The Incidence and Significance of Pseudoparaproteins in a Community Hospital

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Abstract. Pseudoparaproteins were observed in 129 (10.5%) of 1,229 high resolution protein electrophoretic fractionations of serum (N = 847), urine (N = 368), or cerebrospinal fluid (N = 14) performed in this laboratory during a 12-month period. The pseudoparaproteins identified in serum electrophoretic patterns included fibrinogen, C-reactive protein, hemoglobin-haptoglobin complex, elevated beta-globulins (transferrin and C3), lysozyme (muramidase), and an extended migration artifact. In the electrophoretic patterns of urine, the pseudoparaproteins consisted of nonspecific gamma zone bands of varying intensity. Gamma zone trace protein in the cerebrospinal fluid was often of sufficient intensity to cause potential confusion with oligoclonal bands. Awareness of the characteristic electrophoretic migration positions of these pseudoparaproteins helps to avoid unnecessary ancillary testing and expense.

Keywords: Pseudoparaproteins, monoclonal protein, protein electrophoresis, immunofixation electrophoresis

Introduction

High resolution protein electrophoresis (PE) is the primary laboratory screening test for detecting monoclonal paraproteins (M-proteins) in serum [1-3]. Suspected M-proteins are then confirmed with a more specific test, such as immunofixation electrophoresis (IFE). In some settings, however, normally occurring proteins may mimic M-proteins in PE patterns. These pseudoparaproteins (P-proteins) have characteristic migration positions and appearances that permit their differentiation from M-proteins in most cases [4], obviating the need for further laboratory or clinical evaluations.

Commonly identified serum P-proteins include: fibrinogen, C-reactive protein, hemoglobin-haptoglobin complex, transferrin, C3, lysozyme (muramidase), and an extended migration artifact. Their presence in PE patterns may imply elevated concentrations in the serum, often corresponding to significant disease states. Characterization of these proteins may, therefore, have intrinsic clinical utility.

Urine and cerebrospinal fluid (CSF) P-proteins generally denote technical artifacts, owing to the increased concentrations of the samples used for routine PE. Nonspecific bands occur in the gamma region of some urine PE patterns, which may represent polymerized immunoglobulin light chains, urinary tract proteins, or bacterial contaminants. In CSF, gamma trace protein is frequently evident in the mid-gamma region as two faint bands, and must be distinguished from the clinically significant oligoclonal bands.

Awareness of these P-proteins in various body fluid PE patterns is mandatory for accurate interpretation. A systematic comparison of each protein fraction in patient samples with a known control provides the most effective strategy for the detection of these often subtle aberrations. Their incidences may vary among healthcare facilities, depending on the demographics of the patient population. The intent of this study was to identify and establish the incidences of P-proteins encountered in a large community hospital.
Methods and Materials

The Paragon SPE-II Electrophoresis System (Beckman Instruments, Inc., Fullerton, CA) was used for all PE and immunofixation electrophoreses (IFE). Serum samples were evaluated at standard dilutions according to the manufacturer's guidelines. Urine and CSF samples were concentrated according to the manufacturer's specifications prior to electrophoresis. The Beckman Appraise Densitometer was used to quantitate protein fractions. All PE and IFE patterns were interpreted by a clinical pathologist. IFE tests were performed on all samples with atypical patterns. Antisera used for IFE was initially tested against normal controls to ensure the absence of reactivity against normally occurring proteins. Identified M-proteins in excess of 0.5 g/dL were excluded from study, so that only small monoclonal gammopathies of undetermined significance (MGUS) were used for comparison.

Results

One thousand two hundred twenty-nine PE patterns (847 serum, 368 urine, 14 CSF) were reviewed for the presence of P-proteins (Figs. 1 and 2; Tables 1-3). The patient population consisted of 628 females and 601 males, with ages from 23 to 92 yr. The serum P-proteins that were identified included: fibrinogen, 25 cases (3%); extended migration (cathodal) artifact, 22 cases (2.6%); C-reactive protein, 17 cases (2%); elevated transferrin-complement (beta-globulins), 14 cases (1.7%); hemoglobin-haptoglobin complex, 5 cases (0.6%); and lysozyme, 1 case (0.1%). The elevated fibrinogen, C-reactive protein, transferrin, complement (C3), and lysozyme concentrations were confirmed by quantitative analyses. Faint, nonspecific gamma-zone bands were evident in 37 urine samples (10%). Gamma trace protein of sufficient intensity to cause potential confusion with oligoclonal bands was evident in 8

Fig. 1. High resolution agarose gel electrophoretic patterns of pseudoparaproteins (P-proteins). The migration position of each P-protein is designated by an arrow: (1) normal (control) serum protein electrophoretic pattern (beta-lipoprotein designated by an arrow), (2) fibrinogen, (3) C-reactive protein (note corresponding acute phase response), (4) increased beta-globulins (C3-A, transferrin-B), (5) hemoglobin-haptoglobin complex, (6) extended migration artifact or lysozyme, (7) non-specific gamma zone banding in urine, and (8) prominent gamma zone trace protein in cerebrospinal fluid.

