The serological diagnostic challenges of multiple myeloma

Serological screening tests for multiple myeloma are commonly requested by physicians in both primary and secondary care to investigate patients presenting with anaemia or renal impairment of unknown cause. This article reviews the interpretation of these tests.

The latest statistics from Cancer Research UK (2014) show that there were 2742 deaths in 2012 from myeloma. Although the 5-year survival rate has tripled over the last 35 years as a result of advances in treatment, it still remains low at 37%, with a 10-year survival rate of only 17%. Myeloma can present as general medical emergencies, with hypercalcaemia, acute renal failure or spinal cord compression, so general physicians must know how to initially investigate any suspected myeloma appropriately to avoid delay in referral to specialist haematology care. This article summarizes current guidelines on myeloma diagnosis and gives a brief outline of new diagnostic techniques.

A review of current guidelines

Current guidelines for the diagnosis of myeloma are comprehensively reviewed in the updated 2014 British Committee for Standards in Haematology document (Bird et al, 2014) which is based on criteria laid out by the International Myeloma Working Group. When there is a clinical suspicion of myeloma (e.g. unexplained normocytic anaemia, hypercalcaemia, renal failure or lytic lesions on plain X-ray), general blood tests should include full blood count, erythrocyte sedimentation rate, urea, creatinine, calcium and albumin.

Following this, the International Myeloma Working Group recommendations, as excerpted from Palumbo et al (2009), suggest serum and 24-hour urine collection for protein electrophoresis and immunofixation, quantification of monoclonal protein in the urine, i.e. Bence–Jones protein, and nephelometric quantification of immunoglobulins. In patients with light chain disease and suspected non-secretory myeloma, the British Committee for Standards in Haematology guidelines recommend measuring the serum free light chain and calculating the kappa/lambda (κ/λ) ratio (Bird et al, 2009).

A diagnosis of myeloma is confirmed with bone marrow biopsy. Classification of myeloma into different groups based on cytogenetic abnormalities predicts response to therapy and therefore survival (Sawyer, 2011).

This article uses a case example to illustrate the typical diagnostic pathway for multiple myeloma.

Step 1: serum protein electrophoresis

'An 81-year-old man presents with progressive dyspnoea and back pain. Routine blood tests show normocytic anaemia with haemoglobin 88 g/litre, erythrocyte sedimentation rate 58 mm/hr and creatinine level 375 μmol/litre. Serum protein electrophoresis showed a monoclonal band in the gamma region, with a paraprotein level of 4 g/litre with global immunoglobulin suppression.'

Serum protein electrophoresis is a well-established, first-line screening technique when myeloma is suspected. The patient's serum is placed on a specific support medium and an electric current applied, separating the serum protein components into five fractions based on size and charge. Moving from the positive to the negative electrode, the first fraction, which represents the largest peak and hence the largest protein component in serum, is albumin. The next four fractions comprise various groups of globulins, and are termed alpha 1 (α1), alpha 2 (α2), beta (β) (which can be sub-divided into β1 and β2) and gamma (γ) (*Figure 1*). The gamma peak lies closest to the negative electrode and is the area of greatest interest, as immunoglobulins, particularly the IgG isotype, migrate to this area.

Different pathologies will give characteristic patterns. The results are read by scan densitometry, whereby the density of the band is calculated as the area under the monoclonal peak in relation to the total amount of protein produced by that patient. This is usually reported as the monoclonal paraprotein level in g/litre.

It is important to differentiate between monoclonal and polyclonal increases in immunoglobulin on serum protein electrophoresis. Polyclonal gammopathies are commonly driven by an underlying infectious or inflammatory process. They consist of a mixture of different heavy and light chain subtypes, characterized as a diffuse pattern on electrophoresis.

In contrast, monoclonal gammopathies signify an underlying malignant process with clonal expansion of plasma cells producing one or more types of immuno-

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globulin isotypes (such as IgGκ or IgAλ), free light chains and in rare cases heavy chains only. These usually appear as discrete sharp spikes or bands on serum protein electrophoresis (*Figure 1*). Common causes of monoclonal gammopathies are listed in *Table 1* (Attaelmannan and Levinson, 2000).

