4.2 Detection and quantification of serum monoclonal proteins

4.2.1. Serum protein electrophoresis

Serum protein electrophoresis (SPE) is performed in agarose gels, resulting in the separation of serum proteins according to their size and charge and producing two major fractions: albumin and globulins. Albumin is the most abundant serum protein and forms a dense band close to the anode. The globulin fraction is subdivided into five regions, each containing several serum proteins. These are $\alpha_1$, $\alpha_2$, $\beta_1$, $\beta_2$, and $\gamma$, with the $\gamma$-region being closest to the cathode (Figure 4.1).

After proteins have been separated, they are fixed in the gel with an acid fixative and generally stained with Coomassie Brilliant Blue or Amido Black. Stained gels may be examined by eye and are often scanned with a densitometer to produce an electrophoretogram. This is a digital interpretation of the stained gel and is similar in appearance to electrophoretograms generated by capillary zone electrophoresis (CZE; Figure 4.2). Monoclonal proteins may appear as a peak in any region on the electrophoretogram, and may co-migrate with other serum protein bands. Serum monoclonal proteins are typed by sIFE (or immunosubtraction; Section 4.3), while the concentration of each band is determined by scanning densitometry of the SPE gel in combination with serum total protein measurements. Monoclonal protein quantification is a subjective technique, in which the operator demarks the edges of the peak (typically using a perpendicular drop). Measurements of monoclonal proteins that migrate in the $\gamma$-region may be overestimated when polyclonal immunoglobulins are present at the same position. IgA monoclonal proteins typically migrate in the $\beta$-region, and may also present difficulties for accurate quantitation. These issues and other challenges are further discussed in Sections 17.4 and 18.3.5.

Other features suggestive of a monoclonal gammopathy may be detected by SPE e.g. hypogammaglobulinaemia (identified by reduced staining in the $\gamma$-region). If a monoclonal gammopathy is suspected, further investigation is warranted, including serum immunofixation electrophoresis (sIFE; Section 4.3.1) [3][4].

4.2.2. Capillary zone electrophoresis

Capillary zone electrophoresis (CZE) offers an alternative to agarose gel electrophoresis for the detection and quantification of
serum proteins. Protein separation is performed in a liquid buffer system running through narrow-bore capillaries made of fused silica. The separated proteins pass an ultraviolet detector that measures absorbance at 200 to 215 nm to determine the protein concentration.

Electrophoretograms generated by CZE are similar in appearance to those produced by SPE, and can be used for monoclonal protein quantitation (in combination with serum total protein measurements) (Figure 4.2). Katzmann and colleagues [5] reported a good correlation between monoclonal protein concentration obtained by CZE and SPE for values <20 g/L, but above 20 g/L, values for CZE tended to be greater.

The main advantage of CZE over SPE is that it is an automated technique with faster throughput. In addition, most [5][6][7], but not all [8] reports conclude that capillary-based methods have slightly higher sensitivity than agarose gel-based electrophoresis (Table 4.1). The superior sensitivity of CZE over SPE has been shown to identify additional cases of monoclonal serum free light chains (sFLCs) and small monoclonal protein peaks in either the β-region or on a polyclonal background [5][6][7].

<table>
<thead>
<tr>
<th>Method</th>
<th>Analytical sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPE</td>
<td>~0.5 g/L</td>
</tr>
<tr>
<td>sIFE</td>
<td>0.1 g/L</td>
</tr>
<tr>
<td>UPE</td>
<td>20 mg/L</td>
</tr>
<tr>
<td>uIFE</td>
<td>3 - 5 mg/L</td>
</tr>
<tr>
<td>CZE</td>
<td>0.25 g/L</td>
</tr>
<tr>
<td>Total κ and λ</td>
<td>~4 g/L</td>
</tr>
<tr>
<td>sFLC</td>
<td>0.25 - 3 mg/L*</td>
</tr>
</tbody>
</table>

Table 4.1. Detection limits for various methods used to detect monoclonal immunoglobulins. Based on manufacturers’ information and published studies [5][6][8]. UPE: urine protein electrophoresis. * This is the analytical sensitivity of FLC measurement and not the limit for detection of monoclonal FLC, which is dependent upon the presence of an abnormal κ/λ sFLC ratio.

