

Heavy/Light-Chain Analysis of Monoclonal Gammopathies

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Monoclonal gammopathies (M proteins) are associated with an extraordinarily broad array of clinical conditions that range from monoclonal gammopathy of undetermined significance (MGUS)² to multiple myeloma. Three basic goals of the methods used to investigate M proteins are detection, immunochemical characterization, and quantification. Serum protein electrophoresis (SPE) techniques have undergone considerable improvements in the past few decades and now provide crisp resolution (especially in the β region) to detect many subtle electrophoretic differences in M proteins not readily distinguished by older 5-band gel techniques. Immunofixation (IFE) has replaced immunoelectrophoresis to become the gold standard for detecting an M protein and characterizing its isotype.

Despite these advances, however, problems in detecting and especially in quantifying M proteins persist. By SPE, M proteins can migrate anywhere from the α to the γ region. They can be obscured by large proteins, such as haptoglobin in the α_2 region or more commonly by transferrin and complement component 3 (C3) in the β region (1). Occasionally, IgM M proteins self-aggregate, creating problems with detection and measurement. Although most cases of multiple myeloma feature relatively large M protein peaks that are readily detected, even with low-resolution 5-band methods, 15%–20% of patients with multiple myeloma produce only free immunoglobulin light chains that, because of their relatively small molecular weights, are rapidly cleared into the urine, often producing no detectable M protein spike after SPE. Indeed, many cases of monoclonal free light chains (Bence Jones protein) cannot be identified by IFE of serum; however, IFE of concentrated early morning void (or 24-h urine) remains a reliable means to detect such small molecular weight M proteins. Unfortunately, urine samples too often are not submitted along with serum samples for the initial evaluation.

With the development by Bradwell et al. in 2001 of highly specific assays for free light chains (FLCs) in the serum, the latter problem is disappearing (2). In addition to showing that an initial urine sample to rule out the presence of monoclonal FLCs is unnecessary, a large number of studies have demonstrated these anti-FLC reagents to be of value for the initial assessment of prognosis for MGUS and multiple myeloma, detecting and following the monoclonal FLCs in patients with amyloid, detecting and following response to therapy in many cases of nonsecretory (oligosecretory) multiple myeloma, and detecting M proteins in patients with B-cell lymphoproliferative disorders (3–7).

In this issue of *Clinical Chemistry*, Bradwell et al. (8) detail their development and deployment of reagent antisera with specificity for unique epitopes that form at the junction between the heavy- and light-chain constant regions of each immunoglobulin molecule. Although some cross-reactivity remained in their current batches of specific anti-heavy/light-chain (HLC) reagents, the antisera created were specific enough for IgG κ , IgG λ , IgA κ , IgA λ , IgM κ , and IgM λ to establish a ratio for each HLC class in healthy blood bank donor sera and to demonstrate a potential use for these reagents in detecting and measuring the HLC molecules and their ratios in serum samples from patients with multiple myeloma.

In comparing the sensitivity of HLC assays with the sensitivities of SPE and IFE, Bradwell et al. performed dilution studies on 9 sera containing M proteins. The end point for detection by electrophoresis was visualization by 3 experienced observers of a relatively low-resolution (5-band pattern) gel vs HLC results falling outside of the 95% CI. Under these conditions, HLC assays had a slightly lower analytical ability to detect IgG M proteins than SPE but had an equal or greater sensitivity for IgA and IgM M proteins (particularly those that comigrated with proteins in the β region of their gel). Although, the authors reported no comparison dilution studies with IFE alone for sensitivity of detection, IFE and HLC results were concordant for 31 of the 40 SPE-positive or suspicious samples. A “minor” IgA λ by IFE was called an IgG κ by HLC assay, and Bradwell et al. suggested that this case might have reflected “IgG subclass dysregulation.” IFE detected 2 “minor” IgG bands that were not detected by HLC assay, and 4 samples with abnormal HLC results did not have M proteins according to IFE. The

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² Nonstandard abbreviations: MGUS, monoclonal gammopathy of undetermined significance; SPE, serum protein electrophoresis; IFE, immunofixation; C3, complement component 3; FLC, free light chain; HLC, heavy/light-chain.

latter result was not particularly surprising because 1 of 20 nonaffected samples will fall outside of a designated 95% CI by chance. One case of a biclonal IgM λ and IgM κ according to IFE was interpreted only as IgM λ by HLC assay, and one case of FLC M protein according to IFE was correctly negative by HLC assay (and positive for an increased serum FLC ratio).

