

National Academy of Clinical Biochemistry Guidelines for the Use of Tumor Markers in Monoclonal Gammopathies

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Abbreviations: AL, amyloid light-chain; HCT, hematopoietic cell transplantation; IgA, IgD, IgG and IgM, immunoglobulin A, D, G and M respectively; MGUS, monoclonal gammopathy of undetermined significance;

INTRODUCTION

The monoclonal gammopathies, also called paraproteinemias or dysproteinemias, are a group of disorders characterized by the proliferation of one or more clones of differentiated B lymphocytes that each produce an immunologically homogeneous immunoglobulin commonly referred to as a paraprotein or monoclonal (M) protein. The circulating M-protein may consist of an intact immunoglobulin, the light chain only, or (rarely) the heavy chain only. The heavy chain is from one of the five immunoglobulin classes G, A, M, D or E, while the light chain is either kappa or lambda in type.

The monoclonal gammopathies encompass a number of diseases including plasmacytoma, monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma, Waldenströms macroglobulinemia, and systemic amyloid light-chain (AL) amyloidosis.

Multiple myeloma has been sub-classified (1) into the indolent form smoldering multiple myeloma, SMM, and symptomatic systemic disease, often involving end-organ damage to the kidneys, bones and bone marrow. Indolent disease is characterized by a lack of symptoms, few or no bone lesions, and stable concentrations of M-protein (1). Waldenströms Macroglobulinemia is characterized by elevated levels of monoclonal immunoglobulin M (IgM) in the serum, excess lymphoplasmacytoid cells in the bone marrow, and, in contrast to multiple myeloma, the involvement of visceral organs including the liver and spleen. Finally, in systemic AL amyloidosis, although the number of monoclonal plasma cells in the bone marrow is usually low, the protein they produce has an affinity for forming deposits (amyloid) in visceral organs such as the heart, kidneys liver and spleen, and these protein deposits cause end-organ dysfunction and early death (2).

Multiple myeloma arising from plasma cell dyscrasia accounted for approximately 1.1% (~15,270) of new cancer cases and 10% to 15% of all hematologic malignancies in the United States in 2004, and was responsible for 11,070 (2.0%) of cancer deaths, representing the worst ratio of deaths per new cases for any cancer (1)) In a recent review of 1027 patients, the median duration of survival was approximately 33 months [3]. Other sources cite a median survival of 4 years [4].

Although new therapies have recently been introduced, multiple myeloma remains incurable. Autologous hematopoietic cell transplantation (HCT) is standard therapy especially in younger patients.[3-5] A recent phase III trial showed a survival advantage of 10 months in patients who underwent HCT, with a trend toward improved survival in patients with high-risk disease (β_2 microglobulin >8.0 mg/L). [6] The goals of therapy include improving overall survival as well as quality of life. The use of bisphosphonates and

recombinant erythropoietin have proved useful in reducing skeletal related events as well as symptomatic anemia in these patients. [7]

Laboratory findings that should raise suspicion of a plasma cell dyscrasia or Waldenström's macroglobulinemia include elevation of the erythrocyte sedimentation rate or serum viscosity, anemia, renal insufficiency with a bland urine sediment, heavy proteinuria in a patient over age 50, Bence-Jones proteinuria, hypercalcemia, hypergammaglobulinemia and immunoglobulin deficiency. Clinical symptoms that are suspicious for a plasma cell disorder include back pain, weakness or fatigue, osteopenia, osteolytic lesions, spontaneous fractures and recurrent infections (1). However, many patients are asymptomatic at presentation and are brought to medical attention by mild anemia or an elevated total protein on routine blood work.