Step 2: immunofixation electrophoresis

'The monoclonal band detected in the gamma region by serum protein electrophoresis was typed as a monoclonal lambda free light chain using immunofixation electrophoresis. Measurement of total immunoglobulins also showed immune paresis.'

On identifying a possible monoclonal gammopathy on serum protein electrophoresis, immunofixation electrophoresis is used to type the composition of this M-protein, in terms of heavy and light chain involvement. This consists of an electrophoresis step as before, and a fixation stage. Once serum proteins are separated in terms of size and electrical charge by electrophoresis as before, antisera corresponding to the major immunoglobulin heavy chains (G, A, M), as well as kappa and lambda light chains, are applied to the gel in individual lanes. Immune complexes form where corresponding reactions occur. These can be visualized by staining.

Polyclonal reactions are represented by diffuse homogenous staining within all lanes, whereas monoclonal immunoglobulins are seen as discrete dark, narrow bands in the corresponding heavy and/or light chain lanes (*Figure 2*). *Table 2* compares serum protein electrophoresis with immunofixation electrophoresis. Immunofixation electrophoresis techniques can only be used to qualitatively detect the presence or absence of immunoglobulin heavy and light chains, and as such cannot be used to monitor response. Immunofixation electrophoresis is also subject to operator variability as the result is visually interpreted. Free light chain detection with immunofixation electrophoresis is ten times more sensitive than that using serum protein electrophoresis.

Figure 1. Serum protein electrophoresis from a patient with a monoclonal band in the gamma region.

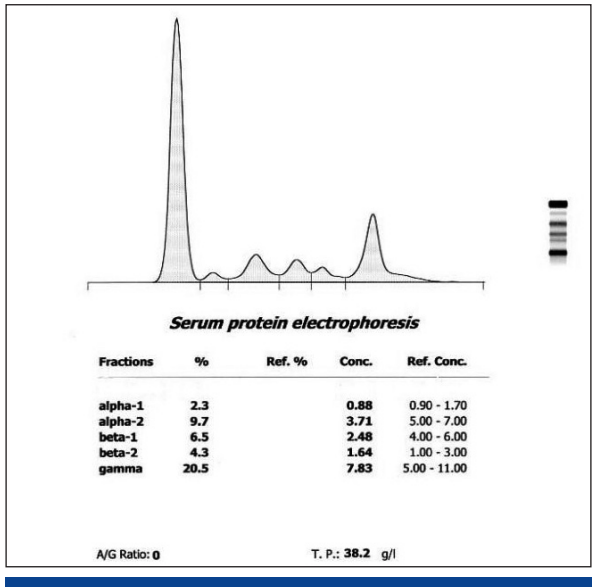

Figure 2. Serum immunofixation electrophoresis with monoclonal band in* Figure 1 *typed as monoclonal IgG kappa (arrowed).

