Detection and Characterization of Monoclonal Components in Serum and Urine

Detection of a monoclonal component (the immunoglobulin product of a clone of plasma cells) in the serum and/or urine is an important part of the laboratory evaluation of a patient with a plasma cell or B-lymphocyte proliferative disease. Usually, large quantities of monoclonal components are present in the serum of patients with multiple myeloma or Waldenström’s macroglobulinemia. Small quantities of monoclonal components in serum may signify light chain disease, a B-lymphocytic lymphoma or leukemia, amyloid AL (amyloidosis associated with immunoglobulin light chains), a neuropathy, or the predisposition to develop a malignant plasma cell/B-lymphocyte proliferative disorder in a patient with no currently evident clinical problem (monoclonal gammopathy of undetermined significance–MGUS). About 25% of individuals who fall into the MGUS category develop myeloma or a related B-lymphocyte proliferative disorder after 20 to 35 years of follow-up (1). Because of this, annual follow-up is prudent, even for patients with relatively small monoclonal components in their serum.

For the past half-century, protein electrophoresis using either cellulose acetate or agarose gels has been the mainstay in screening serum and urine for monoclonal components. Most of the currently used electrophoretic techniques require a considerable amount of manual labor. Thus, although the reagents are relatively inexpensive, the time involved in applying the samples, fixing, staining, and washing the gels, and performing the densitometric scans of the gels make electrophoresis rather costly. Furthermore, there is considerable variation in the resolution of the major protein fractions from one manufacturer’s product to another. Some relatively simple and inexpensive techniques allow one to recognize only five bands that often have fuzzy borders. Those techniques make it difficult to detect small monoclonal components or those that migrate in the beta region, where transferrin, C3, and beta-lipoprotein may obscure a subtle monoclonal band. Not surprisingly, techniques that provide a high-resolution electrophoretic pattern with crisp resolution of the beta region bands are more complex and costly than the five-band techniques. The degree of resolution to optimize detection of quantitatively small monoclonal components or those with migration in the alpha, beta, or extreme slow gamma regions remains controversial. Objective data on the issue of resolution and detection of monoclonal components is sparse. However, some quality-assurance survey results suggest that gels capable of providing a distinct separation of transferrin (beta 1 region) from C3 (beta 2 region) may facilitate detection of subtle monoclonal components (2).

Capillary electrophoresis (CE) has been reported to improve the resolution of serum protein bands, whereas automation permits processing of up to 50 sera per hour with minimal labor (3). With CE, the separation of protein fractions depends on the charge differences of the proteins. Serum is injected into narrow fused-silica capillary tubes, where the negative charge on these walls and the buffer conditions result in a strong endosmotic flow that pulls serum proteins toward the cathode, with gamma globulins leading the way. Peptide bonds are measured by their absorbance of ultraviolet light (~200 nm) at the cathodal end of the capillary. Ten serum proteins may be recognized in the electropherograms that closely resemble densitometric tracings. Some systems provide software that convert electropherogram data to virtual gel images on a video monitor. Resolution provided by the electropherograms and virtual gel images obtained from CE rival some of the better commercially available high-resolution gel electrophoretic systems.

Identification of the heavy and light chain type of monoclonal components detected by CE may be achieved by immunosubtraction (IS-CE). IS-CE is performed by preincubating the patient’s serum with Sepharose beads coated with antibodies against specific heavy or light chains. All immunoglobulins that contain, for instance, a kappa light chain would bind to the Sepharose beads coated with anti-kappa reagent. When that “immunosubtracted” serum is reassayed on the CE, a spike due to an IgGk monoclonal component will no longer be detected.

In this issue of Clinical Chemistry, Henskens et al. (4) compare the ability of their high-resolution agarose and immunofixation (IFE) technique with that of the P/ACE System 5000 CE technique to detect and identify 74 monoclonal proteins. The quantities of the monoclonal components they identified varied widely, from 0.6 g/L to 50.9 g/L. Some of the smaller monoclonal components detected by CE could not be measured by the available software. They were able to detect 70 of the 74 monoclonal components by CE and 73 of the 74 by their agarose technique. Overall, there was good agreement in detection and identification of the monoclonal components by the two techniques. The one monoclonal gammopathy that eluded their agarose technique migrated in the beta region, where the better resolution provided by CE is credited, by the authors, with allowing detection of this quantitatively small (presumably–no quantitative information is provided) beta-migrating IgA lambda molecule.

The four monoclonal components missed by the CE technique were quite varied in both their quantity and isotypes. The quantity of monoclonal component in one of these four cases was quite small, 0.6 g/L, two were somewhat larger, 1.1 and 2.2 g/L, but one was impressively large at 20.3 g/L. Although the latter monoclonal protein had a rather cathodal migration on the agarose gel, it was obvious by that method. The authors note that by changing the ionic strength or the pH of their buffer, they were able to detect the large component on CE. We are not told if these changes affected detection of the other three missed monoclonal components. Problems detecting monoclonal components in the cathodal end of the...
gamma region of CE electropherograms have been reported previously \((5)\). Similarly, others have shown that use of a second buffer may improve detection of monoclonal components in CE systems \((5)\). Indeed, Henskens et al. suggest that use of more than one buffer condition in CE systems may be needed to improve detection of patients with monoclonal components.

