Challenges With Serum Protein Electrophoresis in Assessing Progression and Clinical Response in Patients With Waldenström Macroglobulinemia

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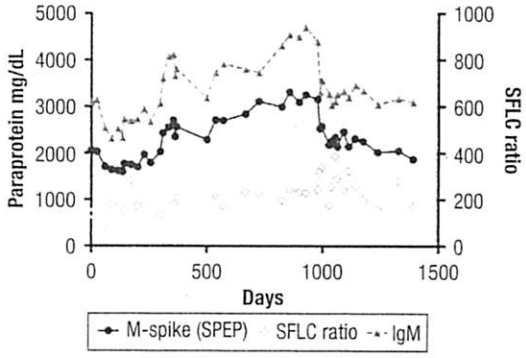
Abstract

Accurate determination of the immunoglobulin (Ig) M paraprotein concentration is crucial to evaluating response in patients with Waldenström macroglobulinemia (WM). In most clinical laboratories, M-spike quantitation is performed by serum protein electrophoresis, which is the same method used to quantitate IgG and IgA paraproteins in patients with multiple myeloma (MM). However, the migration pattern and propensity of IgM paraproteins to form higher-order complexes in serum makes laboratory evaluation of samples from patients with WM especially challenging. We review examples of patients whose IgM paraprotein is particularly ill-suited to M-spike quantitation by serum protein electrophoresis: a case of "sticky M," a case of IgM multimers that cannot be resolved, and a case of an IgM in the β region. In these and similar cases, a method other than M-spike quantitation, such as IgM heavy chain nephelometry, should be considered in laboratory evaluation of paraprotein concentration.

Introduction

Paraprotein-based methods for evaluating tumor burden in Waldenström macroglobulinemia (WM) are an important part of assessing response and progression over time.[1] For a population of isogenetic tumor cells, changes in the paraprotein level reflect changes in the total number of tumor cells throughout the body and thus are excellent markers of disease burden. Quantitation of specific serum paraproteins is routine in a clinical laboratory, yet no single method of paraprotein measurement has been endorsed for assessment of disease progression and response in WM or multiple myeloma (MM).[1,2] This is in part because, unlike other protein biomarkers of disease, each patient's paraprotein is different and may exhibit clone-specific behaviors.

Figure 1 Measurements of Immunoglobulin M Kappa (IgMκ) Paraprotein Concentration in a Single Patient Over a Period of Years. M-spike (black circles) and IgM (gray triangles) are in mg/dL. The Ratio of the Involved to Uninvolved Light Chain (κ/λ ratio) Is Plotted on the Right Axis (open gray circles)



Current standard laboratory methods for the quantitation of IgMκ and IgMλ paraproteins include electrophoresis and nephelometric or tubidimetric assays. In the nephelometric assay for IgM, antibodies against IgM are added to a patient's sample, and the resulting light scatter from the protein-protein complexes is proportional to the concentration of IgM. The obvious advantage of nephelometric assays is that they can be easily automated. Disadvantages include the inability to distinguish between background IgM and disease-associated IgM, as well as variable reactivity with different paraprotein clones. In addition, these assays are vulnerable to overestimation of the paraprotein, particularly in the case of IgM paraproteins,[3] a phenomenon that we have also seen in our laboratory (Figure 1). Errors in these measurements are greater in patients with very low or very high serum IgM levels.[4] The serum free light assay is

