Quantitative Analysis of Serum Free Light Chains

A New Marker for the Diagnostic Evaluation of Primary Systemic Amyloidosis

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Key Words: Amyloid; Amyloidosis; Monoclonal light chains; Immunoglobulins; Stem cell transplantation; Monoclonal gammopathy; Nephelometry

DOI: 10.1309/LYWM47K2L8XYFFB3

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Abstract

Primary systemic amyloidosis is a plasma cell dyscrasia characterized by the accumulation of excess free immunoglobulin light chains (FLCs) as amyloid. One of the diagnostic features of amyloidosis is the presence of circulating monoclonal FLCs in the serum and urine of the patients. The FLC usually is present in small amounts, and immunofixation is required for detection. A nephelometric method for quantitating FLCs in serum has been described using antibodies that recognize only FLC not bound to heavy chain. We describe a retrospective study using this quantitative FLC method for assessing monoclonal FLCs in 95 patients with amyloidosis. The sensitivity of nephelometric serum FLC measurements is particularly useful in patients with negative immunofixation results for serum, urine, or both. In addition, the FLC assay can be used for follow-up of patients with amyloidosis who have undergone stem cell transplantation.

Primary systemic amyloidosis is characterized by bone marrow plasmacytosis, but the plasma cell clone, compared with that seen in multiple myeloma, is small, and marrow clonality is not always apparent.[1] Amyloidosis may be a primary phenomenon or associated with multiple myeloma (5% to 10%) or some other form of monoclonal gammopathy, such as Waldenström macroglobulinemia.[2] Primary systemic amyloidosis is the most common type of amyloidosis seen in the United States. The amyloid fibrils are formed from the amino-terminus of the immunoglobulin light chain variable region and are 8 to 10 nm in diameter.[1] The extent of plasma cell clonality is correlated inversely with survival.[3] The morbidity seen in the disease is due to the infiltration of amyloid fibrils and subsequent dysfunction of the major organs of the body, such as heart, kidney, liver, and nerves. The median survival time ranges from 12[4] to 18[5] months. Peripheral blood autologous stem cell transplantation (SCT) has been used for the past 5 years for the treatment of amyloidosis and may be more effective than conventional chemotherapy.[6]

One of the laboratory hallmarks of primary systemic amyloidosis is the detection of a monoclonal immunoglobulin light chain in serum, urine, or both by immunofixation. However, some patients do not have free light chains detectable by immunofixation in serum or in urine.[4] Larger proportions of patients have no M-protein peak detectable in serum or urine protein electrophoresis, which makes the follow-up more difficult. The standard for response to therapy is monitoring reduction of the monoclonal immunoglobulin protein. Because of the comparatively low sensitivity of immunofixation in patients with primary systemic amyloidosis and its qualitative nature and the lack

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of an M-protein peak in many of the patients, it has been difficult to monitor hematologic responses. A method for quantitative estimation of serum free light chains (FLCs) by nephelometry was described recently.[7] This method permits detection only of free immunoglobulin light chain not bound to heavy chain. Katzmann et al[8] established reference ranges for kappa and lambda serum FLCs for people age 20 to 90 years and demonstrated that the serum FLC assay recognizes monoclonal light chains in some patients in whom immunofixation results are negative. The objective of the present study was to determine whether the serum FLC assay was more sensitive than immunofixation in the diagnosis of primary systemic amyloidosis and whether it could be used to monitor patients following treatment.

Materials and Methods

FLC and Immunofixation Comparison and Sensitivity Studies

Serum samples from 95 patients with primary systemic amyloidosis (group 1) obtained from the Dysproteinemia Serum Bank of the Mayo Clinic, Rochester, MN, were tested in a retrospective analysis for serum FLC by nephelometry. The FLC data were compared with the results of serum and urine immunofixation studies. Bone marrow plasma cell clonality was used to classify patients with negative immunofixation results.