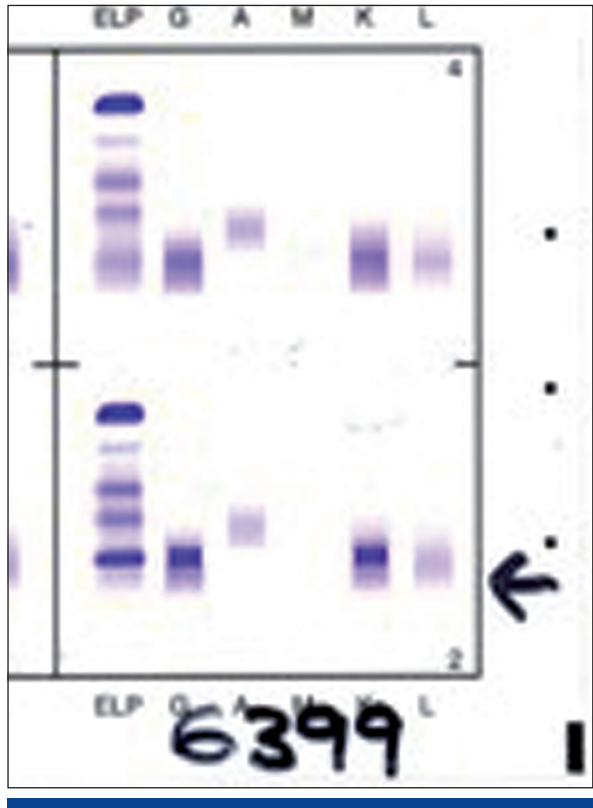

Table 1. Common causes of monoclonal gammopathy

Multiple myeloma
Monoclonal gammopathy of unknown significance (MGUS)
Plasmacytoma
AL amyloidosis
POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy and skin abnormalities)
Lymphocytic diseases, e.g. Waldenstrom's macroglobulinaemia

Table 2. Comparison of serum protein electrophoresis and immunofixation electrophoresis

	Serum protein electrophoresis	Immunofixation electrophoresis
Result output	Quantitative	Qualitative
Automation	Semi-automated or fully-automated by capillary electrophoresis	Semi-automated
Sensitivity	Less sensitive	More sensitive
Cost	Relatively cheap	Expensive

From Bradwell (2010)

Step 3: immunoglobulin measurement

'Measurement of total immunoglobulin levels showed severe immune paresis in this patient.'

Measurement of serum IgG, IgA and IgM by nephelometry principally identifies those patients with potential immune paresis, i.e. abnormally low levels of uninvolved immunoglobulins. These patients may be considered for treatment with intravenous immunoglobulins to prevent recurrent infections.

Nephelometric methods have the advantage of producing objective results that are analytically accurate to low concentrations. However, they do not distinguish between background polyclonal immunoglobulin and tumour-associated immunoglobulins, and as such they overestimate the monoclonal immunoglobulins and are not recommended for monitoring.

Step 4: urinary Bence–Jones protein detection and quantification

'In parallel to the above blood tests, a 24-hour urine collection sent for this patient showed significant Bence–Jones proteinuria at 9.7 g/day.'

Serum samples sent for a myeloma screen require a paired urine sample to investigate for Bence–Jones protein. Initially a plain urine sample (20 ml) is required for urine electrophoresis. This process is similar to that of serum protein electrophoresis. An identical immuno-fixation step is then used to identify the composition of the Bence–Jones protein. The urine protein electrophoresis should be repeated on a 24-hour urine collection if the initial urine electrophoresis and immunofixation electrophoresis yield positive results to quantify the Bence–Jones protein. There has been a move away from 24-hour urine measurements recently in favour of serum free light chain analysis for reasons discussed below.

However, despite the added inconvenience posed by 24-hour urine collections, Singhal et al (2007) explored the relationship between proteinuria and serum free light chain in 174 samples taken from 135 patients and found that renal impairment correlated with the level of proteinuria present, not the serum free light chain ratio. They therefore suggest that serum free light chain assay, although useful in some patients who secrete little or no monoclonal paraprotein, cannot be a replacement for 24-hour urine measurements.

Step 5: serum and urinary free light chain analysis

'Serum free light chain analysis resulted in a very abnormal κ/λ ratio of 0.001, corresponding to a kappa chain of 10.18 mg/litre and a lambda chain of 3740 mg/litre. Subsequent bone marrow biopsy showed infiltration by clonal plasma cells with lambda light chain restriction.

This patient was diagnosed with light chain multiple myeloma.'

In approximately 80% of patients with myeloma, monoclonal intact immunoglobulins are secreted and will be detected at significant levels by serum protein electrophoresis and/or immunofixation electrophoresis. In one retrospective study involving 488 patients with intact immunoglobulin myeloma, Mead et al (2004) reported that 96% of patients with a diagnosis of myeloma had elevated kappa or lambda light chains, or an abnormal κ/λ ratio. As such, serum or urine free light chain investigations are indicated for baseline measurement at diagnosis and in some cases are useful for monitoring the course of disease. The very short half-life of free light chain (2 hours for monomeric kappa and 4–6 hours for dimeric lambda) compared to intact IgG (21 days) is useful in monitoring early response to treatment. This can be especially useful in monitoring non-secretory myeloma, defined as the absence of M-protein in serum and urine by serum protein electrophoresis and immunofixation electrophoresis. This accounts for 2–3% of all myeloma cases.