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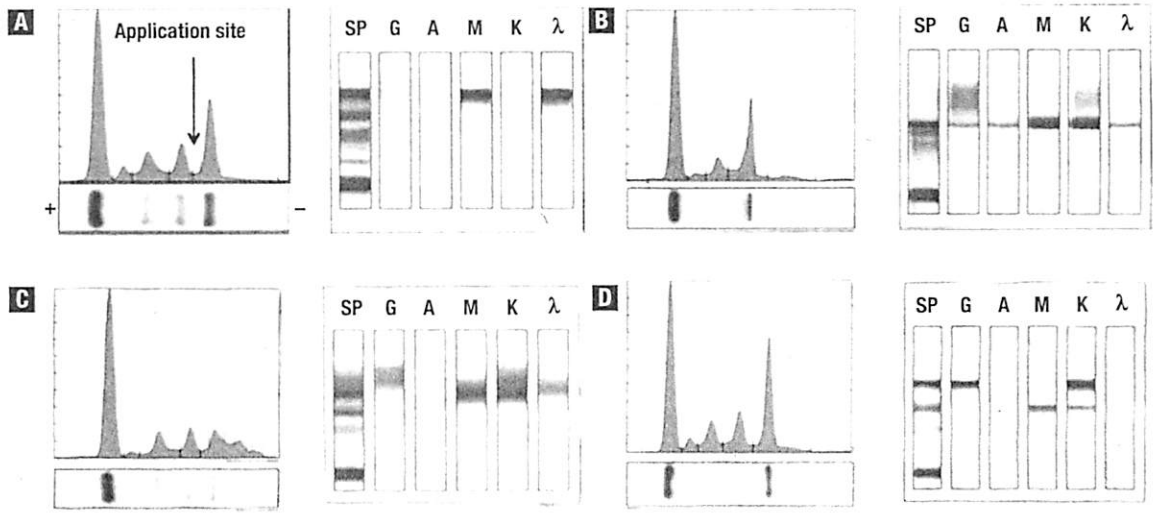
Figure 2 Four Examples of Immunoglobulin (Ig) M Paraprotein Electropherograms With Corresponding Protein Stain (left) and Immunofixation Gels (right). (A) IgMλ in the γ Region With a Straightforward Quantitation. Position of Anode (+) and Cathode (−) Are Shown. (B) "Sticky M" IgMκ Sample Showing Precipitation in Application Site and Atypical Peak Shape on Electropherogram. (C) IgMκ Paraprotein Running as 3 Peaks, Most Likely Corresponding to Higher Order Complexes. (D) A Case of 2 Paraproteins in a Single Sample, in Which IgMκ Paraprotein Is Masked by Background in the β Region, Whereas IgGκ Paraprotein Has a Straightforward Quantitation. This May Be a Case of Biclonal Gammopathy or of Class Switching



a turbidimetric assay for serum free light chains that has proved especially valuable in hyposecretory MM.[5,6] However, the value added by serial serum free light assay measurements for patients with intact immunoglobulins on serum electrophoresis (SPEP) remains controversial.[7,8] A new assay, HeavyLite (The Binding Site, San Diego, CA) is under investigation in patients with WM.[9]

In SPEP, serum proteins are separated according to their size and net charge. Most immunoglobulins are in the gamma region. An electropherogram is generated by optical scanning (Figure 2A, right) and the area under the curve represents 100% of total serum protein. The quantitation is made when a pathologist or trained technologist manually delineates the boundary of the M-spike. The heavy and light chain class is determined by an immunofixation gel that is included as part of a diagnostic workup (Figure 2A, left). SPEP is more labor intensive than nephelometric methods but has the advantage of separating paraprotein from background IgM.

Fortunately, many clinicians who treat patients with WM are generally aware of the limitations of IgM nephelometry and of SPEP and immunofixation studies. This awareness, as well as the observation that different WM paraproteins behave differently in vivo, has led to general agreement that the absolute value of the M-spike is less important than the direction and magnitude of the change in response to treatment.[1] Remarkably, despite their limitations, the values from heavy chain nephelometry and SPEP tend to rise and fall in tandem over time. A typical pattern is shown in Figure 1, in which the serum free light assay results are also depicted.

Patients and Methods

To address potential limitations of IgM M-spike determination, we show specific cases in which quantitation of the IgM M-spike is problematic when using SPEP. In our experience, IgM paraproteins are more likely to exhibit problematic behaviors than IgG paraproteins. Therefore, awareness of the limitations of M-spike quantitation is of particular importance to clinicians who treat of patients with WM. Our clinical laboratory uses the SPIFE 3000 system (Helena Laboratories, Beaumont, TX), and the examples here are performed in accordance with the manufacturer's recommendations in the Clinical Laboratories Improvement Amendments (CLIA)-certified clinical laboratories of the Brigham and Women's Hospital (Boston, MA).

Results and Discussion

The Sticky M

The tendency of IgM paraproteins to form aggregates is a problem both in vivo and in the laboratory. Protein aggregates that are insoluble do not enter the resolving gel. Rather, they precipitate in the loading site (Figure 2B). Such precipitation has been reported in a case of an IgM paraprotein that produced a type I cryoglobulinemia in a patient with WM,[10] but we frequently see it in IgM samples not associated with cryoglobulinemia. In addition to complicating quantitation, precipitation in the immunofixation gel can complicate determination of the class of a paraprotein (Figure 2B). Not surprisingly, this behavior appears to be dependent on both the identity of the paraprotein and the concentration, which shows sample-to-sam-

ple variation even in the same patient. Sometimes a reducing agent, such as β-mercaptoethanol, can be added to the sample to help break up the aggregates and coax the protein into the gel, as has been reported in capillary electrophoresis systems.[11] However, routine addition of reducing agent is not practical in most clinical laboratories.