SCT Studies

A group of 34 patients with primary systemic amyloidosis (group 2) was identified who underwent SCT during the previous 5 years and for whom both pretransplantation and posttransplantation serum samples were available for analysis. These patients had biopsy-proven amyloidosis and clonal bone marrow plasma cells. Serum FLC and immunofixation studies were done on pretransplantation and posttransplantation samples. Patients gave written consent for the use of data. The institutional review board of the Mayo foundation approved the study.

FLC Measurements

Serum samples from the patients in groups 1 and 2 were assessed for serum FLCs by using a BNII nephelometer (Dade Behring, Deerfield, IL). The FLC reagent sets (FREELITE) were a gift (The Binding Site, Birmingham, England).

Immunofixation

Immunofixation was performed with Sebia Hydragel kits on the Sebia Hydrasys electrophoresis system (Sebia, Norcross, GA), using agarose gels. The immunofixation assay used antisera to gamma, alpha, mu, kappa, and lambda immunoglobulins to fix specific proteins after electrophoretic separation, and the precipitated protein was visualized with acid violet stain. In addition, protein electrophoresis was performed on all samples.

Bone Marrow Biopsy

Bone marrow biopsy results were obtained from a hematopathologic evaluation of bone marrow from patients with amyloidosis by cytoplasmic immunofluorescence staining with kappa- and lambda-specific antibodies.[9]

Results

Sensitivity of Serum FLC Compared With Immunofixation

Kyle and Gertz[4] reported that immunofixation detected a monoclonal protein in 72% of serum samples and 73% of urine samples from patients with primary systemic amyloidosis at the Mayo Clinic.[4] Either serum or urine was positive in 89%.[4] To evaluate the sensitivity of serum FLC measurement for the detection of monoclonal light chains in amyloidosis, selected serum samples from group 1 patients were tested by the nephelometric FLC method. The group 1 patients were subdivided into 5 groups by immunofixation results: (1) kappa (n = 18) or (2) lambda (n = 19) M protein in both the serum and urine; (3) kappa (n = 20) or (4) lambda (n = 20) M protein with negative serum immunofixation, but positive urine immunofixation for M protein; and (5) M protein negative by serum and urine immunofixation (n = 18). Bone marrow biopsy immunostain results for clonal populations of plasma cells were used to stratify these 18 patients into kappa (n = 8), lambda (n = 6), and indeterminate (n = 4) groups. In the subgroups with serum monoclonal light chains detectable by immunofixation, the serum kappa and lambda FLC assays were marginally less sensitive than immunofixation **Figure 1**. The subgroups with negative serum immunofixation results but positive urine immunofixation had high serum FLC levels with a high sensitivity of detection of monoclonal light chains by the nephelometric FLC assay. In the subgroup of patients in whom both serum and urine immunofixation results were negative, the serum FLC assay detected a monoclonal light chain in 12 patients. In the patients classified by their bone marrow biopsy findings (8 kappa and 6 lambda), the serum FLC was increased in 7 (88%) of 8 and 5 (83%) of 6, respectively. In the indeterminate group of 4 patients, the serum FLC values were not diagnostic (Figure 1).

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Serum FLC as a Marker for Post-SCT Monitoring

One of the primary objectives of this study was to ascertain the usefulness of the serum FLC method as an adjunct to immunofixation and M-protein quantitation for monitoring hematologic responsiveness. In a manner similar to the studies described in the preceding sections, serum FLC values were measured in a retrospective analysis of pretransplantation samples of patients (9 kappa and 25 lambda) who had undergone SCT for amyloidosis. The sensitivity of the serum FLC method (88% [30/34]; both kappa and lambda) was higher than that with serum immunofixation (76% [26/34]) **Table 1**. Of the 34 patients, 26 were positive by serum immunofixation, 28 by urine immunofixation, and 24 by both serum and urine immunofixation. Although 26 patients were positive by serum immunofixation, the serum M-protein peak could be used to evaluate only 19 patients and the urine M-protein peak to evaluate 17 patients; the peaks were too small to measure in the remaining patients. Therefore, only about half of the total number of patients who had undergone transplantation could be evaluated by serum or urine M-protein levels. There were 6 patients who could not be monitored by protein electrophoresis owing to the absence of an M-protein peak in the serum or urine. However, serum FLC measurements could be used for all 34 group 2 patients, although 4 of these patients had nondiagnostic amounts of FLCs (within the normal range).

