Laboratory Workup for Initial Diagnosis of Monoclonal Gammopathies

SYNOPSIS AND RELEVANCE

Monoclonal gammopathies (MGs) can be seen associated with a variety of disorders, the management of which varies dramatically depending on subclassification. Properly utilizing tests for detection and characterization enables:
1. Prompt and accurate subclassification to guide monitoring of disease progression and/or treatment.
2. Meaningful quantification of biomarkers which can be used in monitoring and treatment.
3. Reduction of unnecessary or obsolete testing.

INSIGHTS

1. Initial evaluation for suspected MG should include both serum free immunoglobulin light chains (sFLC) and serum protein electrophoresis (SPEP) to maximize sensitivity for detection of a monoclonal protein.
2. If there is an abnormal sFLC ratio or a monoclonal protein is detected on SPEP, serum immunofixation (sIFE), or an equivalent test such as immunosubtraction, should be performed to identify the involved heavy chain (if present), and light chain, in the case of a monoclonal protein detected by SPEP.
3. If a light chain is found on sIFE without a corresponding heavy chain (negative for IgA, IgM, and IgG by sIFE), test for IgD and IgE heavy chains before reporting a light-chain only MG.
4. In suspected primary/light chain (AL) amyloidosis, it is recommended to screen with SPEP, sFLC, sIFE, and urine immunofixation (uIFE) because the monoclonal protein may be scant in these cases, and maximal sensitivity is required for timely initiation of therapy.
5. Total/intact serum light chain assays are not sufficiently sensitive for the initial diagnosis of MG such as multiple myeloma (MM) and AL. Serum free light chain assays (sFLC) are more sensitive and biologically relevant; therefore, these tests should be used instead of total/intact light chain assays whenever possible.

BACKGROUND

Monoclonal gammopathies (MGs) are conditions arising from the secretion of monoclonal proteins, which are immunoglobulin proteins or subunits secreted by clonal plasma cells or B-cells. They include a variety of disorders, most commonly the premalignant condition monoclonal gammopathy of undetermined significance (MGUS) and the malignant condition to which it progresses in a subset of cases, multiple myeloma (MM). Monoclonal proteins (M proteins) are also seen in disorders such as lymphoplasmacytic lymphoma/Waldenström macroglobulinemia (LPL/WM), IgM-related disease, light chain deposition disease, and AL (primary/light chain) amyloidosis. The management of these disorders range from incremental monitoring to aggressive immunochemotherapy to potential organ transplantation. Certain conditions, such as AL amyloidosis, can result in significant systemic disease despite relatively small amounts of M protein, so more sensitive methods of detection, or multiple tests, are warranted in patients with symptoms related to this entity.

Serum protein electrophoresis (SPEP) and urine protein electrophoresis (UPEP) are widely available and have long been used as methods to both detect MGs and quantify the amount of monoclonal protein present. However, quantification of the monoclonal protein can be overestimated due to comigration with normal serum proteins, most typically IgA monoclonal proteins, as a third of these will migrate in the beta region, or M proteins migrating into the gamma region in the setting of reactive hypergammaglobulinemia. Furthermore, therapeutic antibodies may also confound the detection and quantification of monoclonal proteins.

The addition of anti-heavy chain and anti-light chain antibody testing by serum immunofixation electrophoresis (sIFE) increases the sensitivity for detection of the monoclonal protein and provides identification of the monoclonal component. Capillary electrophoresis has been developed as an alternative to agarose gel electrophoresis to detect M proteins and quantitate serum protein, and immunosubtraction can be used to subclassify the monoclonal gammopathy, analogous to SPEP and IFE, with comparable sensitivity and specificity and potential automation of the processes. Heavy chain isotypes and light chains can also be quantitated overall in solution.

Some MGs consist of light chains without an associated heavy chain (kappa or lambda light chains only). This is more common in AL amyloidosis than in MM. SPEP may not detect light chain-only MGs as these are cleared relatively quickly from the serum by the kidneys. Thus, methods to detect the light chains in urine were previously essential to detecting these MGs, such as UPEP and IFE. In the early 2000s, methods were developed that allow detection of free kappa and lambda light chains (serum free light chains, sFLCs) with increased sensitivity. sFLC assays are also used to stratify risk of progression in patients with MGUS and smoldering MM (SMM). In addition,

sFLC assays can be used for disease monitoring and treatment assessment, and do not experience interference with therapeutic monoclonal antibodies, which can confound SPEP quantification of MG. Because the free light chain assays are more sensitive and widely available, it is recommended that total/intact light chain assays should no longer be used for the quantitation of M proteins in patients with suspected myeloma.[1]

Because of the increased sensitivity for sFLC, especially for light chain-only disease, it is recommended to perform both SPEP and sFLC for the initial evaluation for MG. In the event of a monoclonal protein detected by SPEP or abnormal sFLC ratio, sIFE or a similarly sensitive method (immunosubtraction or mass spectrometry) to determine the heavy chain (if present) and light chain should be performed.[1] Identifying the involved light and heavy chains is important for a few different reasons. In MGUS, the isotype or absence of the heavy chain are associated with different risks of progression: light chain-only MGUS has the lowest risk of progression, at approximately 0.3% of cases per year, followed by IgG and IgA with approximately 1% of cases per year, and then by IgM MGUS, with the highest rate of progression, at approximately 1.5% of cases per year.[2]

It is also recommended to perform testing for potential IgD or IgE isotypes if a heavy chain is not identified by IgG, IgA and IgM testing; these diseases are rare but may carry a worse prognosis compared to other MM subtypes. IgM monoclonal proteins are more often seen in the B-cell disorder LPL/WM, which carries a different prognosis and potentially warrants a different treatment strategy than MM. The presence of an IgM monoclonal protein may also affect the hematopathologist's approach to the case, such as further analyzing the study for a B-cell clone by flow cytometry and ordering mutation analysis for *MYD88* L265P with/without *CXCR4* mutation analysis. Alternatively, fluorescence in situ hybridization (FISH) testing for recurrent chromosomal alterations is an important prognostic factor for plasma cell neoplasms and should be performed when MM is suspected.

Finally, if the M protein identified on SPEP migrates outside of the gamma region, it may be best to perform quantitative IgG, IgA and IgM at diagnosis to serve as a baseline measurement of the M protein content. Using these levels to monitor monoclonal protein levels over time may be more accurate since they are without interference from other endogenous proteins. If mass spectrometry is used, this is not necessary.

The increased sensitivity of the sFLC assays has largely replaced urine studies for both detection and monitoring of MG, with rare exceptions of disease associated with low-level MG such as AL amyloidosis and in patients with significant MG-related renal disease. Urine studies are most informative using 24-hour-collection specimens; however, testing performed on a first morning voided sample may be helpful.

Because timely detection and treatment of AL amyloidosis is clinically important, and these patients may have very small M proteins, it is recommended to perform SPEP, sIFE, sFLC, and uIFE as initial screening tests when a diagnosis of AL is under consideration.[1] This is a relatively rare diagnosis; however, using a comprehensive testing approach may facilitate earlier detection in centers where AL is not frequently encountered.

The most recent addition to the field of monoclonal gammopathy assessment is mass spectrometry. This technique has the benefit of being able to simultaneously quantitate and subclassify MG, without interference from other endogenous proteins or therapeutic monoclonal antibodies. These methods are not currently widely available, but overall are equivalent in sensitivity to sIFE for diagnostic purposes. Recommendations may change as more information is gathered regarding the utility of this technology.

REFERENCES

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