A study by Drayson et al (2001) in 28 patients with non-secretory myeloma (diagnosed on the basis of monoclonal plasma cells in bone marrow biopsy and clinical symptoms, but lacking detectable M-protein by serum and urine electrophoresis) showed 19 had elevated concentrations of kappa or lambda free light chain and an abnormal κ/λ ratio.

The main use of serum free light chain measurement, however, is for diagnosis and monitoring of light chain multiple myeloma (Bradwell et al, 2003). This accounts for approximately 15% of myelomas, and is defined by monoclonal light chain secretion in the serum, but with no intact monoclonal immunoglobulin produced by the malignant plasma cells. Conventional serum protein electrophoresis techniques detect serum free light chain in only a small number of light chain multiple myeloma cases, and despite the superior sensitivity of immunofixation electrophoresis for detecting free light chain, it is not quantitative, and therefore has limited use for monitoring purposes (Pratt, 2008). Traditionally a plain urine sample is required for urine electrophoresis and immunofixation, although a 24-hour urine collection is necessary to quantify Bence–Jones protein for disease confirmation and monitoring. There are several challenges associated with this.

First, under normal circumstances serum free light chain is produced by the plasma cells of the bone marrow and lymph node, cleared by the glomeruli, and reabsorbed by the proximal tubules. Bence–Jones protein is detected in the urine when the absorption capacity of the proximal tubules is exceeded by production of serum free light chain. However, normal serum production of free light chains is estimated at approximately 500 mg/day, whereas the absorptive capacity of the proximal tubules is around 10–30 g/day (Bradwell et al, 2003). Consequently, urinary free light chain will only be detected when the production of free light chain in the serum is significantly raised and/or if the renal function is significantly impaired, which can be light chain driven or otherwise.

Second, a 24-hour urine collection may be difficult for some patients, in particular those who are old and frail. Unreliable 24-hour urine collection results in inaccurate measurements of free light chain excretion. Urine sample collected may need to be concentrated before analysis, although high resolution electrophoresis is now readily available. In addition, heavy proteinuria resulting from other causes may also impede the interpretation of the true free light chain.

Initially serum free light chain detection was limited by the lack of distinction between free light chain and those bound to intact immunoglobulins. However, Bradwell et al (2001) reported the successful use of an automated immunoassay technique which was able to detect an epitope that is revealed in free light chain, but hidden in whole immunoglobulins. This technique forms the basis of serum free light chain analysis, which gives values for the amounts of free kappa and lambda light chains, and the resulting κ/λ ratio by nephelometry measurements.

Kappa light chains are produced at a rate that is twice that of lambda. However, as a result of the smaller monomeric size of kappa, they are cleared 2–3 times faster than the dimeric lambda molecules. Therefore, kappa exists at a concentration which is approximately half that of lambda. There have been numerous published data attempting to determine the normal values of serum free kappa, free lambda and the κ/λ ratio.

One of the most detailed studies reported by Katzmann et al (2002) demonstrated that for participants from 21 to 90 years of age, a κ/λ ratio range of 0.26–1.65 represented a 100% reference interval for all samples tested. Owing to the faster renal clearance of kappa, the κ/λ ratio for urine samples is often quoted at 1.9 with a 95% confidence interval of 0.46–4. The larger confidence interval for urine measurements, possibly taking into account variations in renal filtration rate and urine concentration, may be another reason to use serum over urinary free light chain measurements.

The κ/λ ratio is also the most important determinant in distinguishing monoclonal from polyclonal rises in serum free light chain, whereby in polyclonal disorders, both kappa and lambda light chains increase in proportion to each other, and so the κ/λ ratio remains constant. It is therefore an excellent marker for clonality (Bradwell, 2010).

