Using LC-ESI-Q-TOF of Immunoglobulin Light Chains to Resolve Ambiguous Serum Protein Electrophoresis Cases

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Background

A monoclonal gammopathy is diagnosed by the detection a monoclonal immunoglobulin (M-protein) at concentrations exceeding the polyclonal background. In clinical practice, the M-protein is routinely detected by protein gel electrophoresis (PEL) and immunofixation electrophoresis (IFE). Occasionally, disease modifying factors and technical artifacts result in PEL and IFEs which appear to have M-proteins but clinical history and other laboratory testing suggest these could be artifacts. Recently, we have described a LC-ESI-Q-TOF method which can profile the mass distribution of serum immunoglobulin light chains. This method we have termed monoclonal immunoglobulin rapid accurate mass measurement (miRAMM). miRAMM results in improved resolution of immunoglobulins in comparison to PEL which aids in the discrimination of PEL/IFE ambiguities and artifacts. Although not yet clinically available, our lab has been reflexing difficult cases to the miRAMM for over a year. During this time we have accumulated several clinical cases in which the miRAMM light chain mass distribution resolved ambiguities in PEL/IFE resulting in a change in clinical management for the patient. Four clinical cases that typify the benefits of miRAMM will be presented.

Methods

Serum immunoglobulins were enriched and then reduced with DTT, and samples were separated using an Eksigent-Ekspert LC system using a Poroshell C3 1x75mm column running at 25 uL/min with gradient of aqueous (0.1 % formic acid) to organic (90:10 ACN/IPA) over 24 minutes. The accurate mass of monoclonal light chains is determined by deconvolution of the mass spectra of multiply charged ions across the retention times of immunoglobulin light chains. SPEP was performed on the SPIFE SPE system (Helena Laboratories, Beaumont TX) and IFE on Sebia 9IF gels (Sebia, Norcross GA). The total protein concentration was determined by

colorimetric assay using biuret reagents from Roche and a Roche Hitachi 912 chemistry analyzer system (Roche Diagnostics, Indianapolis IN).

Results

Case 1 is a 24 year old male who presented with a diagnosis of a monoclonal gammopathy with a 7.7 g/dL M-protein and with pericardial effusions and a right atrial mass. miRAMM analysis (Figure 1) of the patients serum revealed no evidence of a M-protein but rather a skewed kappa polyclonal Ig response. Follow up bone marrow biopsy was negative for clonal plasma cells. This patient was later determined to have IgG4-related disease (IgG4-RD). Case 2 is a 27 year old HIV positive male with lymphadenopathy who by PEL and IFE examination had a restricted IgG kappa band which upon miRAMM analysis revealed was actually an oligoclonal response (Figure 2). This oligoclonal response was most likely from clonal B-cell proliferation secondary to an infection. Case 3 was from a 54-year-old man who presented with a past medical history significant for hepatitis, pancreatitis, rheumatoid arthritis, and Sjogren's syndrome. PEL and IFE examination revealed a hypergammaglobulinemia with restricted IgG kappa and lambda bands. The patient had a remarkably elevated rheumatoid factor. miRAMM analysis demonstrated a normal polyclonal background. Follow-up bone marrow biopsy did not reveal any evidence of a plasma cell disorder. Case 4 was from a 74 year old female who by PEL had no restricted gamma bands. IFE revealed an intense lambda polyclonal distribution in comparison to the kappa band. In this case, miRAMM demonstrated a large monoclonal lambda light chain which was consistent with multiple myeloma.

Summary

These cases demonstrate the ability of mass spectrometry to resolve ambiguous electrophoresis patterns. Future work will focus on the validation and clinical implementation of this method into our clinical practice.

