Laboratory Workup for Initial Diagnosis of Monoclonal Gammopathies

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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.

OBJECTIVES
1.   Define the best initial screening tests for detection and subclassification of MGs.
2.   Ensure that the appropriate biomarkers are detected/quantified at baseline to follow disease progression and/or therapeutic impact.
3.   Facilitate the appropriate utilization of tests for MG detection, characterization, and subclassification.
4.   Decrease inappropriate ordering of individual tests.

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 (amyloid light chain or primary) 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, traditionally analyzed in agarose gels. However, quantification of the monoclonal protein can be overestimated due to co-migration 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 by using turbidimetry or nephelometry.

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. The assay developed by The Binding Site was the first developed and is the best studied; however, several other assays are currently available. 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. While it is recommended to report free kappa, free lambda, and free kappa/free lambda ratios, for the purposes of this text, sFLC will refer to the ratio. 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 with light chain-only MGUS having 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 also 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.

INSIGHTS

1. Initial evaluation for suspected MG should include both sFLC and SPEP to maximize sensitivity for detection of a monoclonal protein.
2. If there is an abnormal sFLC or a monoclonal protein is detected on SPEP, 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 AL, it is recommended to screen with SPEP, sFLC, SIFE, and 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 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.

INTERVENTIONS

1. <u>Develop an order set or best practice alert for patients with suspected MG which includes SPEP and sFLC, including reflex testing if either are abnormal.</u>
Using both of these tests in conjunction provides the best sensitivity for detection of MG. Reflex testing would include sIFE (of an equivalent assay such as immunosubtraction) to identify the involved heavy and light chains, which is important for the reasons discussed in the background. Laboratories could also consider reflexing send-out testing for IgD and IgE for light chain-only cases and the quantitative IgG, IgA, and IgM if the M protein migrates outside of the gamma region.

2. <u>Implement clinical decision support tools in the electronic medical record/laboratory information system to reduce individual inappropriate individual orders,</u> such as <u>sFLC and sIFE without SPEP.</u> Potential methods to reduce inappropriate ordering could include a best practice alert, which indicates that the using the recommended set of screening tests is best for initial identification of MG and/or AL, and an option to add SPEP. the individual tests should remain separately orderable for monitoring.

3. <u>Develop an order set or best practice alert for patients with suspected AL amyloidosis including initial testing for SPEP, sFLC, sIFE (or equivalent testing) and uIFE.</u>
Given the clinical importance of early detection and pharmacologic intervention in AL amyloidosis, as well as the fact that the level of monoclonal protein may be relatively small in these patients, more extensive testing (initial sIFE and uIFE) is recommended to maximize sensitivity.

QUESTIONS AND ANSWERS

QUESTION 1 OBJECTIVE
Identify the most appropriate initial testing for MG in patients with suspected MM.

QUESTION 1
Which of the following assays is recommended for initial detection of MG in patients with suspected MM?
A.   Urine protein electrophoresis (UPEP)
B.   Serum calcium levels
C.   Serum free light chain assay (sFLC)
D.   Total/intact light chain assay
E.   Serum IgG, IgA, and IgM quantitation

The correct answer is C. Serum free light chain assay (sFLC). sFLC is adequately sensitive for detection of light chain-only MGs, which can be missed on SPEP.
A is incorrect. UPEP is most useful when performed on a 24-hour urine sample, which is difficult to collect. sFLC is equivalently sensitive for detection of light chain MGs in patients with MG, and has largely replaced this test.
B is incorrect. Serum calcium levels are not always elevated in MM patients and can be seen in other disorders.
D is incorrect. Total/intact light chain assays are not sensitive enough for detection of light-chain only MM and are no longer recommended for diagnosis or monitoring.
E is incorrect. These tests do not specifically detect MGs and can be elevated in a variety of conditions. They should not be used for initial detection of MG.

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QUESTION 2 OBJECTIVE
Understand the impact of heavy chain isotype in MG-related disorders.

Identifying the isotype of an MG is important for which of the following?
A.   Most IgA MGs are associated with B-cell lymphomas
B.   Presence/absence and isotype of heavy chain is associated with risk of progression in some diseases
C.   Determining the heavy chain isotype is not necessary, only the light chain needs to be identified
D.   Quantifying the isotype is only important if the MG migrates to the gamma region on SPEP
E.   Prompting ordering of molecular testing (*MYD88* L265P, *CXCR4* sequencing) in IgG MGs

The correct answer is B. Presence/absence and isotype of heavy chain is associated with risk of progression in some diseases. In MGUS and SMM risk of progression is light chain-only with lowest risk, followed by IgG and IgA, and finally IgM with highest risk. Rare IgD and IgE MM may also have a worse prognosis.
A is incorrect. Most IgA MGs are associated with plasma cell neoplasms (eg, MM).
C is incorrect. Determining the isotype is important, for the reasons listed in B.
D. is incorrect. Using quantitative isotype testing to monitor disease is most helpful in MGs which migrate outside the gamma region, such as IgAs which migrate in the beta-2 region.
E. is incorrect. *MYD88* L265P and *CXCR4* sequencing is usually performed din LPL/WM, which is typically associated with an IgM MG.

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QUESTION 3 OBJECTIVE
Understand what testing is appropriate for patients with suspect AL amyloidosis.

QUESTION 3
Which of the following is true regarding initial testing for MG in a patient with suspected AL amyloidosis.
A.   Only SPEP is needed since the quantity of MG is usually large
B.   Urine protein electrophoresis (UPEP) performed on a random daytime sample is the most helpful urine test
C.   Urine IFE is not indicated because these are usually IgM MGs
D.   The same panel for initial diagnosis of MGs should be used in these patients
E.   Tests with additional sensitivity are required as the level of monoclonal protein is often small

The correct answer is E. Tests with additional sensitivity are required as the MG is often small. AL amyloidosis can have extensive involvement of multiple organs, most morbidly the heart, despite a very small quantity of MG.
A is incorrect. AL amyloidosis is often associated with a small quantity of MG, and is more often light chain-only than MM, so it can be missed by SPEP alone.
B is incorrect. UPEP on a random daytime urine sample does not have adequate sensitivity. uIFE on a 24 hour urine sample is considered the most sensitive urine test for MG detection.
C. is incorrect. AL amyloidosis is most often associated with IgG MG, followed by light chain-only and IgA MG. AL amyloidosis can occur with IgM MG but is rarer.
D. is incorrect. Although SPEP and sFLC will detect a majority of these patients, the severity of the disease and importance of early intervention warrant using additional initial testing.

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