The M-protein is a tumor marker specific for monoclonal gammopathies because it reflects the clonal production of immunoglobulin. Diagnosis of multiple myeloma is made by several criteria including the following: a monoclonal M protein in the plasma or urine, infiltration of plasma cells in the bone marrow, lytic lesions on radiographs of the skeleton, anemia, and hypercalcemia. Serial measurements of serum and urine M-protein are also used to characterize response to treatment and to follow the course of disease. A partial response is defined as a decrease in the M-protein level to <50% of the pretreatment level and a complete response as disappearance of detectable M-protein from the serum or urine. The role of immunoglobulin monoclonal protein markers in MGUS is crucial in establishing the diagnosis, prognosis, and response to therapy of this clinical entity.

CURRENTLY AVAILABLE MARKERS FOR MONOCLONAL GAMMOPATHIES

Table 1 lists the mostly widely investigated serum- and urine-based tumor markers for monoclonal gammopathies. Also listed is the phase of development of each marker as well as the level of evidence (LOE) supporting the proposed clinical use. The markers presented in Table 1 are discussed below.

TUMOR MARKERS IN MONOCLONAL GAMMOPATHIES: NACB RECOMMENDATIONS

Table 2 presents a summary of the National Comprehensive Cancer Network (NCCN) and National Academy of Clinical Biochemistry (NACB) recommendations on the use of tumor markers in monoclonal gammopathies.

LABORATORY DIAGNOSIS, SCREENING AND MONITORING

Protein electrophoresis

Protein electrophoresis should be undertaken whenever multiple myeloma, Waldenström's macroglobulinemia, or MGUS is suspected. The monoclonal protein migrates as a single entity in the electric field and is detected by the non-specific protein stain as a more intensely stained band superimposed on the usual protein pattern. Serum is typically investigated first, but urine is particularly informative in cases of light chain myeloma where most of the monoclonal protein is absent from the blood. Protein electrophoresis should always be performed in combination with immunotyping in order to determine clonality. In addition to aiding in the diagnosis of a monoclonal gammopathy, protein electrophoresis is useful in monitoring the course of disease and the response to therapy in these patients.

The concentration of M-protein detected by protein electrophoresis is directly correlated to a patient's tumor burden except in the rare cases of non-secretory myeloma. The M-protein usually migrates in the gamma or beta region of the normal protein pattern, but may appear in the alpha-2 or even alpha-1 region upon occasion. Electrophoretic separation is typically carried out using agarose gels or fused silica capillary tubes. The latter technique is referred to as capillary zone electrophoresis and has a resolution comparable to agarose gel mediated electrophoresis.

Immunotyping

Immunotyping is used to identify the clonality (type) of M-proteins observed on electrophoresis and to probe further for the presence of monoclonal proteins when suspicion persists despite a normal protein electrophoretogram. In addition, screening for systemic AL amyloidosis requires the use of serum and urine immunotyping since the quantity of M proteins in the vast majority of cases is too small to be detected by electrophoresis. Clinical presentations always requiring immunotyping studies include the presence of otherwise unexplained sensory motor peripheral neuropathy, nephrotic syndrome, refractory heart failure, orthostatic hypotension, carpal tunnel syndrome, malabsorption, or strong clinical evidence suggestive of primary amyloidosis [1]. Other indications for immunotyping include detection of low intensity M bands by electrophoresis, negative routine electrophoresis results in patients with previously treated multiple myeloma or macroglobulinemia, and the identification and characterization of biclonal (two M-protein) or triclonal (three M-protein) gammopathies. [8, 9]

Immunotyping is determined by either immunofixation or immunosubtraction. In the former technique, the patient's sample is subjected to agarose gel electrophoresis in at least five separate lanes. Following electrophoresis, each lane is overlaid with an antibody specific for a different class of heavy or light chain. The antibody interacts with the cognate immunoglobulin domains to form a large complex that becomes fixed in the gel. Other non-bound proteins are washed from the gel, leaving only the fixed protein to be visualized after non-specific staining. Monoclonal protein appears as an intense band against a background of polyclonal immunoglobulin of the same class. Immunofixation will detect a serum M-protein down to a concentration of about 0.2 g/L and a urine M-protein down to a concentration of about 0.04 g/L, the latter limit being lower due to decreased background staining arising from polyclonal immunoglobulin of the same class co-migrating with the monoclonal protein [10] It is prudent to test specifically for the presence of IgD and IgE monoclonals when no reactivity to anti-G, anti-M, or anti-A is detected in the serum or urine by immunotyping.