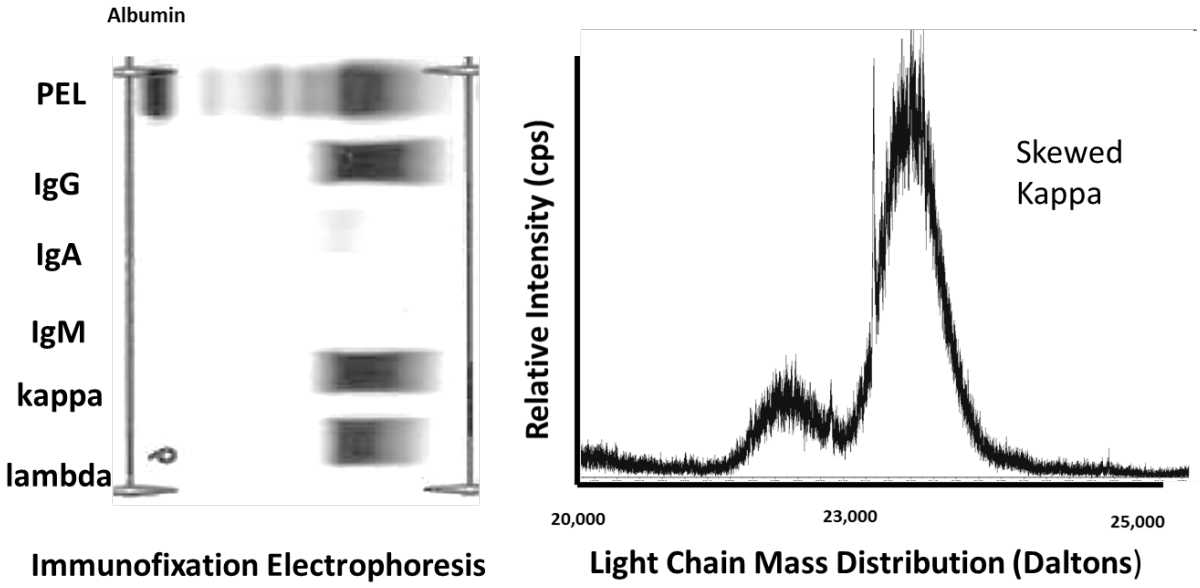

Figure 1: Comparison of IFE and miRAMM mass distribution in a 24 y.o. patient with IgG4 RD

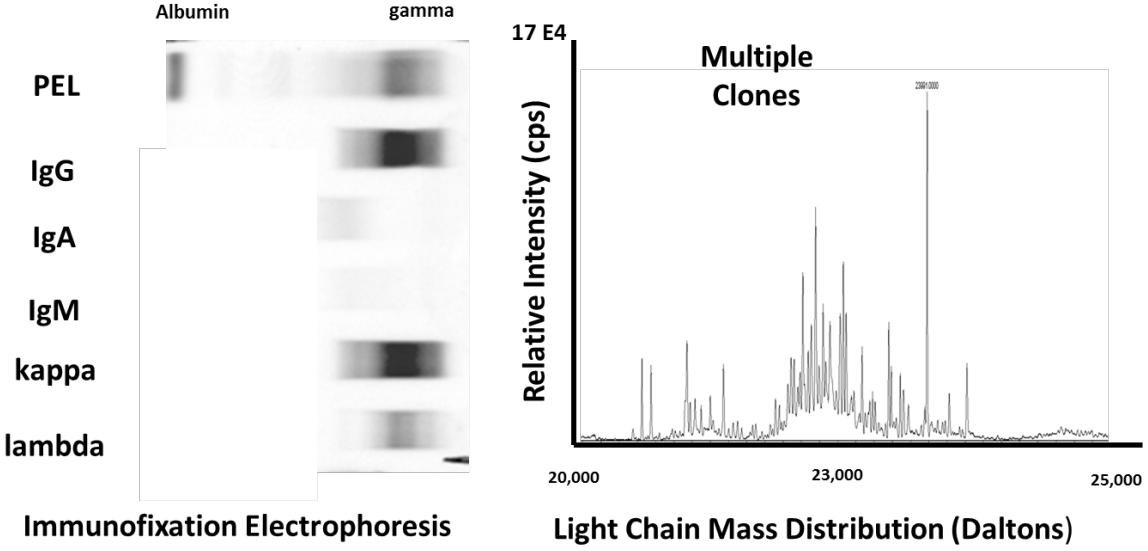

Figure 2 – Comparison of IFE and miRAMM mass distribution in a 27 y.o. HIV positive patient.