4.2.3. Diagnostic sensitivity of SPE and CZE compared with other laboratory techniques

The analytical sensitivity of SPE, CZE and other routine laboratory tests for monoclonal immunoglobulin detection is summarised in Table 4.1. In practice, the detection limit of electrophoretic techniques is dependent on a number of factors including: 1) the position of the monoclonal protein; 2) the level of polyclonal background immunoglobulins (in the γ-region); and 3) the width of the monoclonal protein peak [9]. Not all monoclonal proteins can be accurately quantified by SPE due to co-migration or dye saturation issues (Section 17.4).

When the diagnostic sensitivity of CZE and SPE is compared with that of sIFE, both methods fail to detect a small percentage of monoclonal proteins (Table 4.2). A high proportion of these samples are monoclonal IgA, IgM or sFLCs [8]. Such monoclonal proteins represent a diagnostic challenge as they may be small and co-migrate with other serum protein peaks, making detection and accurate quantification challenging (Section 17.4). In some cases, an abnormal κ/λ sFLC ratio may prompt sIFE to be performed, and reveal the presence of a hidden monoclonal intact immunoglobulin (Chapter 13) [10]. The diagnostic specificity of SPE and CZE are similar, although the reported values are variable between different studies (Table 4.2).
SPE & CZE Study Diagnostic sensitivity (%) Diagnostic specificity (%) Diagnostic sensitivity (%) Diagnostic specificity (%)

Bossuyt 1998[12] 86.0 Not reported 93.0 Not reported

Katzmann 1998[5] 90.7 98.9 94.9 98.6

Poisson 2012[6] 89.9 75.4 97.4 (or 92.3)* 57.6 (or 72.2)*

Yang 2007[8] 90.0 100 81 100

<table>
<thead>
<tr>
<th>Study</th>
<th>SPE</th>
<th>CZE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bossuyt 1998[12]</td>
<td>86.0</td>
<td>Not reported</td>
</tr>
<tr>
<td>Katzmann 1998[5]</td>
<td>90.7</td>
<td>98.9</td>
</tr>
<tr>
<td>Poisson 2012[6]</td>
<td>89.9</td>
<td>75.4</td>
</tr>
<tr>
<td>Yang 2007[8]</td>
<td>90.0</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 4.2. Comparison of the diagnostic sensitivity of SPE and CZE to detect monoclonal proteins identified by sIFE. *Values refer to Sebia CAPILLARYSTM-2 (or Helena V8TM) instruments.

4.2.4. Serum free light chain analysis

Freelite® sFLC assays are quantitative, latex-enhanced immunoassays that are performed on routine nephelometric or turbidimetric instruments (Chapters 5 and 37). Of all the methods used to detect monoclonal immunoglobulins, sFLC analysis has the highest analytical sensitivity for identifying monoclonal sFLCs (Table 4.1)[13][14][15].

The high clinical sensitivity of sFLC assays is dependent upon assessing the individual sFLC concentrations and the κ/λ sFLC ratio. Tumour suppression of the normal plasma cells in the bone marrow reduces the concentration of polyclonal uninvolved sFLCs, and thereby enhances the sensitivity of the κ/λ ratio.

Katzmann et al.[16] concluded that a combination of sFLC and SPE provided a simple and efficient initial diagnostic screen for the high-tumour-burden monoclonal gammopathies such as MM, Waldenström’s macroglobulinaemia (WM) and smouldering MM (SMM) (Chapter 23). International guidelines recommend that sFLC analysis in combination with SPE and sIFE is sufficient to screen for all pathological monoclonal plasmaproliferative disorders other than AL amyloidosis, which requires IFE of a 24-hour urine sample in addition to the serum tests (Chapter 25).