Immunosubtraction differs from immunofixation in that prior to electrophoresis, aliquots of the serum or urine sample are incubated with Sepharose™ beads coupled to anti-heavy chain or anti-light chain antibody. Each aliquot is incubated with a coupled antibody of different specificity that binds to immunoglobulins containing the appropriate epitopes, removing them from solution (hence, the term immunosubtraction). Aliquot supernatant is subjected to electrophoresis, typically through fused silica capillary tubes, and the densitometric trace of each aliquot is compared to that of the patient sample not incubated with any of the Sepharose beads. The immunotype is identified by matching to the antiserum that removes the M-protein abnormality observed in the sample not incubated with any of the Sepharose beads. This procedure is available in an automated format, making it a convenient option to employ in immunotyping M-proteins. [11, 12]

Quantitation of Immunoglobulins

Quantitation of IgG, IgA and IgM, is routinely carried out by nephelometric and turbidimetric procedures that measure the light scattered by the macromolecular lattices formed from the reaction of immunoglobulin heavy chains with polyvalent class-specific heavy chain antisera. These procedures are currently fully automated which makes them convenient to use both in serial monitoring of disease progression in monoclonal gammopathy and in monitoring for hyperviscosity syndrome. In addition, quantitation is clinically useful in detecting and monitoring the polyclonal hypogammaglobulinemia that results from functional impairment of the normal immunoglobulin producing cells of the bone

marrow by excessive expansion of the malignant clone(s). Class-specific quantitation by light scatter provides a more accurate assessment of disease burden than densitometric quantitation of the electrophoretic scan, particularly in patients with high levels (>50 grams/L) of M-protein (12) but, unlike electrophoresis, will fail to recognize biclonal gammopathies when the two clones are comprised of the same isotype. IgM 7S monomers, polymers of IgA, and aggregates of IgG are all accurately measured by the light scattering technique.[12]

Radial immunodiffusion is a different technique used in the past to quantitate immunoglobulins. This method is tedious, time consuming, manual, technically demanding and potentially misleading because the relative proportions of pentameric and monomeric IgM and of dimeric and monomeric IgA influence quantitation. Given these limitations, radial immunodiffusion is no longer recommended for routine immunoglobulin quantitation.

Automated light scattering techniques using appropriate antisera can be employed to quantitate kappa and lambda light chain and to monitor any imbalance in their ratio arising from clonal production of one or other light chain isotype by B-cell malignancy. The method detects light chain whether complexed to heavy chain or free, unless antisera specific for free light chain is employed. In addition, the light chain may be from either monoclonal or polyclonal immunoglobulin. Light chain quantitation may fail to produce abnormal results when an M-protein is present in low concentration against the variable background of normal polyclonal immunoglobulins; the concentration of each isotype as well as the kappa to lambda ratio may fall within the reference range. Traditionally, kappa and lambda quantitation has been used less frequently than IgG, IgA and IgM quantitation in the work up of suspected monoclonal gammopathy and the management of established disease.

Recently, however, there has been renewed interest in quantifying free light chains in the serum. This is due to the availability of specific assays of greatly improved sensitivity [18, 20] that can generate an accurate free kappa to lambda ratio that, in turn, provides a reliable numerical index of clonality. This development has significantly changed the way in which patients with so-called non-secretory myeloma and AL amyloidosis are diagnosed and subsequently monitored during therapy. [13-16]

Historically, 3% of myeloma patients have presented with no evidence of monoclonal protein in the serum and urine by traditional immunofixation studies and have been considered to have "non-secretory" disease. It is now evident that most of these patients have low, but nevertheless abnormal, concentrations of serum free light chains. [15] In one typical study, 19 of 29 non-secretory myeloma patients were found to have abnormally elevated serum free light chains with abnormal ratios (12 kappa excess, 7 lambda excess)

and a median plasma cell infiltration of the bone marrow of 50% (range, 6 to > 90%). [15] The free light chain levels in these patients varied in accordance with disease activity.