Bradwell et al. provide convincing evidence that use of anti-HLC reagents could help with the vexing issue of detecting and providing reproducible measurements of M proteins that comigrate with other major serum components, such as transferrin or C3 (8). Although IFE is the current gold standard for detecting and immunochemically characterizing M proteins, many laboratories do not perform IFE as part of their routine SPE screening. Some workers have suggested adding an IFE test when subtle abnormalities are apparent in the β region on an SPE gel or when the fractions of the β_1 or β_2 region are increased without an obvious explanation (such as a β - γ bridging pattern, seen in cirrhosis) (9). Unfortunately, IFE is not a quantitative technique. Currently, the M protein spike is measured by densitometric scanning or by delineating the M protein with capillary electropherograms. In such measurements, there is always a component of the measurement that contains polyclonal antibodies. In addition, as noted above, when a relatively small M protein overlays a typical serum component, it can be extremely difficult to obtain a precise measurement with these techniques. Direct measurement of the HLC fractions involved gives a ratio that may fare as well as the free κ/λ ratio in providing useful information, provides greater sensitivity than SPE alone, and renders a value that should be reproducible from one laboratory to another (8).

Along these lines, Bradwell and colleagues propose that use of the HLC ratio could provide a sensitive means to follow the course of a neoplasm and its response to therapy (8). They reason that the M protein type will be similarly affected by changes in blood volume and the half-life of the affected heavy-chain class. Therefore, the HLC ratio will theoretically provide more dynamic information than measurement of total M protein alone. Although these investigators suggest that similar success has been obtained with free κ/λ ratios in serum, the most recently reported International Myeloma Working Group guidelines do not specifically endorse the use of the FLC assay in place of protein electrophoresis of 24-h urine samples for monitoring patients with plasma cell disorders or for following patients who have measurable disease by SPE or urine protein electrophoresis (3). Yet these guidelines may evolve with time as these issues are evaluated in prospective studies that compare the power of the HLC ratio with protein electrophoresis of good-quality res-

olution. I suspect that the unique advantage of SPE to document the precise electrophoretic position of the M protein may remain valuable for following patients who receive stem cell transplantation therapy. Oligoclonal bands often appear after chemotherapy and transplantation (9–11). It may be helpful to have information about the migration position of the M protein to help distinguish between a recurrence of the original M protein and oligoclonal bands of the same HLC type, but with a clearly different electrophoretic migration.

HLC assay is not a replacement for the highly sensitive and flexible IFE technique. IFE's strength is that the technique exhibits no interference caused by comigration of M proteins with serum components and readily detects biclonal gammopathies, IgD and IgE M proteins, and γ , α , and μ heavy-chain diseases. The utility of the HLC technique will benefit from further evaluations and perhaps some tweaking of the cutoffs used to demonstrate its utility in detecting and measuring M proteins under typical clinical laboratory conditions. Under these circumstances, many M proteins will fall into the MGUS end of the clinical spectrum with a relatively small quantity of M protein (typically IgG). With the 95% CI, furthermore, some cases characterized by small quantities of M protein may be difficult to distinguish from a maze of nonpathologic sera with borderline results or from polyclonal increases in immunoglobulins, which are known to produce relative increases in the free κ/λ ratio (12). The HLC assay is an important addition to our armamentarium for detecting and measuring M proteins. It brings a reproducible, quantifiable number to the evaluation of M proteins, together with the ability to view the M protein HLC type.

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