Pseudo-monoclonal gammopathy: a report of four cases

Hypergammaglobulinemia can result from both polyclonal and monoclonal immunoglobulin production. Polyclonal hypergammaglobulinemia is usually associated with inflammatory conditions and detected on serum protein electrophoresis (SPEP) as broad indistinct band. In contrast, monoclonal gammopathy is diagnosed based on the appearance of relatively restricted bands on SPEP or serum immunofixation (IFX) or by abnormal serum immunoglobulin free light chain (FLC) ratios and is associated with malignant (i.e., multiple myeloma) or premalignant (i.e., monoclonal gammopathy of undetermined significance (MGUS)) disease.[1-3] Once a monoclonal protein is detected, an extensive workup including bone marrow biopsy may be necessary. Therefore, it is critical to have a reliable method to differentiate between these two conditions. Recent publications have shown that mass spectrometry (MS) can be used to monitor the monoclonal protein with higher accuracy compared to traditional methods (SPEP/IFX/FLC). This is based on the fact that these immunoglobulins secreted from clonal plasma cells have a unique amino acid sequence and, consequently, a unique molecular mass.[4] Herein, we describe four patients with hypergammaglobulinemia ascribed to a pseudo-monoclonal gammopathy diagnosed by traditional methods, but were found to have polyclonal hypergammaglobulinemia using MS.

Patient 1: A 30-year-old female with a past medical history of rheumatoid arthritis, vasculitis and MGUS presented to our institution with fatigue. Laboratory evaluation revealed iron deficiency anemia with hemoglobin of 9.1 g/dL (normal, 12 to 15.5 g/dL). A repeat SPEP was interpreted as a monoclonal (M) spike of 6.1 g/dL. Serum IFX showed a biclonal gammopathy (monoclonal IgG kappa (κ) and IgG lambda (λ)). The κ and λ FLCs were 8.50 mg/dL (normal, 0.33 to 1.94) and 2.82 mg/dL (normal, 0.57 to 2.63), with an abnormal κ/λ ratio of 3.01 (normal, 0.26-1.65). Bone marrow biopsy revealed 8% plasma cells that were polyclonal by flow cytometry. Due to the discrepancy between the presence of M-spike and absence of clonal bone marrow plasma cells, the patient's serum was further tested using Hevylite® testing, which is an immunoassay to measure the amount of intact κ and λ immunoglobulin pairs.[5] Hevylite® testing revealed IgG κ of 4450 mg/dL and IgG λ of 1890 mg/dL, with a normal heavy chain ratio of 2.35 suggesting the absence of M-protein. To further confirm these results, MS-based miRAMM (monoclonal immunoglobulin Rapid Accurate Mass Measurements) test was performed. The miRAMM LC mass distribution (Figure 1B) Patient 1) revealed a markedly skewed polyclonal κ to λ light chain ratio (~8) and a polyclonal background seen in other hypergammaglobulinemia patients.[6] Typical IgG κ to λ ratios for normal patients is between 1.17-3.61.[7] Given the patient's rheumatoid arthritis, the patient was treated with prednisone which led to resolution of symptoms and polyclonal hypergammaglobulinemia. However, on subsequent follow up at 15 months, the patient presented with symptoms of hyperviscosity syndrome, including epistaxis and hematuria. On evaluation, she had an elevated serum viscosity of 6.6 centipoise (normal, ≤1.5). She was treated with therapeutic plasma exchange and rituximab with marked improvement of symptoms.

Patient 2: A 54-year-old male with rheumatoid arthritis and Sjögren syndrome presented with fatigue, fever, weight loss and headache. Laboratory studies showed normocytic anemia with hemoglobin of 11.7 g/dL, with rouleaux formation and increased gammaglobulin of 4.7 g/dL (normal, 0.6 to 1.6). Serum viscosity was elevated at >5.6 centipoise. His κ and λ FLCs were both elevated at 63.0 mg/dL and 7.25 mg/dL, with an abnormal κ/λ ratio of 8.7. Additional tests were positive for anti-nuclear

