Amyloidosis and Waldenström’s Macroglobulinemia

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Primary systemic amyloidosis is an immunoglobulin light chain disorder that is 1/5th as common as multiple myeloma. Amyloidosis is regularly seen in the practice of a hematologist and has recently undergone major advances in terms of the ability to evaluate responses as well as new therapeutic options that were not available when this topic was covered as an education session at the American Society of Hematology meeting 5 years ago. Waldenström macroglobulinemia (WM) is rarer than amyloidosis (1500 per year WM versus 3000 per year amyloid in the US), and recent consensus panels have established the definition of the disease, the diagnostic criteria, criteria for initiation of therapy and a new classification scheme. In this session, new developments in amyloid and macroglobulinemia, from suspicion of the diagnosis to treatment, are covered.

In Section I, Dr. Morie Gertz answers four specific questions: (1) When should amyloidosis be suspected? (2) How does one heighten one’s index of suspicion for amyloid? (3) How is the diagnosis confirmed and the type classified as primary? (4) What is the prognosis and how is it accurately assessed? Recent findings on cardiac biomarkers, presenting features and use of the free light chain assay are reviewed. Staging for amyloid and recently proposed criteria of response and progression are covered.

In Section II, Dr. Giampaolo Merlini comprehensively reviews therapy of amyloidosis from the use of standard melphalan/prednisone to the recently described standard dose therapies including dexamethasone, thalidomide/dexamethasone, melphalan/dexamethasone and IV melphalan/dexamethasone. An extensive discussion of the role of high-dose therapy with stem cell reconstitution follows and includes patient selection, predictors of immediate morbidity and mortality, and survival expectation. Finally, a therapeutic strategy is proposed.

In Section III, Drs. Steven Treon and Giampaolo Merlini review the most current information on WM. The consensus panel results and recommendations of the clinical pathologic definition of WM, the prognostic markers and the indications to initiate therapy in WM, the uniform response criteria in WM and available treatments for the disease are reviewed. Drs. Treon and Merlini cover recently published treatment protocols that use rituximab, purine nucleoside analogs, and alkylating agents. The current data on thalidomide, alpha interferon, and high-dose therapy are also covered.

I. AMYLOIDOSIS: DIAGNOSIS AND PROGNOSIS

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Amyloidosis is a rare systemic disorder that results from tissue deposition of amyloid protein. Amyloid protein is defined by its resistance to proteolysis and its three-dimensional configuration as a beta pleated sheet. There are several subtypes of amyloidosis including primary amyloidosis, also known as light chain amyloidosis, secondary and familial amyloidosis. The incidence of amyloidosis is 8 patients per million per year. The structural subunits of the amyloid protein in light chain (AL) amyloidosis are the fragments of monoclonal immunoglobulin heavy chains or light chains (Table 1). The symptoms of amyloidosis are vague and include fatigue, edema, and weight loss and are not helpful in formulating the correct differential diagnosis. Occasionally, patients are recognized because of their monoclonal protein and are diagnosed as atypical multiple myeloma because they have a light chain present but less than 10% bone marrow plasma cells. Since there is no diagnostic blood test, radiograph, or scan procedure, awareness of the diagnosis is essential to correctly identify patients early in the course. Below is a typical patient.

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Illustrative Case
A 79-year-old man had a 1-year history of dyspnea on exertion, lower extremity edema, and a 10-kg weight loss. An initial evaluation in a primary care setting including echocardiography and electrocardiography were interpreted as being nondiagnostic. The patient was referred for evaluation of noncardiac dyspnea to a pulmonologist. A computed tomography (CT) scan of the abdomen showed shoddy retroperitoneal lymphadenopathy, and laparoscopic biopsy of the nodes showed sinus histiocytosis. After the patient left Mayo Clinic, a monoclonal G-lambda protein was detected in the serum with a peak of only 0.5 g/dL. The 24-hour urine protein showed 330 mg but consisted of lambda light chain and no albumin. A repeat echocardiogram showed increased wall thickness and restrictive diastolic filling consistent with amyloid. A subcutaneous fat aspirate demonstrated amyloid. Amyloid stains subsequently performed on the abdominal lymph nodes showed vascular amyloid deposits.