Fig. 2. Free monoclonal lambda light chains in urine, seen as multiple gamma zone bands. The upper pattern (UPE = urine protein electrophoresis) shows several bands in the gamma region, which were identified as free monoclonal lambda light chains by immunofixation electrophoresis (lower pattern).
Table 1. Incidence of pseudoparaproteins (P-proteins) identified in 847 serum protein electrophoretic patterns

<table>
<thead>
<tr>
<th>Identity of P-protein</th>
<th>No of samples with P-protein</th>
<th>% of samples with P-protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen</td>
<td>25</td>
<td>3.0%</td>
</tr>
<tr>
<td>Extended migration artifact</td>
<td>22</td>
<td>2.6%</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>17</td>
<td>2.0%</td>
</tr>
<tr>
<td>Elevated beta globulins (transferrin-complement C3)</td>
<td>14</td>
<td>1.7%</td>
</tr>
<tr>
<td>Hemoglobin-haptoglobin complex</td>
<td>5</td>
<td>0.6%</td>
</tr>
<tr>
<td>Lysozyme (muramidase)</td>
<td>1</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

Table 2. Incidence of pseudoparaproteins (P-proteins) in protein electrophoretic patterns of 368 urine samples and 14 CSF samples

<table>
<thead>
<tr>
<th>Identity of P-protein</th>
<th>No of samples with P-protein</th>
<th>% of samples with P-protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonspecific gamma-zone bands(a)</td>
<td>37</td>
<td>10%</td>
</tr>
<tr>
<td>Gamma-zone trace protein(b)</td>
<td>8</td>
<td>57%</td>
</tr>
<tr>
<td>Oligoclonal bands(b)</td>
<td>5</td>
<td>36%</td>
</tr>
</tbody>
</table>

\(a\) urine samples only
\(b\) CSF samples only

Table 3. Samples with M-proteins identified with electrophoretic migration positions similar to P-proteins

<table>
<thead>
<tr>
<th>Identity of M-protein</th>
<th>Serum samples</th>
<th>Urine samples</th>
<th>CSF samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG-kappa</td>
<td>(2^a)</td>
<td>0</td>
<td>1(^c)</td>
</tr>
<tr>
<td>IgA-kappa</td>
<td>(2^b)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IgA-lambda</td>
<td>(1^b)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Free lambda</td>
<td>0</td>
<td>1(^d)</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\)corresponds to P-protein position of fibrinogen
\(^b\)corresponds to P-protein position of beta globulins
\(^c\)corresponds to P-protein position of gamma trace protein
\(^d\)corresponds to P-protein position of multiple gamma zone bands

(57%) of CSF PE patterns. Oligoclonal bands were identified in 5 (36%) of the CSF specimens.

The M-proteins (identified by IFE) that migrated in P-protein positions were: IgG-kappa, 2 cases, migrating in the fibrinogen position in serum samples; IgA kappa, 2 cases, and IgA-lambda, 1 case, migrating in the beta-globulin region in a serum sample; free lambda light chains mimicking nonspecific gamma banding in one urine sample (note, the corresponding serum sample was negative for monoclonal IgD and IgE); and IgG-kappa in the gamma trace region of a CSF sample from a patient with a corresponding serum IgG-kappa M-protein.

Discussion

Pseudoparaproteins (P-proteins), commonly encountered in body fluid PE patterns, can be misinterpreted as potentially clinically significant M-proteins. Fortunately, the characteristic migration positions and appearances of P-proteins in PE are generally sufficient to permit their identification and avoid unnecessary clinical workup and expense. Identification of these P-proteins is best achieved by a systematic interpretation scheme for PE of each body fluid type. Interpretation of densitometer scans alone is insufficient for detection of small P-proteins in many cases, and should be used only to quantitate protein fractions confirmed by visual inspection of the electrophoretic gel. Accurate visual interpretation is best achieved by comparing the location and intensity of specific bands in the alpha-1, alpha-2, beta, and gamma zones with a control serum PE pattern. This permits consistent identification of migration position and staining intensity aberrations, which are often subtle and may easily be overlooked if examined in isolation. Knowledge of the body fluid type directs the interpretation, as P-proteins differ in serum, urine, and CSF.