The advantage of measuring free light chains in serum rather than urine is that the variable reabsorption and subsequent destruction of filtered light chains by tubular cells of the proximal nephron is not a factor. The serum kappa to lambda ratio better reflects the relative rates of production and is a more dependable indicator of clonality. During chemotherapy of 270 patients presenting with light chain multiple myeloma [20, 23], urine tests frequently normalized, whereas serum tests remained abnormal, indicating superior sensitivity for residual disease in this patient group. Urinary testing is also more difficult in diseases where large amounts of polyclonal light chains are excreted in the urine (e.g., systemic lupus erythematosus, renal insufficiency, tubular dysfunction), potentially obscuring the monoclonal light chains of interest. However urinary light chains assays are more sensitive than urinary immunofixation and provide quantitative results. [16, 17]

As is the case for “non-secretory” myeloma, 5% to 10% of light-chain amyloidosis patients present with no evidence by immunofixation of a monoclonal gammopathy although by careful inspection of immunohistochemically stained marrow specimens the presence of clonal disease can be inferred in most cases. In contrast, the quantitative free light chain assay appears more sensitive than immunofixation for clonal disease in a proportion of these patients. Most importantly for the treatment of patients with systemic AL amyloidosis, the free light chain assay provides a direct measure of the fibril-precursor protein in both plasma cell dyscrasias and non-Hodgkin’s lymphoma. [13, 14] Since the response of amyloid organ disease lags behind the reduction in the fibril-precursor protein, ability to measure the latter resolves what has hitherto been a major problem in treating AL amyloid patients, i.e. assessing response and titrating therapy. With the free light chain assay, stable declines after treatment of >50% from baseline values have been shown to be associated with prolonged survival in systemic AL amyloidosis. [14]

Serum viscosity

Serum viscosity is measured relative to the viscosity of water using one of several commercially available viscometers. The reference interval for serum viscosity is 1.5 to 1.8 centipoise (cp). Hyperviscosity symptoms are rarely clinically evident until the viscosity exceeds 3 cp.

Determination of serum viscosity is clinically indicated in any patient with a monoclonal gammopathy and symptoms of oronasal bleeding, blurred vision, dilatation of retinal veins, flame-shaped retinal hemorrhages, unexplained congestive heart failure, or neurologic

symptoms such as headaches, vertigo, nystagmus, deafness, tinnitus, ataxia, diplopia, paresthesias, disorientation, stupor, or somnolence.[18] Serum viscosity should be measured when the concentration of monoclonal IgM exceeds 40 g/L and that of monoclonal IgA or IgG exceeds 60 g/L. [18]

Hyperviscosity occurs less frequently in patients with high concentrations of monoclonal IgG and more frequently in Waldenström's macroglobulinemia associated with elevated monoclonal IgM. [19] The relationship between serum viscosity and IgM concentration is nonlinear, as the former depends upon the molecular characteristics of the monoclonal protein and its degree of aggregation.

The magnitude of the viscosity correlates poorly with clinical symptoms. Although some patients remain asymptomatic until the viscosity reaches 6 to 7 cp, or even higher, many exhibit symptoms when the viscosity is at a level of 4 cp, perhaps because they are more susceptible due to concurrent organ-specific disease of the microvasculature, increased hematocrit or compromised cardiac status.

