Multiple Myeloma with Systemic Amyloidosis: Serum Free Light Chain Dimerization Analysis in the Diagnosis of an Equivocal Case of Plasma Cell Dyscrasia

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The term amyloid light-chain (AL) amyloidosis refers to a disorder characterized by the deposition of misfolded light-chains that are secreted from clonal plasma cells (PCs). The monoclonal protein fibrils accumulate in certain organs, disrupt their tissue architecture, and impair the function. The organ recovery is strongly associated with the depth of the hematological response achieved. The deeper the hematological response (i.e., the greater the reduction of the amyloidogenic light chains), the more likely organ response will occur and the longer the survival [1].

Automated nephelometric immunoassay for immunoglobulin free light chains (FLC) in serum measures the total levels of polyclonal and monoclonal FLC, and the kappa/lambda (κ/λ) FLC ratio is a surrogate measure of clonality. Both polyclonal and monoclonal antibody-based immunochemical methods have been developed to quantify FLC. The FLC antibodies must recognize only epitopes, which are hidden in intact immunoglobulins (Ig) to avoid falsely elevated FLC from cross-reaction.

Although the FLC assay has become an established test in the diagnosis and monitoring of monoclonal gammopathies, the limitations of this assay are now well recognized. First, monoclonal FLC contain unique combinations of hypervariable regions that may affect the availability of the limited number of epitopes to antibodies used in the assay. Second, FLC are usually monomers and dimers, but higher molecular polymeric FLC forms often exist, acting as multi-antigenic targets and leading to overestimation of antigen concentration [2]. In addition, overestimation of the FLC concentration due to non-specific interference has been reported.

In a previous study [3] we used the sodium dodecyl sulfate electrophoresis-based Western blotting to analyze circulating FLC in AL amyloidosis. In contrast to the nephelometric FLC assay, this method allowed us to study FLC monomers and disulfide-bound dimers in patient sera. We found that AL amyloidosis patients demonstrated abnormally increased levels of monoclonal FLC dimers and/or abnormal κ/λ ratios of dimeric FLC. Moreover, the results showed that FLC monomer-dimer patterns in sera of AL and MM patients were different from those of monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma (SMM). We found that our technique was diagnostically helpful in cases of suspected AL amyloidosis.

We showed the utility of this Western blot-based technique in clarifying the amyloidogenic properties of a suddenly elevated FLC-κ on nephelometry in a patient diagnosed with IgG-λ multiple myeloma and systemic AL amyloidosis.

This study was approved by the Helsinki Committee of Assuta Medical Center, approval number AAA-0071-19.

PATIENT DESCRIPTION

A 63-year-old man was admitted to our hospital with a diagnosis of IgG-λ MM and systemic AL amyloidosis. His medical history included gout, hypertension, and hyperlipidemia.

The patient had been well until approximately one year earlier, when bloody diarrhea and abdominal pain crisis developed. Colonoscopy and computed tomography enterography findings supported the diagnosis of inflammatory bowel disease, and mesalazine was empirically started. During follow-up, the patient underwent abdominal computed tomography that demonstrated the presence of lytic lesions in the pelvic bones and was referred to our institution for further evaluation.

At admission, the serum protein electrophoresis and immunofixation assays revealed an IgG lambda (λ) M-spike, with a FLC-λ level of 1620 mg/dl. Bone marrow (BM) biopsy showed lambda-restricted plasma cell infiltration of 90% and positive Congo red staining. The fluorescence in situ hybridization (FISH)

analysis of BM plasma cells demonstrated 100% t(11;14).

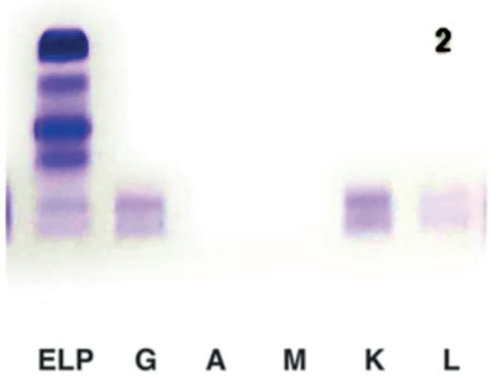
Cardiac magnetic resonance imaging showed an interventricular septum 14–15 mm, left ventricular posterior wall 13 mm, and left ventricular end diastolic diameter 46 mm. The perfusion test demonstrated a diffuse subendocardial diffusion defect in all segments of the heart, compatible with amyloidosis. During the diagnostic workup, the patient incurred sigmoid perforation and underwent partial colectomy with permanent colostomy. The pathology highlighted extensive deposits of birefringent (green) amyloid (Congo red staining), mainly in muscularis propria.

Bortezomib-based therapy was then started. The involved-to-uninvolved free light chain difference (dFLC) was still higher than 40 mg, considering the systemic involvement of amyloidosis. At this point, we decided to change the protocol to lenalidomide and dexamethasone. One year later, the FLC lambda level was normalized, but the κ/λ ratio became abnormally high (κ/λ = 2.91) due to an unexpected increase in FLC-κ level (46.3 mg/L). Serum protein electrophoresis and immunofixation detected 30 mg/L IgG-λ. Interestingly, we were able to observe the appearance of a second monoclonal fraction of IgG-κ [Figure 1A], highlighting the amyloidogenic potential of the monoclonal κ light chains.

