were classified according to reference intervals and compared with IFE results. RCV values for sFLC levels were calculated, and sensitivity and specificity were compared between assessments based on reference intervals and those based on RCVs. For patients under follow-up, the light chain type involved at diagnosis was used for evaluation (e.g., λ results were interpreted according to RCV values in patients with λ -type monoclonal gammopathy).

RCV-down values were used to assess remission (when a decrease from the previous result was expected), while RCV-up values were applied to identify recurrence. Sensitivity and specificity were calculated using standard diagnostic performance formulas.

RESULTS

In this study, a total of 254 paired IFE and sFLC results from 143 patients were evaluated. Fifty patients had repeated measurements available for follow-up analysis. The calculated RCVs for increases and decreases in κ and λ light chains ranged from 26.5% to 39.9% (Table 1).

Among patients with follow-up data who were not receiving monoclonal antibody-based therapy, nine achieved remission, one experienced relapse, while disease status remained stable in 40 patients during the observation period. Detection of disease-related changes using κ/λ ratio-based interpretation and RCV-based evaluation in patients with dynamic results is summarized in Ta-

Table 1. Calculated RCVs for sFLC measurements based on analytical and biological variation data

	CV_I (%)	CV_G (%)	CV_A (%)	RCV up (%)	RCV down (%)
Kappa free light chain	5.90	14.60	12.00	36.10	26.50
Lambda free light chain	5.60	21.90	13.41	39.90	28.50

Abbreviations: CV_I – within-subject biological variation, CV_G – between-subject biological variation, CV_A – analytical variation, RCV up – Reference Change Value (increase), RCV down – Reference Change Value (decrease), sFLC – serum free light chain. CV_I and CV_G values were retrieved from the EFLM Biological Variation Database, and calculations were performed using the EFLM BV RCV calculator [9].

ble 2. As shown in Table 2, among patients who exhibited an IFE-defined change in disease status during follow-up (n=10), reference interval-based κ/λ ratio interpretation detected changes in 5 patients, predominantly reflecting decreases consistent with remission. In contrast, RCV-based interpretation of serial serum free light chain measurements identified significant changes in 8 patients, including one relapse detected as an RCV-defined increase and multiple remission cases detected as RCV-defined decreases. These findings indicate that RCV-based monitoring showed higher detection of IFE-defined changes compared with reference interval-based evaluation.

In patients without prior follow-up measurements (n=93), 24 (25.8%) were IFE-positive. In this subgroup, κ/λ ratio interpretation using reference intervals identified 11 of 24 IFE-positive cases and yielded a specificity of 90% among IFE-negative cases.

DISCUSSION

Current International Myeloma Working Group (IMWG) recommendations emphasize the importance of sensitive laboratory tools for monitoring treatment response and detecting minimal residual disease, highlighting the need for improved methods for longitudinal assessment. In multiple myeloma, a negative IFE result is accepted as part of a complete response and indicates remission [11]. However, IFE remains a qualitative method and may not adequately reflect subtle disease dynamics during follow-up. In this context, the integration of quantitative tools capable of capturing clinically meaningful changes over time is of increasing interest [12,13]. This study suggests that RCV-based interpretation may have potential clinical utility as a complementary approach for monitoring monoclonal gammopathies using sFLC measurements.

The present study highlights the added value of sFLC testing when combined with RCV analysis for monitoring monoclonal gammopathies. The clinical relevance of RCV was not evaluated in isolation, but rather in relation to

established electrophoretic definitions of remission and relapse, supporting its potential role as a complementary monitoring tool. While evaluation based solely on the κ/λ ratio demonstrated limited sensitivity (50%) for detecting disease-related changes, incorporation of RCV improved sensitivity to 80%. However, these findings should be interpreted with caution, given the limited number of patients with IFE-defined status changes during follow-up (n=10).

Biological variation data play a central role not only in defining analytical performance specifications but also in enabling the calculation of RCV, which determines whether observed differences between consecutive measurements are statistically and clinically significant. In addition, assessment of the index of individuality provides insight into the appropriateness of population-derived reference intervals for individual patient follow-up [14]. Together, these concepts support a more individualized and dynamic interpretation of laboratory results.