In addition, Bradwell et al (2003) demonstrated the increased sensitivity of serum free light chain measurements compared to urine electrophoresis in a sample of 224 patients with light chain myeloma. In this study, 100% of patients were identified to have abnormal concentrations of the corresponding serum free light chain and κ/λ ratio, and the results indicate that serum free light chain analysis is a reliable method of detecting and monitoring light chain myeloma, particularly for identifying those patients in complete disease remission compared to urinalysis.

Starting treatment and monitoring response

'The patient was started on thalidomide-based chemotherapy, with measurements of serum free light chain to monitor disease response. Following three cycles of chemotherapy, the patient's symptoms improved with a corresponding reduction in the free lambda chain burden.'

Future directions: Hevylite

So far, current techniques and their limitations have been discussed. For example, the inaccuracy of serum protein electrophoresis to measure proteins at low concentrations, the inability of immunofixation electrophoresis methods to quantify the protein level despite its higher sensitivity, and the failure of serum free light chain to detect those patients with myeloma who do not produce monoclonal free light chain. There is therefore a need for new techniques.

A method reported by Bradwell et al (2009), known as immunoglobulin heavy chain/light chain immunoassays or 'Hevylite', has shown promise in addressing these limitations. Hevylite uses an automated immunoassay technique to measure both kappa and lambda light chain subsets of the different immunoglobulin classes using nephelometry. Specific polyclonal Hevylite antibodies, produced in sheep, target unique epitopes situated at the junction between the heavy and light chain constant regions. Hevylite antibodies are therefore capable of recognizing and measuring the immunoglobulin heavy chain/light chain subsets, i.e. IgGκ and IgGλ, IgAκ and IgAλ, and IgMκ and IgMλ.

The resulting ratio of involved (abnormal/malignant) monoclonal gammopathy *vs* the isotype-specific polyclonal background uninvolved immunoglobulin, Hevylite κ/λ, is similar to the free light chain κ/λ ratio of serum free light chain, as it enables monoclonal intact Ig production to be both detected and accurately measured. In addition, as four unique epitopes exist between the heavy and light chain constant regions, there is good formation of antigen–antibody immune complex, resulting in very accurate measurement of involved/uninvolved immunoglobulin ratios by nephelometry.

Studies have shown Hevylite to be more sensitive than serum protein electrophoresis and serum immunofixation, in particular for proteins at low levels, with the added advantage of producing quantitative results for those patients who are only positive or even undetectable by standard immunofixation electrophoresis techniques. In addition, as well as identifying the traditional immune paresis of the uninvolved immunoglobulins which are isotypically different to the monoclonal gammopathy, Hevylite is able to measure the Hevylite-pair suppression, i.e. the degree of suppression of the uninvolved light chain of the same immunoglobulin isotype. This has been shown to be a strong predictor of both progression-free survival and overall survival in myeloma at diagnosis. Hevylite may possibly be of higher predictive

value than many other traditional measurements (e.g. β2-microglobulin, albumin, lactate dehydrogenase) (Bradwell et al, 2013).

Conclusions

The diagnosis of myeloma is a multistep process. Serum protein electrophoresis and immunofixation electrophoresis, although well established, are generally time-consuming and limited by operator expertise. There is therefore an ongoing need to establish more automated and sensitive tests. The breakthrough of the serum free light chain immunoassay has revolutionized disease monitoring of light chain myeloma in the last decade. Likewise, the new Hevylite assay can offer greater sensitivity and quantitative information of the involved/uninvolved immunoglobulin pairs. BJHM

Conflict of interest: none.

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KEY POINTS

- The diagnosis of multiple myeloma is a complex and multi-step process that involves a number of key immunological tests.
- Key immunological investigations include serum and urine electrophoresis, immunofixation electrophoresis, and free light chain assays.
- Hevylite is emerging as a new diagnostic test with possible greater sensitivity compared to traditional electrophoresis and immunofixation techniques.