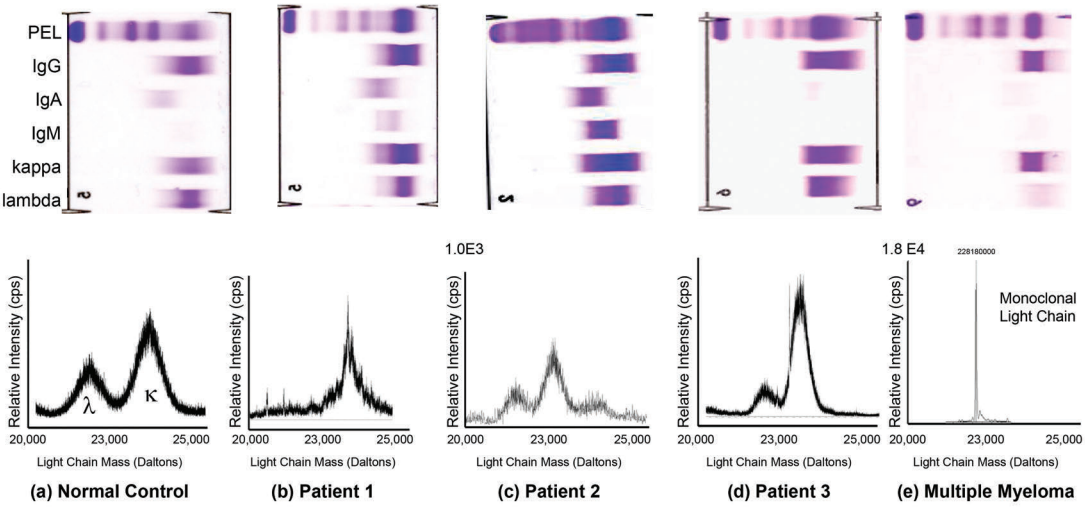

Figure 1. Comparison between serum protein electrophoresis (SPEP) and immunofixation (IFX) *versus* mass spectrometry (MS). (a) normal control, b) patient 1 with biclonal gammopathy [IgG kappa (κ) and IgG lambda (λ)] with substantial portion of the IgG is restricted polyclonal, c) patient 2 with IgG κ and very restricted polyclonal immunoglobulins, d) patient 3 with polyclonal immunoglobulins and e) M-spike in a patient with multiple myeloma. PEL: protein electrophoresis

antibody, rheumatoid factor, and anti-cyclic citrullinated peptide antibody. SPEP was interpreted as an M-spike of 2.2 g/dL. Serum IFX demonstrated an IgG κ monoclonal protein. Bone marrow biopsy showed only reactive changes. He underwent several sessions of therapeutic plasma exchange. To determine the presence of a monoclonal protein, a serum sample was analyzed using miRAMM, which showed a polyclonal background with no monoclonal immunoglobulin (Figure 1C) Patient 2). The patient was diagnosed with polyclonal hypergammaglobulinemia, probably of autoimmune origin, causing hyperviscosity syndrome. He was treated with rituximab which led to improvement of hypergammaglobulinemia and resolution of hyperviscosity.

Patient 3: A 24-year-old male with a history of recurrent urticaria was found to have lymphadenopathy and hypergammaglobulinemia. Other complaints included weight loss, chest pain and arthralgia in the wrists and knees. At onset of symptoms, imaging studies at an outside facility revealed lymphadenopathy in the groin and mediastinum, and the biopsies were read as reactive. The patient was seen at our institution and his complete blood counts were normal. A repeat SPEP was interpreted as M-spike of 7.7 g/dL, consistent with outside records. Serum IFX showed biclonal IgG κ and IgG λ. Other laboratory findings revealed an elevated κ and λ FLCs of 55.5 mg/dL and 8.9 mg/dL, respectively, with an abnormal κ/λ ratio of 6.2 and elevated serum viscosity of 2.7. Infectious disease serologies were negative. Echocardiography and cardiac magnetic resonance imaging showed pericardial effusion and a right atrial mass. Bone marrow biopsy showed no evidence of clonal plasma cell disorder. Given his unexplained monoclonal gammopathy, the patient's serum was analyzed using miRAMM, which showed that his hypergammaglobulinemia was mainly polyclonal with a skewed κ/λ ratio of ~5 (Figure 1D) Patient 3). Subsequently, on repeat biopsy of the mediastinal lymph nodes and pericardium, he was diagnosed with IgG4 disease. The patient was started on rituximab with improvement of symptoms.

Patient 4: A 51-year-old female with past medical history of Hodgkin's lymphoma, currently in remission, presented with dyspnea, periorbital swelling and anemia. Imaging studies revealed bilateral extraocular soft tissue deposits and bilateral interstitial pulmonary infiltrates, the latter was consistent with lymphocytic interstitial pneumonia on lung biopsy. Labs revealed a serum viscosity of 2.1 centipoise, IgM-4320 mg/dL (normal, 37-286), IgG-2370 mg/dL (normal, 767-1590), κ-32.9 mg/dL and λ-5.13 mg/dL, respectively, with an abnormal κ/λ ratio of 6.41. SPEP and IFX were suspicious but not conclusive for a monoclonal IgM. Because of an abnormal FLC ratio and elevated IgM, miRAMM was performed on an IgM enriched sample from the patient. The resulting LC mass distribution (Figure 2) did not show monoclonality but contained a κ skewed polyclonal IgM with the presence of a higher mass IgM κ polyclonal distribution. Additional tests showed normal rheumatoid factor, antinuclear antibody and IgG4 (8.7 mg/dL, range: 2.4-121) levels. Bone marrow biopsy was negative for a clonal B-cell population. However, lacrimal gland biopsy revealed extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) and patient continues to be on treatment with steroid and rituximab.