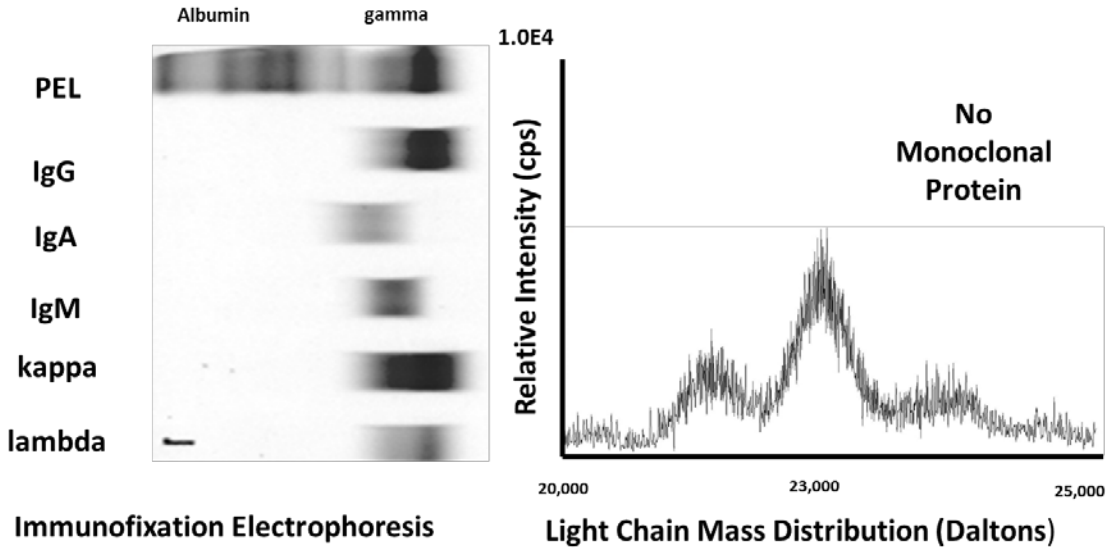

Figure 3 – Comparison of IFE and miRAMM mass distribution in a 54 y.o. patient with active disease

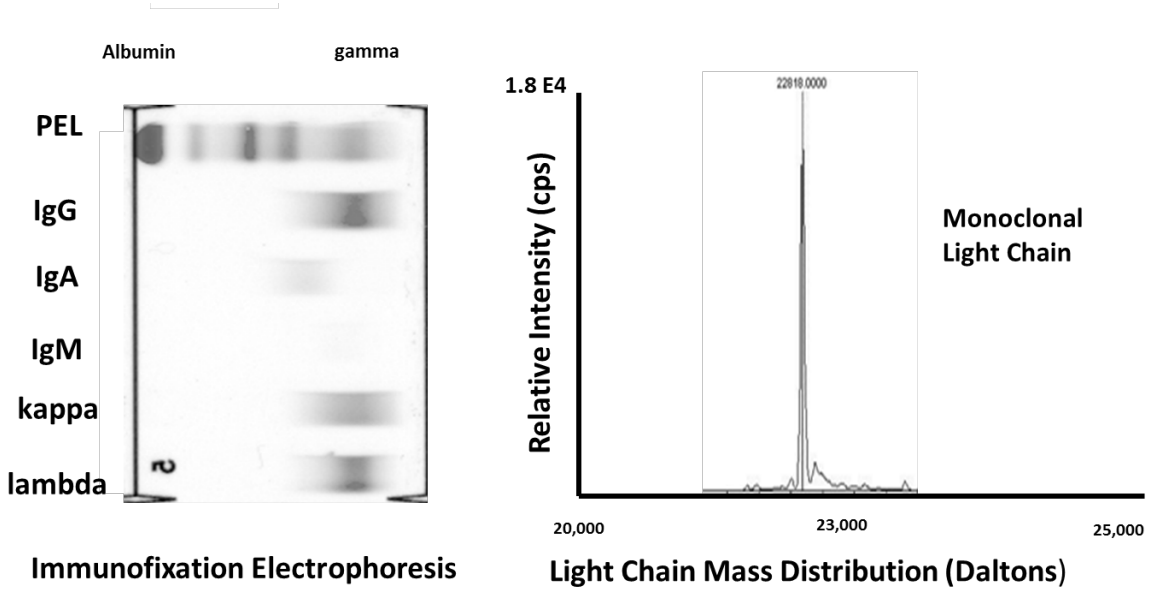

Figure 4 - Comparison of IFE and miRAMM mass distribution in a 74 y.o. patient with no distinct gel M-spike