Chapter 8: Clinical Tests for Monoclonal Proteins

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INTRODUCTION

Monoclonal gammopathy is a hallmark of plasma cell dyscrasias and some B-cell lymphoproliferative disorders (1). They cover a wide spectrum of diseases from the premalignant condition monoclonal gammopathy of undetermined significance (MGUS) to symptomatic multiple myeloma, malignant lymphomas, and chronic lymphocytic leukemia (CLL). The monoclonal (M) proteins can be the entire immunoglobulin, light chain only, or, rarely, heavy chain only. Their ability to cause kidney disease is another characteristic they have in common. In a disease such as multiple myeloma, the risk of AKI correlates with the severity of disease and can be as high as 50% (2,3). In one study, 87% of patients with AKI had the most advanced stage (III) of disease according to the Durie Salmon classification (4). In fact, only 44% of the patients with stage III disease had normal renal function. Other less common causes of AKI in this population include interstitial nephritis and acute tubular necrosis (5–7).

A number of glomerular and tubular lesions have also been described in myeloma patients; however, these lesions are actually more common in patients where the diagnostic criteria for multiple myeloma or lymphoma have not been met and are diagnosed with monoclonal gammopathy of renal significance (MGRS) (8). Patients with MGRS-related kidney disease are more likely to present with proteinuria, hematuria, and mild renal impairment than rapid-onset AKI as in cast nephropathy. In either situation, the identification of a monoclonal protein changes the diagnosis, pathophysiology, and prognosis and directs the clinician toward a hematologic evaluation (9). Monoclonal protein testing should be a part of any workup of AKI, as well as proteinuria with mild reduction of renal function in adults. This article will review the current available tests for monoclonal proteins.

SERUM PROTEIN ELECTROPHORESIS

Serum protein electrophoresis (SPEP) is the most commonly used laboratory test for the detection of monoclonal proteins. Serum proteins are loaded on to a gel or a capillary tube and are separated by an electrical current based on charge and size. The proteins are then stained for visualization. The proteins migrate into five zones or fractions. These are albumin, $\alpha 1$, $\alpha 2$, β, and γ. The β fraction often has two peaks. Albumin is the most abundant protein in the serum and should make up the largest peak in normal serum. When a monoclonal (M) protein is present, a sharp band appears often in the γ region. However, M proteins can migrate to the β or even the α fractions. This often occurs when the M protein is comprised of an IgA or free light chain (FLC). In some cases, no band is detected but instead there is a decrease in the γ peak (10). The hypogammaglobulinemia is due to the monoclonal gammopathy.

Currently, SPEP is the most commonly used test for M proteins globally because of its ease of use and relatively low cost. Fully automated systems are available for both the agarose gel and capillary tube methods, which have increased reproducibility and efficiency. Because SPEP is quantitative, it is used in both diagnostic and response criteria in multiple myeloma (11,12). Despite its utility, its detection limit is not sensitive enough as a single screening test, especially in low-burden diseases like MGRS. The detection limit for an M protein is 0.3–0.5 g/dL in the γ region and up to 0.7 g/dL in the α or β region (13). SPEP is positive in 87.6% of multiple myelomas but only 73.8% of immunoglobulin light chain (AL) amyloidosis and 55.6% of light chain deposition disease (LCDD) (14). In addition, the M-band on the SPEP only indicates an M protein but it does not distinguish the isotypes. To find out the type, immunofixation is required.

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Table 1. Screening panel

Condition	SPEP	SIFE	SFLC	UPEP/IFE
MGRS	X	X	X	X
AL/MIDD	X	X	X	X
WM	X		X	
Multiple myeloma	X		X	

Immunofixation should be performed if a screening test is positive to help type the monoclonal protein. SPEP, serum protein electrophoresis; SIFE, serum immunofixation; SFLC, serum free light chain; U, urine; MGRS, monoclonal gammopathy of renal significance; AL, AL amyloidosis; MIDD, monoclonal immunoglobulin deposition disease; WM, Waldenström macroglobulinemia.

URINE PROTEIN ELECTROPHORESIS

The first person to recognize that monoclonal proteins have special properties in the urine was Dr. William MacIntyre (15). Urine of myeloma patients turns opaque when boiled, clears with the addition of acid, and turns opaque again as it cools. The property was attributed to a protein that was named after the physician who misidentified it: Dr. Henry Bence Jones. It was not until later that Bence-Jones protein was identified as immunoglobulin FLC. Urine protein electrophoresis (UPEP) uses the same principle as SPEP and has the same advantages and disadvantages. The urine M-spike is used for diagnosis and response determination in multiple myeloma (16). However, because M proteins are not always present in the urine of patients with monoclonal gammopathy, the sensitivity of UPEP is the lowest of all the tests and should never be used alone for screening. In a study with 2,799 patients of which 4.4% had a plasma cell dyscrasia, SPEP was positive in 94.4% of patients, whereas only 37.7% had a positive UPEP (17). This has led some to suggest that the serum FLC assay should replace the UPEP for screening (see below). However, despite the low sensitivity, UPEP does provide additional information. In patients with renal impairment, the presence of an M protein and low albumin concentration on the UPEP is highly suggestive of cast nephropathy, whereas a high albumin concentration is more likely the results of LCDD or AL amyloidosis (18). In addition, the presence of a monoclonal light chain in the urine significantly increases the risk of renal injury in myeloma patients (19). Finally, urine M-spike is still used in response determination in multiple myeloma (11). Therefore, UPEP remains a useful supplemental test in patients with paraproteinemia.

