

Comparison of Serum Immunofixation Electrophoresis and Free Light Chain Assays in the Detection of Monoclonal Gammopathies

Prescilla B. Wood, Yolonda G. McElroy, Marvin J. Stone

Abstract

Background: Diagnosis of monoclonal gammopathies (MGs) is determined by demonstration of a monoclonal immunoglobulin molecule or chain in serum and/or urine. Previous results on immunofixation electrophoresis (IFE) and quantitative free light chain (FLC) measurements have been conflicting. **Patients and Methods:** The purpose of this study was to compare IFE with serum FLC assays in the detection of MG. Between November 2006 and November 2007, results on routinely ordered serum IFE specimens were compared with independently conducted FLC assays. **Results:** Monoclonal gammopathies were identified in 144 specimens by IFE; 73 patients (50.7%) had a normal κ/λ ratio in the FLC assay. Also, 44.6% of samples with IgG and IgA M-proteins had a normal κ/λ ratio. Of 357 sera that showed no M-protein by IFE, 95.8% exhibited a normal κ/λ ratio. Out of 11 patients with light chain disease, 9 had abnormal κ/λ ratios. It is unclear why half of the patients with MG by IFE had normal κ/λ ratios. Lower M-components in these patients suggested that some had monoclonal gammopathy of undetermined significance. **Conclusion:** For screening purposes, serum IFE should be carried out in patients with suspected MG.

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Introduction

Our previous study on light chain disease (LCD) in patients with multiple myeloma (MM) and light chain (AL) amyloidosis indicated that 80% had detectable Bence-Jones proteinemia.¹ However, free monoclonal light chains could not be accurately measured. The advent of the serum free light chain (FLC) assay has made quantification possible.²⁻⁴ These assays have become widely available and are used for diagnosis and management of certain patients with monoclonal gammopathies (MGs).⁵⁻⁷ It is unclear how this new method compares with standard tests for identifying MGs. The purpose of this report is to compare the results of routinely ordered immunofixation electrophoresis (IFE) with independently conducted serum FLC assays in a single laboratory in a hospital that serves as a primary care and referral center.

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Departments of Pathology and Oncology, Baylor Sammons Cancer Center, Baylor University Medical Center, Dallas, TX

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Address for correspondence: Marvin J. Stone, MD, MACP, Baylor Sammons Cancer Center, 3500 Gaston Ave, Dallas, TX 75246

Fax: 214-820-2780; e-mail: marvins@baylorhealth.edu

Patients and Methods

Between November 2006 and November 2007, results on 501 routinely ordered serum IFEs were compared with independently conducted serum FLC assays. Approximately 52% of specimens came from community hospitals. Each assay was performed on the same day, and the results interpreted by an experienced medical technologist and physician. Immunofixation electrophoresis was performed with Paragon IFE kit (Beckman Coulter, Brea, CA) and serum FLC assays (Freelite™, The Binding Site Ltd, Birmingham, UK) were performed on a Dade Behring BNII automated nephelometer (GMI Inc, St. Paul, MN), as previously described.^{2,3} Results were reported as the FLC concentration and the FLC κ/λ ratio (normal range, 0.26-1.65). Serum protein electrophoresis (SPE) was performed by agarose gel electrophoresis with the SPIFE 3000 (Helena Laboratories, Beaumont, TX) instrument. An abnormal SPE was defined by the presence of a quantifiable M-spike, hypogammaglobulinemia, or suspicious contour in the α_2 , β , or γ regions. Over 95% of the IFEs were unequivocally positive.

This study was approved by the Baylor University Medical Center Institutional Review Board.

Results

Monoclonal gammopathies were identified in 144 specimens by IFE. Seventy-three (50.7%) had a normal κ/λ ratio by the



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serum FLC assay. Results are shown in Table 1. Forty-five of 101 (44.6%) of the IgG and IgA M-protein specimens had a normal κ/λ ratio. The overall magnitude of the M-spike by SPE was lower in the normal κ/λ ratio group compared with the abnormal κ/λ ratio patients ($P < .0001$; Wilcoxon test, χ^2 analysis). Of 357 sera that showed no M-protein by IFE, 95.8% exhibited a normal κ/λ ratio. Nine of 11 patients with LCD had abnormal κ/λ ratios, whereas abnormal ratios were unusual in IgM or biclonal patients (6 of 32). The IFE was ordered mainly by primary care physicians or medical subspecialists other than Hematologists-Oncologists (6.7%). A separate group of specimens, ordered for "serum FLC only," was requested by predominantly Hematologists-Oncologists. This group consisted of 946 samples, of which 587 (62.1%) had abnormal κ/λ ratios. Of these, 454 showed an increase and 133 a decrease in the κ/λ ratio.