Evaluation of Hematologic Responses

Further stratification of group 2 patients by changes from before transplantation to after transplantation in levels of urine total protein, serum M protein, and serum FLCs indicated that the decrease in serum FLC levels was comparable to the decrease in urine M-protein levels for 10 (29%) of the patients and did not add additional information **Figure 2A**. However, in 13 (38%) of the patients, the serum FLC levels decreased earlier than the levels of urine total protein or serum M protein **Figure 2B**. In another 8 patients (24%), serum FLC was the only marker for follow-up for a hematologic response **Figure 2C**. In 3 (9%) of the patients, the urine total protein decreased before any change was evident in the serum FLC levels **Figure 2D**.

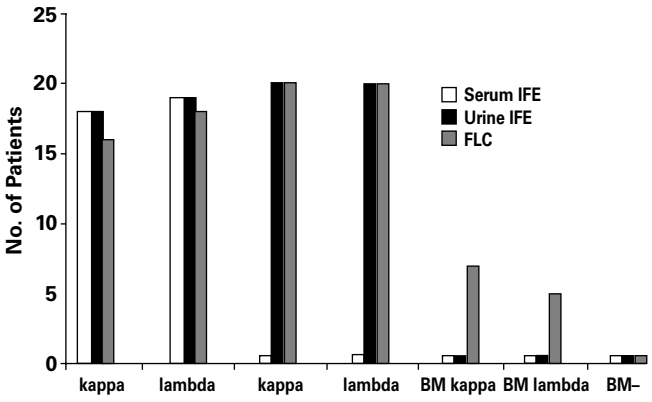

Figure 1 Diagnostic sensitivity of serum free light chain (FLC) compared with immunofixation (IFE) in a cohort of 95 patients with primary systemic amyloidosis. Columns 1 and 2 represent 18 and 19 patients, respectively, who had M-protein spikes in both serum and urine. Columns 3 and 4 represent 20 patients each who had no serum M-protein spike but positive urine M-protein spikes. The last 3 columns represent 8, 6, and 4 patients, respectively, with no serum or urine M proteins. Those who were serum and urine IFE negative were placed in the kappa (8 patients) or lambda (6 patients) groups on the basis of bone marrow immunostaining results. The other 4 patients had indeterminate bone marrow staining results. In the serum IFE-positive groups, the sensitivity of serum FLC was only marginally lower than that of IFE, but in the serum IFE-negative groups, the sensitivity of serum FLC was high, indicating that it has important clinical validity in the latter groups of patients.

Assessment of Hematologic Recovery in the Absence of M Protein

All 6 of the group 2 patients who did not have a serum or urine M protein had increased levels of serum FLCs. Three in this group showed a greater than 50% reduction in light chain level after transplantation, while 1 had a reduction of less than 50% in serum FLC levels. Two patients had an increase in serum FLC levels after transplantation.

Table 1
Diagnostic Sensitivity of Serum Free Light Chain Measurements in Patients With Primary Amyloidosis Before Stem Cell Transplantation*

Light Chain	No. of Patients	Serum Immunofixation	FLC	Median FLC, mg/L	FLC kappa/lambda Ratio, % Abnormal
kappa	9	7 (78)	7 (78)	75.4 (3.3-19.4)†	8.33 (0.26-1.65)‡
lambda	25	19 (76)	23 (92)	84.8 (5.7-26.3)†	0.0374 (0.26-1.65)‡

FLC, free light chain.
* Data are given as number (percentage) positive unless otherwise indicated.
† Reference range.
‡ Diagnostic range.