Cryoglobulins

Cryoglobulins are immunoglobulins and complement components that precipitate upon refrigeration of serum. The major clinical manifestations of mixed cryoglobulinemia include palpable purpura, arthralgias, lymphadenopathy, hepatosplenomegaly, peripheral neuropathy, and hypocomplementemia (with the fall in C4 levels often being most prominent). For patients for whom cryoglobulinemia is a significant clinical problem, plasma exchange and newer therapies such as Rituximab™ can be considered. After phlebotomy, blood must be maintained at 37°C during delivery to the laboratory and prior to clotting and centrifugation in order to avoid premature loss of the cryoglobulins among the separated cells. The serum is then placed in a refrigerator or ice bath and examined at 24 hours for the presence of cryoprecipitate. If no precipitate is observed, the specimen is kept at 4°C for an additional six days, at which time it is examined once again for precipitate. Any precipitate is washed at 4°C, re-dissolved in warmed buffer and subjected to immunoelectrophoresis with monospecific antisera to determine the type of immunoglobulin in the precipitate. [20]

CONCLUSION

Great progress has been made in both the diagnosis and treatment of the monoclonal gammopathies over the last decade. With the advent of an era of molecularly based treatment strategies and diagnostics, understanding of these diseases, their diagnosis, and treatment will be further advanced. Future prospects include the use of the new serum free

light chain assay to aid in the diagnosis and study of light-chain MGUS, a hitherto undiagnosed entity, and improved light-chain tests for systemic AL amyloidosis that provide prognostic information based on measurements of light-chain aggregation *in vitro*.

Table 1. Useful and potentially useful markers for monoclonal gammopathies

Cancer Marker	Proposed Use	Phase of Development	Level of evidence	Ref
Serum-based markers				
Serum protein electrophoresis (agarose gel and capillary zone)	Diagnosis, screening, and follow up of plasma cell dyscrasias	In clinical use	1	1,7,8,9
Serum immunofixation	Diagnosis and identification of clonality in plasma cell dyscrasias	In clinical use	1	8,9
Quantitative immunoglobulin	Diagnosis and follow up of plasma cell dyscrasias	In clinical use	1	10,11
Free light chains	Diagnosis and follow up of non-secretory myeloma, MGUS, and amyloidosis	In clinical use	1	18,19,20
Viscosity	Diagnosis of Hyperviscosity syndrome	In clinical use	1	13
Beta 2 microglobulin	Prognostic evaluation of MM (prognosis is poor if >4.0)	In clinical use	1	21
Urine-based markers				
Urine protein electrophoresis	Diagnosis, screening, and follow up of plasma cell dyscrasias	In clinical use	1	2
Urine immunofixation	Diagnosis and identification of clonality in plasma cell dyscrasias	In clinical use	1	8,9
Urine free light chains	Diagnosis and follow up of non-secretory myeloma, MGUS, and amyloidosis	In clinical use	1	19

Table 2. Recommendations for use of markers in monoclonal gammopathies by NCCN and NACB Expert Groups.

Marker	Application	NCCN	NACB 2005
Serum protein electrophoresis (agarose gel or capillary zone)	Diagnosis, screening, follow up	Yes	Yes
Serum immunofixation	Diagnosis and identification of clonality	Yes	Yes
Quantitative immunoglobulins	Diagnosis and follow up.	Yes	Yes
Free light chains	Diagnosis and follow up of non-secretory myeloma, MGUS and amyloidosis	Yes ¹	Yes
Viscosity	Hyperviscosity syndrome	Yes ¹	Yes
Beta 2 microglobulin		Yes	
Urine protein electrophoresis	Diagnosis, screening, follow up	Yes	Yes
Urine immunofixation	Diagnosis and identification of clonality	Yes	Yes
Urine free light chains	Diagnosis and follow up of non-secretory myeloma, MGUS, and amyloidosis		

NACB, National Academy of Clinical Biochemistry; NCCN, National Comprehensive Cancer Network.

¹ Recommended in certain clinical situations guided by symptomatology and markers of an individual's disease.

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