The high sensitivity associated with RCV-based interpretation may offer practical advantages in the early identification of disease progression or relapse. This approach may help identify patients who could benefit from further evaluation with more sensitive techniques, such as minimal residual disease (MRD) assessment; however, this potential role remains hypothesis-generating and was not directly evaluated in the present study. By accounting for both analytical and intra-individual biological variability, RCV enables a more accurate understanding of patient-specific trends compared with conventional reference interval-based strategies. This observation is consistent with previous studies demonstrating that patient-centered analytical frameworks improve the clinical relevance of serial biomarker evaluation [15]. Although RCV-based interpretation improved sensitivity, a reduction in specificity was observed. This trade-off may lead to false-positive alerts and potentially unnecessary additional investigations. Therefore, RCV-based monitoring should be interpreted in conjunction with clinical findings and other laboratory results.

Table 2. Disease-related changes detected by κ/λ ratio RI and RCV-based evaluation during patient follow-up

ID of patient	Status change (IFE-defined)	Monoclonal type	κ/λ ratio detected change (RI-based)	κ/λ ratio direction	sFLC detected change (RCV-based)
1	Remission	IgA λ	Yes	↓	Yes (RCV↓)
2	Relapse	IgG κ	No	–	Yes (RCV↑)
3	Remission	IgG κ (biclonal)	No	–	No
4	Remission	IgG κ	Yes	↓	Yes (RCV↓)
5	Remission	IgG λ	No	–	Yes (RCV↓)
6	Remission	IgG κ	No	–	Yes (RCV↓)
7	Remission	IgG λ	Yes	↓	Yes (RCV↓)
8	Remission	IgG λ	No	–	No
9	Remission	IgG κ	Yes	↓	Yes (RCV↓)
10	Remission	IgG κ	Yes	↓	Yes (RCV↓)

“Yes” indicates that the corresponding method detected a change consistent with the IFE-defined remission/relapse status. For κ/λ ratio interpretation, “direction” indicates whether the ratio shifted towards normalization/decrease (↓) or increase (↑) between serial measurements; “–” indicates no meaningful change detected by RI-based interpretation. RCV↑ indicates an increase exceeding the calculated RCV threshold (suggesting relapse/progression), whereas RCV↓ indicates a decrease exceeding the calculated RCV threshold (suggesting remission/response). Interpretation was performed using the involved light chain (κ or λ) identified at diagnosis. Abbreviations: IFE – immunofixation electrophoresis, RCV – reference change value, RI – reference interval, sFLC – serum free light chains.

These findings are consistent with guideline-based approaches that encourage individualized interpretation of serial laboratory results rather than reliance solely on population-based reference intervals. Several limitations of this study should be acknowledged. The sFLC assay may not reliably distinguish between polyclonal and monoclonal light chain increases in all clinical settings, potentially affecting specificity. Furthermore, the single-center design limits the generalizability of the findings. Future multicenter studies involving larger and more diverse patient cohorts are warranted to compare RCV-based monitoring directly with MRD assessment and to better define its role in standardized follow-up algorithms for monoclonal gammopathies.

CONCLUSIONS

Reliance on reference intervals alone limits the sensitivity of κ/λ ratio-based monitoring. Incorporation of reference change values into serial sFLC assessment provides a patient-centered framework that may improve the recognition of clinically relevant disease-related changes during follow-up.

ABBREVIATIONS

CV_A – Analytical variation
 CV_I – Intra-individual biological variation
 IFE – Immunofixation electrophoresis
 MRD – minimal residual disease
 RCV – Reference change value
 RI – Reference interval
 sFLC – Serum free light chain
 SPE – Serum protein electrophoresis

ACKNOWLEDGEMENT

None.

AUTHORS' CONTRIBUTION

ST – formal analysis, visualization, writing - original draft.
 HB – data curation, formal analysis, writing - original draft.
 AVÖ – data curation, formal analysis.
 All authors have read and approved the final version of the manuscript.

CONFLICT OF INTEREST

None to declare.

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