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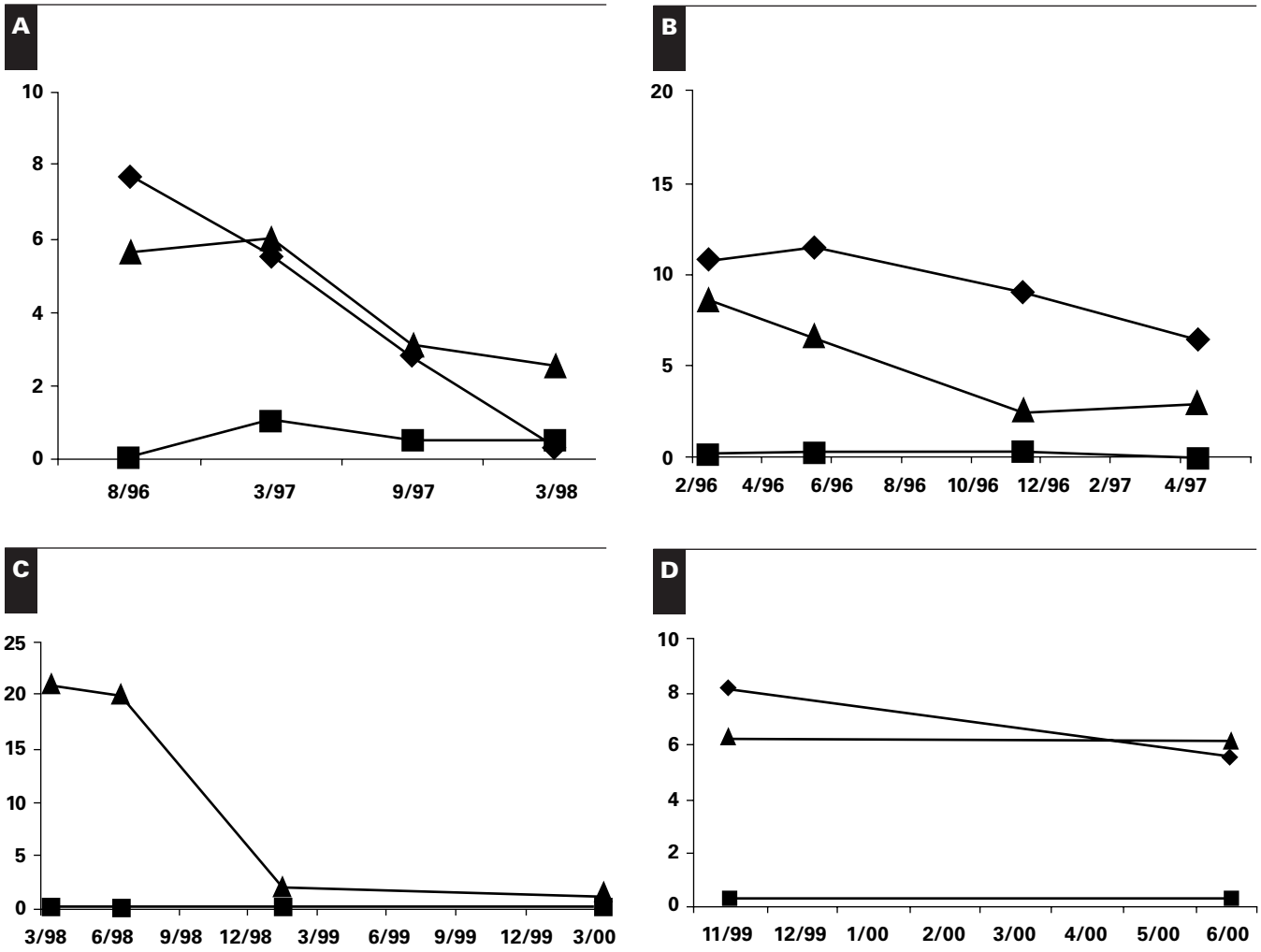