IgM Multimers

IgM paraproteins can form pentamers or other higher order complexes that are soluble but create multiple overlapping peaks (Figure 2C). It is reasonable to quantitate each spike individually and to report 3 values, but it is also reasonable to quantitate the triplet as a single, unresolvable peak. Indeed, it would also be reasonable to quantitate only the peak that can be easily distinguished from background. Each of these approaches gives a different value. The addition of β-mercaptoethanol to the sample can consolidate the peaks into a single peak, presumably by reducing the protein to a monomeric state. Unfortunately, although the M-spike concentration may be more accurate in a reduced sample, flagging all future samples from the same patient for reductant addition is not practical in most clinical laboratories.

The β M-Spike

Most intact immunoglobulins run in the γ region, a region with very little background from other serum proteins (Figure 2A). IgA and IgM paraproteins are more likely than IgG to run in the β region of the gel, which contains normal serum proteins that complicate quantitation. An example of an IgM paraprotein obscured by background is shown in Figure 2D. For internal comparison, the IgG paraprotein in this sample is much easier to quantitate. If only the IgG M-spike is reported, then the tumor burden may appear to increase over time, when it is more likely a case of class switching or a biclonal gammopathy. Conversely, there is no reliable way to quantitate the M-spike in the β region.

Conclusion

Although the M-spike by SPEP provides a reliable quantitation for most paraproteins, it is vulnerable to clone-specific behaviors that affect protein stability and aggregation. IgM paraproteins are more likely to demonstrate variation due to these problematic behaviors. Clinical laboratories, therefore, should strive for consistency in interpreting difficult cases so that serial measurements have meaning. Moreover, in cases of WM, a method other than M-spike quantitation should also be considered in laboratory evaluation of paraprotein concentration.

Disclosure

The authors have stated that they have no conflicts of interest.

References

1. Owen RG, Kyle RA, Stone MJ, et al. Response assessment in Waldenstrom macroglobulinaemia: update from the VIth International Workshop. *Br J Haematol* 2013; 160:171-6.
2. Rajkumar SV, Harousseau JL, Durie B, et al. Consensus recommendations for the uniform reporting of clinical trials: report of the international myeloma workshop consensus Panel. *Blood* 2011; 1:117, 4691-5.
3. Murray DL, Ryu E, Snyder MR, et al. Quantitation of serum monoclonal proteins: relationship between agarose gel electrophoresis and immunonephelometry. *Clin Chem* 2009; 55:1523-9.
4. Tripsas CK, Patterson CJ, Uljon S, et al. Comparative response assessment by serum IgM M-protein and total serum IgM following treatment of patients with Waldenström's macroglobulinemia. *Clin Lymphoma Myeloma Leuk* 2013; 13:250-2.
5. Bradwell AR, Carr-Smith HD, Mead GP, et al. Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. *Clin Chem* 2001; 47:673-80.
6. Drayson M, Tang LX, Drew R, et al. Serum free light-chain measurements for identifying and monitoring patients with nonsecretory multiple myeloma. *Blood* 2001; 97:2900-2.
7. Mori S, Crawford BS, Roddy JV, et al. Serum free light chains in myeloma patients with an intact M protein by immunofixation: potential roles for response assessment and prognosis during induction therapy with novel agents. *Hematol Oncol* 2012; 30:156-62.
8. Uljon SN, Richardson PG, Schur PH, et al. Serial serum free light chain measurements do not detect changes in disease status earlier than electrophoretic M-spike measurements in patients with intact immunoglobulin myeloma. *Clin Chim Acta* 2011; 412:562-8.
9. Leleu X, Koulieris E, Maltezas D, et al. Novel M-component based biomarkers in Waldenstrom's macroglobulinemia. *Clin Lymphoma Myeloma Leuk* 2011; 11: 164-7.
10. Pattenden RJ, Davidson KL, Wenham PR. The value of serum free light chains in a case of Waldenstrom's macroglobulinaemia that produces a type I cryoglobulinaemia. *Ann Clin Biochem* 2009; 46:531-2.
11. Keren DF, Gulbranson R, Carey JL, et al. 2-mercaptoethanol treatment improves measurement of an IgMkappa M-protein by capillary electrophoresis. *Clin Chem* 2001; 47:1326-7.