Fibrinogen was the most commonly identified serum P-protein in this study (Table 1). Electrophoretic migration position of fibrinogen was characteristic, but its intensity of staining varied with concentration. Fibrinogen bands in all cases were sharply defined and were identified in patients who had received anticoagulant therapy (particularly patients from cardiac care units). Visual inspection of many specimens revealed a tiny fibrin clot in the test
tube, which, when removed, eliminated the P-protein band on repeat PE. If a clot was not evident, addition of thrombin to the sample was used to elicit clot formation and indicate the presence of fibrinogen. Awareness of this common P-protein has prompted an increased intensity of sample inspection by our medical technologists, prior to initial electrophoresis, to identify and remove small clots, and thereby avoid the need for repeat PE or possible IFE.

Persistence of a band in the fibrinogen region of the PE, in the absence of a clot in the sample, is indicative of a M-protein with a similar migration position. In this study, two examples of minor IgG-kappa M-proteins (each less than 0.5 g/dL) were identified in the fibrinogen zone (Table 3). In one of the samples, coexistent fibrinogen was detected. IFE is therefore recommended for samples demonstrating a persistent band in the fibrinogen zone following removal or exclusion of fibrinogen (fibrin clot).

C-reactive protein migrates as a band in the mid-gamma zone, associated with acute phase response (decreased albumin, elevated alpha-1 and alpha-2 globulins, decreased transferrin, and increased complement). The C-reactive protein band exhibits varying intensity, depending upon its serum concentration. Low-normal or decreased serum immunoglobulin levels may accentuate its electrophoretic appearance. Faint staining intensity and slightly irregular contour are characteristic, which differ from the more sharply defined, uniform features of a true M-protein. Quantitative measurement of C-reactive protein is easily performed, and can provide assurance if any doubt exists about the identification of the band.

Serum free hemoglobin, when present, binds with haptoglobin, an alpha-2 globulin, to form a complex which shifts the migration of haptoglobin to the slow (cathodal) alpha-2 region or between the alpha-2 and beta-1 zones. This complex is usually observed with hemolyzed specimens due to traumatic venupuncture (iatrogenic hemolysis), but can also be observed with in vivo hemolytic conditions. Visual inspection of the specimen test tube will usually reveal gross evidence of hemolysis. In this setting, the collection of a non-hemolyzed specimen eliminates the P-protein in the PE. Persistence of a band in this region in a non-hemolyzed specimen requires IFE to exclude an M-protein.

Lysozyme (muramidase) migrates as a small band at the extreme cathodal (cationic) edge of the electrophoretic pattern. This protein is rarely present in sufficient quantity to be identified by electrophoresis, and its presence generally indicates an acute myelogenous leukemia with monocytic differentiation (French-American-British Classification type M4 or M5). When such a band is identified in PE, correlation with examination of the peripheral blood smear and quantitative measurement of lysozyme is indicated. Lysozyme may also be elevated in the sera of patients with tuberculosis or sarcoidosis, but the degree of elevation is generally insufficient to produce a detectable electrophoretic band.

A more common cause of a faint band with such extreme cathodal location is an electrophoretic sample that has been allowed to migrate in the gel beyond the recommended time interval (extended migration artifact). In this setting, the artifact is generally evident in most or all of the samples run on the same gel, and may be accentuated in samples with elevated polyclonal immunoglobulins. Repeating the PE with the proper time span eliminates this artifact.

It is important to recognize that M-proteins may also migrate in an extremely cathodal position. In contrast to the previously discussed migration artifact, these M-proteins occur as an isolated abnormality in an electrophoretic run containing several other patient samples. This underscores the importance of visual inspection of all PE patterns within a given sample run to differentiate technical artifacts from potential paraproteins.

Elevated concentrations of beta-globulins (transferrin and complement C3) may enhance the staining intensity of these normally occurring PE bands and simulate M-proteins. Transferrin can be significantly increased with iron deficiency, and complement C3 with an acute phase reaction. This change was observed in 14 (1.7%) of the samples in this study (Table 1). However, three small M-proteins (two IgA-kappa and one IgA-lambda) were also identified by IFE of specimens with this pattern (Table 3). These M-proteins were indistinguishable from elevated transferrin or C3 upon visual inspection of the agarose gel or densitometric pattern. Confirmation by IFE is therefore recommended for all PE fractionations that demonstrate this alteration.
Several additional PE bands that may be confused with serum M-proteins should also be noted:

1. Point of application artifact occurs as an extremely thin, discreet line in the application well which may appear distinct from surrounding polyclonal immunoglobulins. This artifact has the appearance of "being drawn on with a pen or pencil," and, as such, appears too thin for a true M-protein. The point of application artifact is relatively common, and should not routinely illicit any further evaluation.