There was good overall agreement in the characterization of the monoclonal components by IFE and IS-CE. Inexplicably, one IgM monoclonal component was not typable by IS-CE. Of course, the four monoclonal components that were missed by CE could not be typed by IS-CE. This points out one weakness of IS-CE compared with IFE. When a monoclonal component is present in small concentration but not readily detectable on the electrophoretic screen (perhaps obscured by other bands in the beta region), it can be detected by IFE because that technique is inherently more sensitive than the electrophoretic screen. Monoclonal components that occur in some forms of neuropathy often are present in relatively small quantities that require the improved sensitivity of IFE for detection \((5)\). For IFE, following electrophoresis, the serum is reacted with specific antibodies against IgG, IgA, IgM, \(\kappa\) or \(\lambda\) (and uncommonly IgD or IgE). By virtue of the additional protein provided by the reagent antisera and the clear background after washing an IFE gel, even tiny monoclonal components are readily detected. In contrast, if one does not observe a band in the electropherogram, one lacks the requisite frame of reference to "subtract" that band by use of the reagent antisera. Indeed, Henskens et al. note that interpretation of small IgG monoclonal components on a polyclonal background was more difficult using IS-CE than on the standard IFE technique.

Thus, although CE provides a high-resolution technique that is readily automated, it remains to be seen if it is a superior methodology for detecting monoclonal components when compared with currently available high-resolution gel-based systems. Similarly, IS-CE can be automated and seems to be a reliable method to identify obvious monoclonal components in serum, but it is less sensitive and less flexible than gel-based IFE.

Urine is the other major fluid studied for the presence of monoclonal components, usually free monoclonal light chains (Bence Jones proteins—BJPs). The techniques used to screen urine for BJPs and to examine the pattern of proteinuria as an estimate of the location of leakage within the nephron are even less standardized than those of serum electrophoresis. Most of us have long since abandoned using the peculiar thermal characteristics of BJPs to precipitate when heated to \(~56^\circ\text{C}\) and to redissolve upon boiling. The sulfosalicylic acid (SSA) precipitation test is an insensitive screen. When positive, it requires characterization by electrophoretic study; when negative, it is too insensitive to rule out a BJP. Electrophoresis on concentrated urine and immunologic characterization by IFE or immunoelectrophoresis are the most sensitive methods to detect BJPs. Either a 24-h urine sample or an early morning void provide the preferred urine specimens for evaluation \((7)\). Urine is concentrated before it is studied by electrophoresis; however, neither the amount nor the method of concentration is standardized. Many laboratories use ultrafiltration concentrators that provide a concentration from 10- to 100-fold. Electrophoresis of the concentrated urine then stained with one of many standard reagents (Ponceau S, Amido Black, Coomassie Blue, or others) is useful to estimate both the location of protein leakage in the nephron and to detect rather large quantities of BJPs. For optimal sensitivity, however, certainly when there is clinical suspicion of light chain disease, an immunologic technique such as IFE is needed \((8)\).

In this issue of \textit{Clinical Chemistry}, Le Bricon et al. \((9)\) compare the ability of standard agarose gel electrophoresis on concentrated urine (25-fold) with that of sodium dodecyl sulfate-agarose gel electrophoresis (SDS-AGE) on unconcentrated urine to detect BJPs and the pattern of leakage within the nephron. SDS-AGE has the technical advantage of dispensing with the requirement for a concentration step. By providing separation of the urinary proteins by molecular weight, SDS-AGE facilitates the interpretation of the pattern of proteinuria, as compared with standard agarose electrophoresis. One potential disadvantage of SDS-AGE is that one cannot distinguish monoclonal from polyclonal free light chains because they have the same molecular weight. By standard agarose technique, this distinction is readily made because the monoclonal free light chains have a unique pI, producing a spike, whereas polyclonal free light chains have a diversity of pI that produces a diffuse staining pattern. This disadvantage is mitigated somewhat by comparing the relative amounts of the light chains present at 25 kDa to the amounts and molecular weights of the other proteins in the urine. By performing this comparison, Le Bricon et al. detected more true positive BJPs with their SDS-AGE screen on unconcentrated urine than with the 25-fold concentrated urine with their routine agarose screen. The few false positives are not a significant concern because they need to be confirmed by IFE. To quantify the BJPs by SDS-AGE, one needs to perform an additional electrophoresis on samples treated with \(\beta\)-mercaptoethanol to combine the BJP monomers and dimers.

SDS-AGE has some clear advantages for determining the site of leakage within the nephron and seems more sensitive than the routine agarose electrophoresis screen. Yet, there are a few issues that need to be kept in mind when selecting a strategy for BJP detection. First, neither SDS-AGE nor routine agarose using 25-fold urine are as sensitive a screen for BJPs as IFE. In our laboratory, we perform both electrophoresis and IFE for \(\kappa\) and \(\lambda\) chains on all samples submitted for BJP evaluation. If the IFE is positive, it is referred to an IFE that excludes intact monoclonal proteins. Although only one case of BJP was missed by SDS-AGE in the Le Bricon et al. study, it would have been detected with a strategy that employed IFE for the screen. Second, the patterns of SDS-AGE shown seem to have a considerable variation in the migration of light chain monomers even on the same gel. How much
variation is seen from gel to gel and lot to lot? Third, the performance of SDS-AGE is more time consuming than routine AGE. The additional requirement for a second sample treated with β-mercaptoethanol may be a bit more than many routine clinical laboratories will be willing to undertake—especially those that routinely perform IFE as part of the routine screen for BJPs.

SDS-AGE is a promising technique that allows the clinical laboratory to identify the molecular weight of proteins found in the urine. It may provide a screen for BJPs superior to routine electrophoresis on concentrated urine that we may wish to adopt, but for the time being, our laboratory will continue performing IFE for κ and λ as part of our screen for BJPs.

References


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