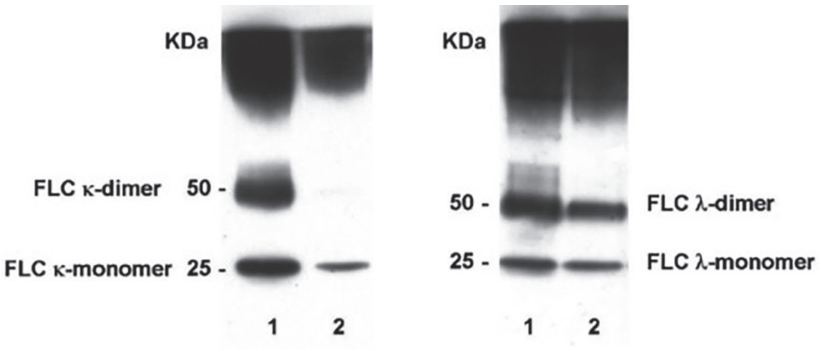
Western blot analysis of FLC monomers and dimers was performed according to the procedure described earlier [3]. As depicted in Figure 1B, the patient's serum sample showed predominance of FLC-λ versus FLC-κ, as compared to that of the healthy serum control. Based on intensity measurements of the immunodetected FLC bands, we found that the κ/λ ratio of the patient's FLC dimers (raw value) was at least 50 times lower than that of a control sample. The calculated clonality index of dimeric FLC was abnormally low (Dκ/λ = 0.018) and thus, compatible with λ type AL amyloidosis. This finding, together with the patient's Congo red positive tissue staining, was consistent with AL-λ type amyloidosis. Moreover, this case showed only trace amounts of FLC-κ dimers, which is not compatible with κ type malignant monoclonal gammopathy. Based on these results, we concluded that the new plasma cell clone may not have amyloidogenic features.

Figure 1. Serum protein electrophoresis and immunofixation

[A] The original IgG-λ monoclonal protein as well as a new IgG-κ monoclonal band were identified by immunofixation



[B] Western blot analysis of serum free light chains (FLC) monomers (25 kDa lane) and FLC dimers (50 kDa lane).
Track 1 = healthy serum (control), track 2 = serum of the patient. Left panel: FLC kappa (κ) analysis. Right panel: FLC lambda (λ) analysis. Patient's serum (track 2) shows extremely high predominance of dimeric FLC-λ over dimeric FLC-κ, in contrast to that observed in control serum (track 1). This finding, together with the patient`s Congo red positive tissue staining, is consistent with AL-λ amyloidosis.



COMMENT

Systemic amyloidosis is caused by tissue deposition of misfolded proteins that result in progressive organ damage. Clinical observations demonstrated that the amyloid precursor (the free light chain) plays an important role in tissue dysfunction. In this sense, it is essential to eliminate the production of newly formed light chains that feed the formation of oligomers and fibrils to obtain sustained improvement of AL amyloidosis-related organ dysfunction and improve survival [1].

Protein electrophoresis and immunofixation, as well as the FLC assay, are used for diagnosis and monitoring of plasma cell dyscrasias (PCD). In some cases, results may be uncertain or even conflicting: the identified monoclonal protein may still be benign and unrelat-

ed to the amyloid deposit. Such contradictory results were indeed obtained in our case of AL-λ amyloidosis. The unexpected appearance of a new IgG-κ plasma cell clone raised questions regarding the nature of the protein. Accordingly, we evaluated whether the newly observed monoclonal IgG-κ had amyloidogenic properties. In this case, change of treatment would be considered.

Based on FLC monomer-dimer pattern analysis (MDPA) technique, we showed that the new IgG-κ clone did not have amyloidogenic properties. These results concur with those from our recent study [3] showing the real-life utility of FLC MDPA in solving diagnostic dilemmas in cases of kidney disease associated with PCD, when the results of immunohistochemical methods and other laboratory tests were inconclusive for determining the precise diagnosis or response to treatment [4]. The advantage of FLC MDPA is that FLC monomer-dimer patterns in Western blotting of AL and multiple myeloma patient sera are different from those of MGUS and SMM [5]. The FLC monomer-dimer pattern analysis of this case revealed abnormally low κ/λ ratio of dimeric FLC in the sera of the patient, thus supporting the diagnosis of AL-λ amyloidosis. Moreover, we were able to show that FLC-κ M-D pattern did not fulfill the criteria of an amyloidogenic clone. This, together with the lack of new target organ damage prompted us to maintain the treatment protocol without adding additional potential drug toxicities.

CONCLUSIONS

Our FLC MDPA technique may be useful in cases where results of nephelomeric FLC assay are inconclusive in determining the amyloidogenic potential of increased FLC. This new assay helps distinguish malignant from premalignant forms of the main monoclonal gammopathies, and might be useful in the management and follow-up of AL amyloidosis.

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Anyone can be passionate, but it takes real lovers to be silly.
Rose Franken (1895–1988), American author and playwright

Capsule

A boost from SARS-CoV2 infection

During clinical trials of severe acute respiratory syndrome coronavirus-2 vaccines, no one who had survived infection with the virus was tested. A year after the pandemic was declared, vaccination of previously infected persons is a reality. **Reynolds** and co-authors addressed the knowledge gap in a cohort of U.K. healthcare workers given the Pfizer/BioNTech vaccine in which half of the participants had experienced natural virus infections early in the pandemic Genotyping indicated that a genetic component underlies heterogeneity in immune responses to vaccine and to natural infection. After vaccination, naïve individuals developed antibody responses similar to those seen in naturally infected persons, but T cell responses were more limited and sometimes absent. However, antibody and memory responses in individuals vaccinated after infection were substantially boosted to the extent that a single vaccine dose is likely to protect against the more aggressive B.1.1.7 variant. It is possible that the messenger RNA vaccine has an adjuvant effect, biasing responses toward antibody generation.

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