Discussion

Monoclonal gammopathies have been identified by SPE and typed by immunoelectrophoresis or immunofixation electrophoresis since the 1960s.¹ The FLC assay, developed during the past decade, permits quantification of FLCs with enhanced sensitivity. Serum FLC assays are valuable in certain patient subgroups with MG, including light chain myeloma, AL amyloidosis, solitary plasmacytoma, and nonsecretory myeloma.⁵⁻⁷ They may also be helpful in risk stratification of patients with monoclonal gammopathy of undetermined significance (MGUS).⁸ Light chain assays also have been useful as prognostic indicators in patients with MM. Some have recommended that these assays be used to determine response to therapy in myeloma.^{7,9,10} However, it is not clear what role serum FLC assays should play in the identification of the general population of patients at risk for MG.

In our study, 50% of patients with MG by IFE had normal serum κ/λ ratios. Other groups have also reported discrepancies between IFE and serum FLC in patients with MG.^{11,12} In addition, nearly 15% of patients with urinary monoclonal proteins reported by the Mayo Clinic group had normal serum FLC ratios.⁶ Jaskowski et al reported that 68.2% with κ -type monoclonal proteins and 68.6% with λ -type monoclonal proteins by IFE had normal κ/λ ratios by turbidimetry.¹¹ Rajkumar et al found that only 33% of MGUS patients had an abnormal serum FLC ratio.⁸ The lower magnitude of the M-spike by SPE in our patients with normal κ/λ ratios suggests that some had MGUS. However, the proportion of patients with MGUS in this subgroup is not certain because of limited clinical information. Other patients, including some with MGUS, may have balanced heavy-light chain synthesis without excess FLCs; this could account for a portion of the discrepancy noted between IFE and serum FLC assays. Renal functional impairment must be taken into account when interpreting FLC results. With renal failure and accompanying nephron loss, FLCs are retained in the serum and their levels will rise.

Our study has several limitations. Clinical information on the patients studied was incomplete, in part because half of the serum specimens came from outlying community hospitals. Further data will be required to ascertain the proportion of MGUS patients. Because MGUS is much more frequent than MM or other malignant MG, this is an important point to establish in deciding when serum FLC assays should be used.

Table 1 Results of Immunofixation Electrophoresis and Free Light Chain Ratios

IFE Status	Number of Patients	Normal κ/λ Ratio	Percent Normal Ratio
IgG, κ	47	19	40.4
IgG, λ	29	14	48.3
IgA, κ	13	7	53.8
IgA, λ	12	5	41.7
IgM, κ	11	10	90.0
IgM, λ	8	6	75.0
LCD (5 κ , 6 λ)	11	2	18.2
Biclonal	13	10	76.9
M-Protein Total	144	73	50.7
No M-Protein	357	342	95.8

Abbreviations: IFE = immunofixation electrophoresis; κ/λ = kappa/lambda; LCD = light chain disease

Several points suggest that the high proportion of MG patients with normal κ/λ ratios is not an artifact. First, 96% of patients with no M-protein by IFE had normal ratios. Secondly, 82% of individuals with LCD had abnormal ratios. Thirdly, the proportion of patients with normal ratios were similar in the IgG and IgA subgroups (Table 1).

The explanation for the large proportion of patients with IFE-demonstrated MG and normal serum FLC ratios requires further study and careful definition of clinical subgroups. A recent study by Landgren et al involving 77,476 healthy adults showed that 71 developed MM. All 71 had a preceding MGUS.¹³ Eight or 9 years before the MM diagnosis, 47% of MGUS patients had abnormal serum FLC ratios. Two years before the diagnosis of MM, 85% had an abnormal serum κ/λ ratio. In a group of 122 myeloma patients, Singhal et al found that the serum FLC ratios were normal in 34% of cases with positive serum IFE.¹⁴ These authors concluded that the role of serum FLC ratio in defining response to treatment is controversial. In considering the use of screening for detection of disease, Katzmman et al reported that a panel of SPE and serum FLC without IFE resulted in an 8% reduction in sensitivity for MGUS, 23% for POEMS, 4% for plasmacytoma and 1% or less for AL amyloidosis and smoldering MM.¹⁵ These investigators found that only 42.4% of patients with MGUS had abnormal serum FLC assays.

It is unclear which technique or combination of techniques is optimal for the identification of different types of MG. Because of the high incidence of MGUS and in view of our results and those of other investigators,^{8,11,12,14} we recommend that IFE be performed with SPE for screening purposes. For those patients who have a diagnosis of MM, Waldenström macroglobulinemia, or AL amyloidosis, SPE, and FLC may be adequate. Discrepancies between IFE and FLC occur in patients with myeloma on treatment.¹⁴ The role that serum FLC assays has in monitoring patients on therapy also requires further study.

Conclusion

Comparison of serum IFE with serum FLC in a large primary care and tertiary referral hospital showed that 50.7% of subjects

with an M-protein demonstrated by IFE had normal κ/λ ratios. These findings suggest that IFE and SPE rather than FLC should be used for screening in the detection of MGs.

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Disclosures

All authors have no relevant relationships to disclose.

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