Figure 2 Evaluation of hematologic responses in patients with primary systemic amyloidosis after transplantation. **A**, Serum free light chain (FLC) follows the same pattern seen with urine total protein in 10 of 34 patients. **B**, In 13 patients, the serum FLC levels decreased before either serum M protein or urine total protein levels. **C**, In 8 patients, serum FLC was the only marker that could be evaluated. **D**, Of 34 patients, 3 had a decrease in urine total protein before changes were observed in the serum FLC or serum M protein level. The urine total protein is expressed in g/24 h, the serum M protein in g/L, and serum FLC in mg/dL. Diamonds indicate urine protein; squares, serum M protein; and triangles, serum FLC.

Discussion

A constant source of the amyloid precursor protein, the FLC, is essential to the pathogenesis of amyloidosis, but what is unclear is exactly how much of the protein is necessary to initiate and perpetuate the process. Because the half-life of FLC is less than 2 hours, the serum or urine concentration reflects only a small fraction of the total FLC being synthesized by the clonal plasma cell population.[10] Patients with primary systemic amyloidosis usually have a low percentage of plasma cells in the bone marrow and small amounts of Bence Jones proteinuria.[4] There is no evidence to suggest that FLC synthesis is different from that seen in other pathologic light chain disorders. It seems, therefore, that in amyloidosis, these light chain molecules have the ability to form amyloid at a high rate. On the other hand, the patients might have high concentrations of cell surface molecules that can bind the amyloidogenic FLC or they might have defective or abnormal clearance of the amyloid deposits. It also has been suggested that patients with amyloidosis may have other tissue-specific aberrations, such as defective processing of soluble Bence Jones proteins by macrophages[11] or inadequate removal of fibrillar amyloid deposits owing to impaired proteolysis or other scavenging functions.[12,13] Association of amyloid fibrils with other proteins or molecules in the circulation also may contribute to defects in fibril degradation.[14-16]

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Both serum and urine protein electrophoresis usually have been used to study the M protein. We used serum FLCs to evaluate hematologic responses, using polyclonal antibodies specific for kappa or lambda FLC, which do not recognize light chain bound to heavy chain. Abraham et al[17] and Drayson et al[18] demonstrated that serum FLC is a useful adjunct to urine total protein for monitoring patients with light chain myeloma. The present study compared the sensitivity of serum FLC assays with serum or urine immunofixation for detecting monoclonal light chains in patients with amyloidosis. We studied a large cohort of patients in subcategories based on their immunofixation status, which provided an opportunity to determine serum FLC levels in patients with immunofixation-negative results in serum and urine. The serum FLC results showed a sensitivity of 95% to 100% in patients whose serum was immunofixation-negative for kappa or lambda (Figure 1). In addition, serum FLC measurement had a sensitivity of approximately 86% in patients whose immunofixation results were negative in both serum and urine (Figure 1). The diagnostic sensitivity of serum FLC measurement in pretransplantation samples from patients who underwent SCT also was higher than that seen with serum immunofixation (Table 1).

Since the larger group of 95 patients with primary systemic amyloidosis was selected specifically to evaluate the sensitivity of the FLC method, the test was assessed further in an unselected group of 34 patients with amyloidosis who had undergone SCT. Of these 34 patients, only about half could be evaluated for a hematologic response by serum or urine M-protein values, whereas all could be assessed by serum FLC measurement. Interestingly, in 21 of 34 patients, serum FLC was the only marker for measurement or the level decreased before a decreased level of urine total protein or serum M protein was evident, and the change in level mirrored changes in urine total protein level in another 10 patients (Figure 2).

The primary objective of this study was to evaluate the sensitivity of serum FLC quantitation in patients with primary systemic amyloidosis. The FLC method was more sensitive than serum or urine immunofixation and was of particular value in the diagnosis and follow-up of patients who had negative results of serum or urine immunofixation.

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Supported in part by grant CA-62242 from the National Cancer Institute, Bethesda, MD, and the Hematologic Malignancies Fund, Mayo Clinic.

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