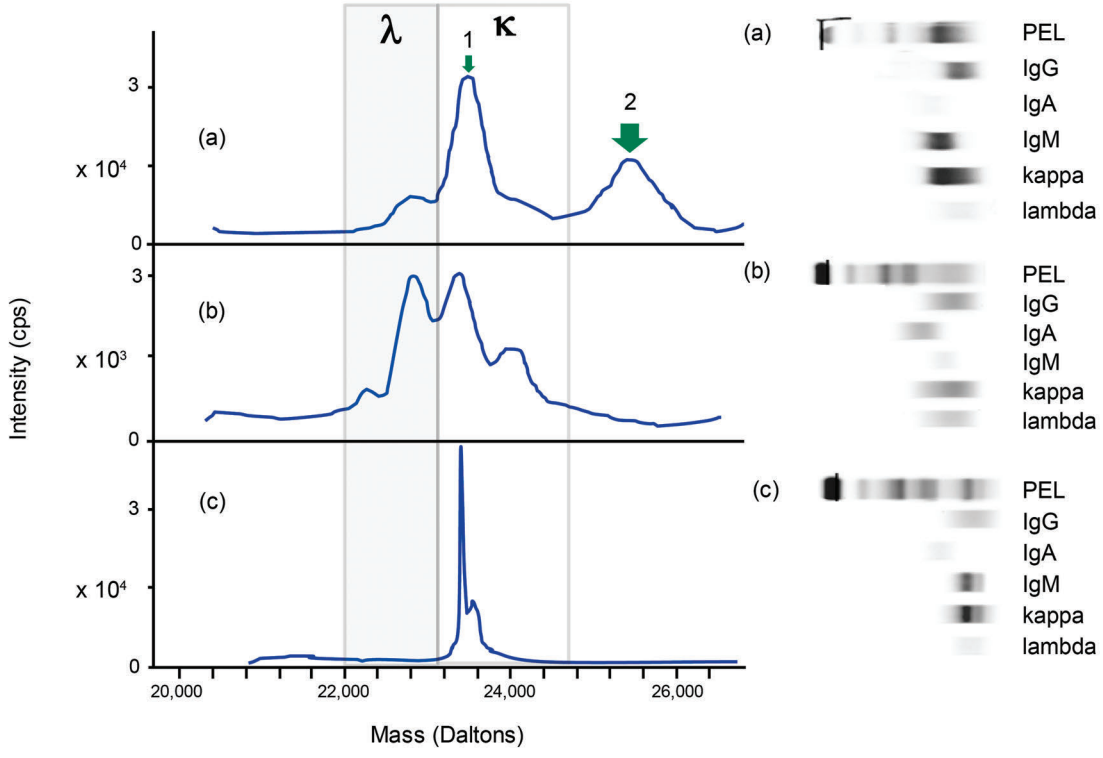

Figure 2. IgM light chain mass spectrometry. The IgM light chain mass distribution and corresponding IFX for (a) Patient 4, (b) healthy control and (c) IgM producing lymphoplasmacytic lymphoma patient. Patient 4 has a polyclonal increase in IgM κ (Arrow 1) and a polyclonal high mass IgM κ (Arrow 2) that most likely represents a post-translationally modified IgM κ population. PEL: protein electrophoresis.