SERUM AND URINE IMMUNOFIXATION

Samples are electrophoresed in parallel lanes in immunofixation (IFE). Antibodies against the heavy and light chains of the immunoglobulin are then applied to each lane separately. A reaction forming a sharp band would suggest the presence of monoclonal immunoglobulin component (Figure 1). The M protein composed of the entire monoclonal immunoglobulin would be positive for both a heavy chain and a light chain. The sensitivity of IFE has a detection limit of ~0.1 g/dL of

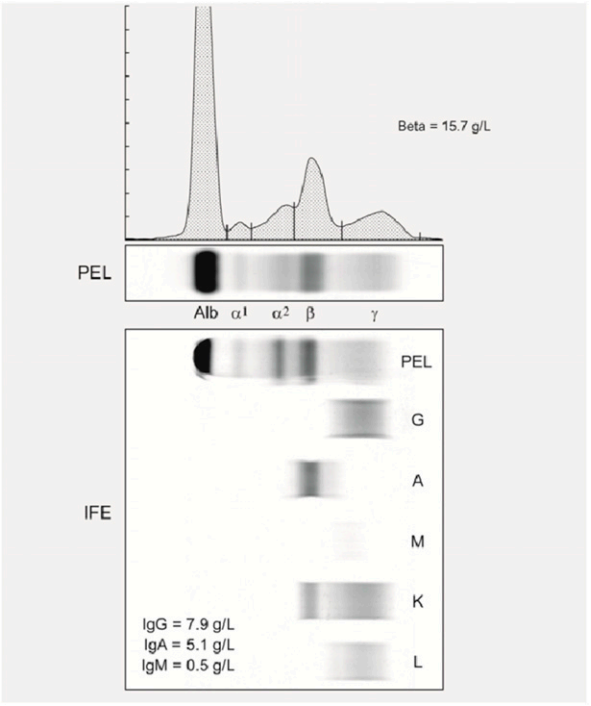

Figure 1. Serum protein electrophoresis (PEL) and immunofixation (IFE). A monoclonal (M) spike was detected in the β region of the protein electrophoresis. The immunofixation identified a band in the IgA and κ lanes that corresponded to the band in the β region of the protein electrophoresis. The M protein in this case is a monoclonal IgA κ. The blurry smudge in IgG lane indicated that the IgG was polyclonal.

monoclonal protein. In multiple myeloma, serum IFE increased the detection rate from 87.6% to 94.4%. Similarly, the sensitivity increases from 65.9% to 73.8% in AL amyloidosis. In a mixed group of patients, the sensitivity increases from 79% to 87%. Not only is the sensitivity improved with IFE, but the type of the monoclonal protein can be identified. IFE is qualitative and not quantitative. Therefore, for response determination in multiple myeloma and AL amyloidosis, it is only used for assessment of complete response. The extra reagents add significantly to the cost, making IFE less affordable.

SERUM FLC ASSAY

In the early 2000s, a new assay was introduced to aid in the detection of monoclonal proteins. Using antibodies against epitopes that are normally hidden in the intact immunoglobulin, the assay detects both κ and λ FLCs. It is quantitative and automated. The assay is not specific for monoclonal light chains but instead infers monoclonality when an abnormal ratio between the κ and λ FLCs is detected. The normal ratio is between 0.26 and 1.65. A high ratio suggests a κ clone, whereas a ratio $<$0.26 suggest a λ clone.

The addition of the serum FLC assay to SPEP and serum IFE has significantly increased the sensitivity of monoclonal protein

testing. Prior to the introduction of the FLC assay, up to 5% of multiple myelomas were thought to be nonsecretory. Using the FLC assay, 19 of 28 nonsecretory myeloma patients were found to have abnormal κ to λ ratios, and 4 had suppression of one or both FLCs (20). The FLC assay was able to identify abnormalities in 82% of patients classified as nonsecretory by serum and urine PEP and IFN. Most of these were light chain–only myeloma. Recently, the serum FLC ratio was added to the diagnostic criteria of multiple myeloma (12). In AL amyloidosis, the serum FLC assay increases the detection rate from 69% (with serum IFE alone) to 99% (21). The addition of UPEP did not identify any additional patients. Another study found the combination of serum IFE and serum FLC detected 100% of the patients with multiple myeloma, Waldenström macroglobulinemia, and smoldering multiple myeloma (14). The addition of UPEP did not increase the sensitivity for the above diagnoses but did assist in the identification of MGUS, extramedullary plasmacytoma, AL amyloidosis, and LCDD.