4.2.5. Hevylite immunoassays

Immunoglobulin heavy/light chain (Hevylite®, HLC) assays separately quantify the different light chain types of each immunoglobulin isotype (i.e. IgGκ, IgGλ, IgAκ, IgAλ, IgMκ and IgMλ, Chapter 9). HLC assays are performed on routine nephelometric or turbidimetric instruments (Chapters 9 and 38). The molecules are assessed in pairs to produce HLC ratios (e.g. IgGκ/IgGλ) in the same manner as κ/λ sFLC ratios. HLC assays provide an alternative tool to aid in the management of diseases associated with monoclonal intact immunoglobulins, including monoclonal gammopathy of undetermined significance (MGUS), SMM and intact immunoglobulin multiple myeloma (IIMM) (Chapters 13, 14, and 18).

HLC assays may overcome many of the known limitations of serum electrophoresis (including co-migration and dye saturation) and are less labour-intensive and less subjective. The sensitivity of the HLC ratio to detect monoclonal intact immunoglobulins is dependent upon the concentration of both the involved and uninvolved immunoglobulin HLC. In a study of 999 patients with MGUS, IgA and IgM HLC assays were generally as sensitive as SPE, whereas IgG HLC assays did not identify a monoclonal immunoglobulin in almost half of patients, presumably due to the lack of polyclonal uninvolved HLC suppression[5]. By contrast, Katzmann et al.[17] demonstrated that whilst IgG HLC assays had a similar sensitivity to SPE for the detection of IgG monoclonal proteins in sera from IgG MM patients (n=155, Table 4.3), the sensitivity of IgA HLC assays was greater than SPE and comparable to that of IFE for IgA monoclonal proteins in IgA MM patient sera (n=149, Table 4.4). The authors concluded that IgA HLC assays can substitute for the combination of SPE, IFE and total IgA quantification for monitoring β-migrating IgA monoclonal proteins.
Table 4.3. SPE and HLC ratio abnormalities in IgG MM. Data are n (%); * Monoclonal protein band or small, fuzzy band.

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>n</th>
<th>Abnormal SPE*</th>
<th>Abnormal IgGκ/IgGλ HLC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presentation</td>
<td>32</td>
<td>31 (97%)</td>
<td>30 (94%)</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>123</td>
<td>104 (85%)</td>
<td>106 (86%)</td>
</tr>
<tr>
<td>All</td>
<td>155</td>
<td>135 (87%)</td>
<td>136 (88%)</td>
</tr>
</tbody>
</table>

Table 4.4. SPE, HLC, IFE and total IgA abnormalities in IgA MM. Data are n (%); * >3.56 g/L; # n=111; † n=141.

<table>
<thead>
<tr>
<th>Serum sample</th>
<th>n</th>
<th>SPE monoclonal protein quantified</th>
<th>Abnormal IgAκ/IgAλ HLC ratio</th>
<th>Positive IFE</th>
<th>Total IgA &gt;upper limit of normal*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presentation</td>
<td>30</td>
<td>23 (78%)</td>
<td>29 (97%)</td>
<td>30 (100%)</td>
<td>26 (87%)</td>
</tr>
<tr>
<td>Post-treatment</td>
<td>119</td>
<td>18 (15%)</td>
<td>54 (45%)</td>
<td>56 (50%) #</td>
<td>48 (40%)</td>
</tr>
<tr>
<td>All</td>
<td>149</td>
<td>41 (28%)</td>
<td>83 (56%)</td>
<td>86 (61%) †</td>
<td>74 (50%)</td>
</tr>
</tbody>
</table>

4.3. Typing of serum monoclonal proteins

4.1 Introduction

Figures

Figure 4.1. Schematic of serum protein electrophoresis.
The approximate position of polyclonal immunoglobulins in normal serum and the anode (+) and cathode (-) are indicated.

View source:
- 4.2.1. Serum protein electrophoresis

**Figure 4.2. Capillary zone electrophoresis.**

**(A)** Normal serum. **(B)** Monoclonal protein peak in γ-region, indicated by an arrow. (These figures were originally published in [1], reproduced with permission from Taylor & Francis Group.)

View source:
- 4.2.1. Serum protein electrophoresis
- 4.2.2. Capillary zone electrophoresis

**References**

1. Keren DF. Protein electrophoresis in clinical diagnosis. Arnold (Hodder Headline), 2003