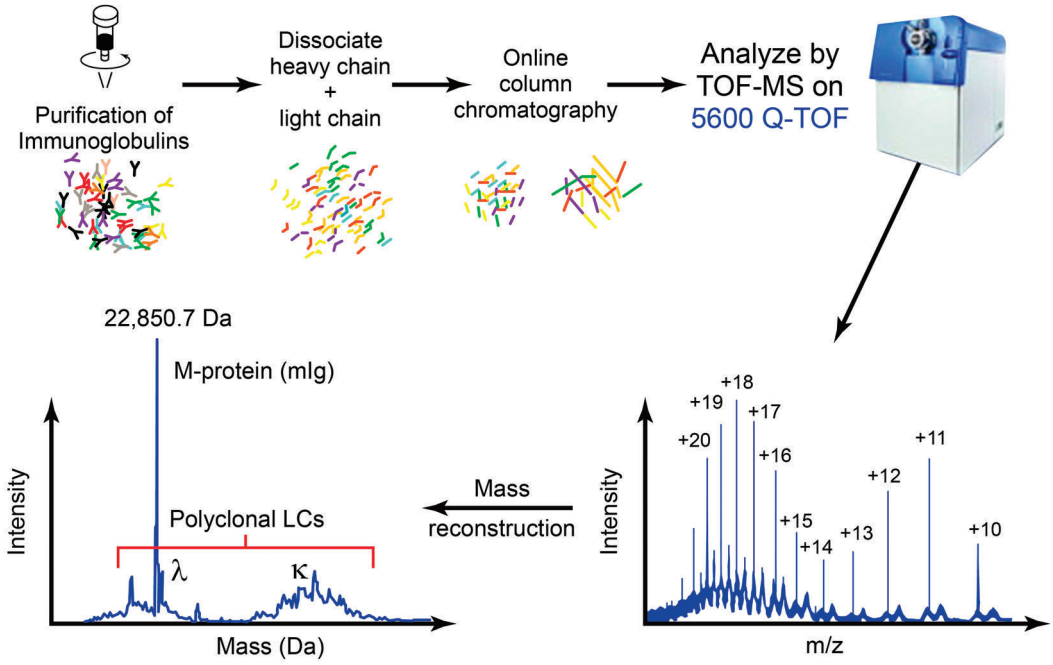

Figure 3. Overview of "monoclonal immunoglobulin Rapid Accurate Molecular Mass" or miRAMM. Immunoglobulins are first purified from human serum and subsequently reduced with dithiothreitol which is sufficient to break inter-chain disulfide bonds. The dissociated mixture of heavy chains (HCs) and light chains (LCs) is then separated by Reverse-Phase Liquid Chromatography (RPLC) using C4 column chromatography prior to analysis by time-of-flight mass spectrometers. The resulting spectra of multiple charged ions are deconvoluted into the accurate molecular mass spectra. Peaks above the polyclonal LCs are indicative of monoclonal immunoglobulin [*Adapted from Mills et al. Methods. 2015;81:56-65; with permission*].

Several interfering factors mimic the appearance of a monoclonal band on SPEP/IFX, leading to false reporting of a monoclonal gammopathy (*Online Supplementary Table S1*):[8] i) cryoglobulins precipitate from serum or plasma at low temperature of blood samples, leading to the appearance of restricted band on SPEP, ii) fibrinogen migrates as a distinct band in the beta-gamma region causing a false identification of a monoclonal band, iii) high polyclonal immunoglobulins in connective tissue disorders, chronic lymphoproliferative disease, or chronic active hepatitis, may be mistaken for monoclonal gammopathy, iv) hemolysis may lead to misreading due to hemoglobin-haptoglobin complexes, which may appear as a large band in the alpha-2 region, v) high transferrin concentration may cause a false positive M-spike in the beta region, vi) nephrotic syndrome patients have an increase in the alpha-2 and beta bands, leading to false reporting of a monoclonal gammopathy, but the albumin and gamma globulin concentrations in such cases will be low, and vii) acute phase reactants may cause an increase in the alpha-1 concentration causing a restricted band on SPEP.

In our study, patients 1, 2 and 3 had ongoing systemic inflammatory processes and patient 4 had extranodal MALT lymphoma of the lacrimal gland. Additionally, patients 1 and 2 had hyperviscosity syndrome, while the rest had elevated serum viscosity. This can be associated with both monoclonal and polyclonal hypergammaglobulinemia.[9] Therefore, a different method with higher sensitivity and specificity could have been used to investigate the nature of hypergammaglobulinemia.

Several studies have suggested the role of mass spectrometry in detecting and monitoring patients with monoclonal gammopathy.[4,10] Each M-protein is comprised of a unique sequence of amino acids that is specific to each clonal plasma cell. The method involves removal of potentially interfering serum proteins (e.g., fibrinogen, hemoglobin), and denaturing of primary structure of the immunoglobulin effectively removes other interferences such as rheumatoid factor. Since the method relies on the light chain profile which is typically not post-translationally modified, the peaks on high resolution mass spectrometers are distinct (Figure 3).

In summary, the finding of restricted band on SPEP/IFX or presence of abnormal FLC studies does not always correspond to a monoclonal protein and can be falsely positive in the presence of confounding factors. The latter should be suspected when bone marrow biopsy does not show monoclonal plasma cells. We are in the final stages of validating the miRAMM for routine clinical use.[11] Therefore, miRAMM, a mass spectrometry based on the molecular mass of the monoclonal protein, is more accurate compared to SPEP/IFX and can be confirmatory in these types of cases.

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doi:10.3324/haematol.2017.171694

Information on authorship, contributions, and financial & other disclosures was provided by the authors and is available with the online version of this article at www.haematologica.org.

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