2. Beta-lipoprotein is present in varying intensities in virtually all serum PE patterns. This protein exhibits a relatively wide electrophoretic mobility, and may be observed anywhere between the alpha-2 and beta-2 regions. The thin, irregular linear appearance of beta-lipoprotein in agarose gel is characteristic, regardless of its migration location. Its occasional comigration with alpha-2 globulins or transferrin, resulting in increased staining of the alpha-2 or beta-1 region, respectively, should be recognized.

3. Nephrotic syndrome produces significant elevations of alpha-2 globulins and transferrin (with corresponding reduction of albumin and gamma globulins), which may mimic M-proteins. Identification of this pattern and correlation with clinical history is generally diagnostic. However, if the reduction in gamma globulins is more than slight, correlation with serum and urine IFE to exclude M-protein/Bence-Jones proteinuria is warranted [5].

4. Gamma zone oligoclonal banding in serum PE is a P-protein pattern that was not observed in this series, but which may potentially be confused with paraproteinemia [6]. Etiologies for this pattern are non-specific, and include lymphoproliferative disease, viral syndromes, immune complex disease, hepatitis, HIV infection, and hemophilia associated with frequent infusions of factor VIII concentrate [7]. Transplant-related oligoclonal (and monoclonal) gammopathies have also been described [8-11]. Identification of this electrophoretic pattern must therefore be correlated with the clinical setting.

Two to four faint bands were seen in the gamma region of 37 of the urine PE patterns (Table 2). The number, distribution, and staining intensity of these bands varied between patients. IFE of these samples were negative for M-protein, although a non-specific pseudo-oligoclonal pattern (so-called "ladder light chain" pattern) [12], due to polyclonal free light chains in the urine, was frequently noted. Free gamma chain fragments, which may also occur in urine IFE, were not observed [13]. One example of Bence-Jones proteinuria was mistaken for this gamma banding pattern on the initial PE. IFE of the sample demonstrated a distinct, dominant free lambda M-protein (Fig. 2). Review of the original PE gel confirmed a distinct gamma band that was visibly different from the non-specific banding pattern observed in the other patients with negative IFE [14].

In CSF, prominent gamma trace protein [15,16] may create confusion with oligoclonal bands. With the Paragon SPE II System, gamma trace exists as two faint bands of variable intensity in the mid gamma region, corresponding to the CSF total protein concentration. True oligoclonal bands were always identified in a migration position cathodal to these gamma trace bands. As an adjunct to CSF electrophoresis, clinicians at our institution calculated the CSF IgG index, CSF albumin index, and CSF IgG synthesis rate [17,18] as part of the diagnostic evaluation for demyelinating diseases (eg, multiple sclerosis). In each of the samples with oligoclonal bands, these ancillary CSF IgG calculation studies were also positive, whereas the calculation results were normal for patients whose PE samples demonstrated gamma trace protein only.

M-protein (IgG-kappa) was identified in a single CSF sample (Table 3). Subsequent serum PE and IFE from this patient also confirmed monoclonal IgG-kappa. This finding emphasizes that the identification of an isolated abnormal band in CSF must be interpreted in conjunction with a corresponding serum PE. M-protein that is limited to the CSF is less common, and suggests primary CNS lymphoproliferative malignancy.

In the future, capillary electrophoresis (CE) may offer advantages over high resolution agarose gel PE for differentiating some P-proteins from M-proteins. In particular, CE provides improved resolution of the beta region, is more sensitive for the detection of small monoclonal light chains, and eliminates the point of application artifact [19]. CE is also reportedly less labor intensive and has the potential for automation. This technique is currently being evaluated in many laboratories.
Conclusions

The characteristic migration locations and appearances of P-proteins generally permit their identification in routine PE and preclude unnecessary further testing and expense. Care must be taken to exclude true M-proteins, which may coexist with or mimic P-proteins, particularly in the beta and gamma regions, corresponding to the migration positions of transferrin-C3 and fibrinogen, respectively. A systematic approach to the visual inspection of specimen samples and PE patterns is the most effective mechanism for consistent identification of these proteins.

Acknowledgments

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References