In addition to diagnostic evaluation, serum FLC is also used in disease monitoring and assessment of response. The degree and speed of serum FLC reduction have been found to be the most important predictors of renal recovery in cast nephropathy (22). In AL amyloidosis, the reduction of serum FLC has been shown to be a better predictor of outcomes than the M-spike. To achieve stringent complete response in multiple myeloma and complete response in AL amyloidosis, the serum FLC ratio has to be normalized (16,23).

Although the serum FLC assay increases the detection rate of monoclonal gammopathy, clinicians should be aware of its limitations. First, the assay does not distinguish between polyclonal FLCs and monoclonal FLCs. The higher (or lower) the κ to λ ratio, the more likely a monoclonal gammopathy exists. However, several conditions are known to cause minor abnormalities. The most common is renal insufficiency. Because FLCs are mostly cleared by the kidney, a reduction in glomerular filtration rate will cause a rise in the FLC levels. This rise, however, is not symmetric because κ FLCs are cleared more readily than λ. Thus, the asymmetric increase in FLCs results in an increase in the ratio. In patients with severe renal impairment, a renal reference range for the κ to λ ratio (0.37 to 3.1) has been recommended (24). Patients with autoimmune diseases can also have mildly abnormality κ to λ ratios. Finally, look for a biclonal gammopathy if there is elevation in both FLCs but the renal function is normal and autoimmune disorders have been ruled out.

URINARY FLC

The measurement and quantification of FLC can also be performed in the urine. An elevated κ to λ ratio suggests a κ clone, and a depressed ratio suggests a λ clone (25). Studies suggest that the urinary FLC can correlate with the serum FLC in an individual patient (26). However, urinary FLC excretion does not always increase even in patients with elevated serum FLC levels (27). Thus, urinary FLC levels do not appear to contribute to diagnostic sensitivity of the current monoclonal testing regimen.

SCREENING AND MONITORING

The screening test(s) for any disease must have sufficient sensitivity to identify as many patients as possible but also cost effective enough to apply to the general population. Currently, no single test is capable of accomplishing these goals in monoclonal gammopathy. This is especially true for diseases with very low levels of monoclonal protein (28). However, in diseases such as multiple myeloma and Waldenström macroglobulinemia, where the monoclonal protein is usually abundant, the combination of serum IFE and serum FLC has been shown to be nearly 100% sensitive (14). In diseases with lower levels of monoclonal gammopathy, urine IFE can increase the sensitivity but at a higher cost (Table 1).

For monitoring, the goals are different. Typically, the type of monoclonal protein is no longer important so IFE is not routinely required. The response is typically based on the reduction of the M protein. Depending on the M protein, this is done with SPEP, UPEP, and/or serum FLC assay. IFE is required when the M-spike is no longer detectable to evaluate for complete response. In multiple myeloma, FLC should be followed even when patient has a M-spike because of the phenomenon light chain escape. This occurs in some heavily treated patients where the clonal evolution produces a clone that makes more FLC than the intact immunoglobulin (29). In these patients, the M-spike would remain low, suggesting persistent response but the involved FLC will rise rapidly.

TAKE HOME POINTS

- Monoclonal protein testing is an important diagnostic tool for the evaluation of AKI and proteinuria.
- The Serum free light chain assay significantly increases the detection rate of monoclonal protein when added to serum immunofixation.
- Urine protein electrophoresis can help distinguish between tubular or glomerular injury in patients with multiple myeloma.

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REVIEW QUESTIONS

1. A 68-year-old man presents with a 1-year history of sudden-onset nephrotic range proteinuria, easy bruising with spontaneous "black eyes," and more recent onset of dyspnea on exertion. Which of the following tests should be performed for screening?

   a. Serum protein electrophoresis
   b. Urine protein electrophoresis
   c. Serum immunofixation
   d. Serum free light chain assay
   e. All of the above

Answer: e is correct. The presentation is concerning for amyloidosis. Because amyloidosis is usually caused by a low burden plasma cell dyscrasia, all of the tests should be performed to avoid false negativity.

2. Which test is most important in the screening of non-secretory multiple myeloma?

   a. Urine immunofixation
   b. Complete blood count
   c. Serum creatinine
   d. Serum calcium
   e. Serum free light chain assay

Answer: e is correct. Prior to the serum free light chain assay, up to 5% of the multiple myeloma cases were thought to be nonsecretory. The serum free light chain assay identifies 80% of these cases as light chain myeloma.

3. A 65-year-old man with hypertension, mild anemia, and CKD stage IV presents with a mildly depressed serum κ to λ free light chain ratio of 0.12 (normal = 0.26–1.65). Serum and urine immunofixation are negative. What is the most likely possible explanation for his abnormal κ to λ free light chain ratio?

   a. CKD
   b. Hypertension
   c. Chronic myelogenous leukemia
   d. Monoclonal gammopathy of undetermined significance
   e. None of the above

Answer: d is correct. CKD and renal impairment result in a mildly elevated κ to λ ratio. This is because the reduction of glomerular filtration reduces the clearance of κ free light chain more than λ. Hypertension does not affect free light chain clearance. Chronic myelogenous leukemia is a myeloid disease and does not produce monoclonal protein. With the history of CKD, the low κ to λ ratio is likely to be due to the presence of a monoclonal gammopathy.