In this section, four questions will be addressed: (1) When should amyloid be considered? (2) If the diagnosis is under consideration, what is an appropriate diagnostic evaluation? (3) If there is a very strong suspicion, how is the diagnosis confirmed? (4) What is the prognosis for patients with proven disease?

The physical findings of amyloidosis include enlargement of the tongue, periorbital purpura, and the shoulder pad sign. Although very specific for the diagnosis, these are easily overlooked and are seen in less than 20% of patients with AL. Reliance on symptoms and signs alone without being aware of the possibility of amyloid will inevitably result in an overlooked diagnosis.

Amyloidosis is a disease that infiltrates organs and causes their dysfunction. The four most common organs involved in amyloid include: heart, kidney, liver, and the peripheral nerve (Figure 1). Amyloidosis should be considered in the differential diagnosis of any patient with nephrotic syndrome without clear alternative explanation. Amyloidosis accounts for 10% of adult non-diabetic nephrotic syndrome. When adult patients are seen with nephrotic syndrome, amyloidosis, as well as nil disease, membranoproliferative glomerulonephritis, and membranous glomerulopathy must be considered in the differential diagnosis. One-half of patients presenting with amyloidosis have demonstrable cardiomyopathy. The symptoms can range from easy fatigability to overt congestive heart failure. This diagnosis must be entertained in any patient with cardiac symptoms of fatigue without a history of ischemia such as exertional angina or a previously documented myocardial infarction. The electrocardiogram (EKG) may show a pseudo-infarction pattern, and patients may be incorrectly diagnosed as having a silent ischemic syndrome. The findings on echocardiography, which include thickening of the heart walls, can be misinterpreted as ventricular hypertrophy as in our illustrative case. Any patient with unexplained cardiac symptoms without valvular disease, coronary artery disease, or long-standing hypertension should be considered for possible amyloidosis. We have seen patients referred to cardi-
ologists with overt heart failure undergo cardiac catheterization, be found to have normal coronary arteries, and then be dismissed from further evaluation with no follow-up.

It is the hematologist’s responsibility to educate specialists at their institution on the proper evaluation of a patient with an unexplained cardiac disorder or unexplained proteinuria. Immunofixation of the serum and of the urine is required to screen for light chain amyloidosis.

Amyloid involving the liver occurs in approximately one-sixth of patients and is characterized by palpable hepatomegaly, elevation of the serum alkaline phosphatase, and no imaging abnormalities by CT or magnetic resonance imaging (MRI). Symptoms may be limited to early satiety and weight loss. The clinician’s responsibility is to obtain immunofixation of the serum and the urine in addition to the usual studies for hepatitis, primary biliary cirrhosis, and other infiltrative liver disorders.

One in 6 patients with amyloidosis presents with symptomatic sensorimotor peripheral neuropathy. The neuropathy can be both axonal and demyelinating. Symptoms occur primarily in the lower extremities, and sensory changes are greater than motor changes. There is often a 2-year delay between the onset of symptoms and the recognition of amyloid. Important clues include: half of the patients have associated carpal tunnel syndrome, and a number of them will have autonomic neuropathy. Autonomic failure manifests as alternating diarrhea and constipation, pseudo-obstruction with vomiting, orthostatic hypotension, and impotence. The neuropathy is frequently painful, requiring analgesics. Gabapentin and amitriptyline often fail to provide benefit. These patients may be recognized to have a monoclonal gammopathy but are often misdiagnosed as having monoclonal gammopathy of undetermined significance (MGUS)–associated neuropathy without proper diagnostic testing to exclude amyloid.

Amyloidosis should be suspected in any patient with nephrotic range proteinuria, infiltrative cardiomyopathy, peripheral neuropathy, hepatomegaly, symptoms of bowel pseudo-obstruction, or atypical multiple myeloma.

**Screening for Amyloid**

Amyloidosis is a plasma cell dyscrasia with a small monoclonal population of plasma cells in the bone marrow, and this knowledge can be used to advantage in screening for the disease. Since the amyloid deposits are composed of monoclonal light chains and heavy chains, most patients will have a detectable immunoglobulin abnormality either by immunofixation of serum, immunofixation of a 24-hour urine specimen, or a detection of an abnormal immunoglobulin-free light chain (Freelite®). Screening electrophoresis is inadequate since 20% of patients with amyloidosis will not have an intact immunoglobulin protein in the serum or a level too low to demonstrate a spike on the electrophoretic pattern (Figure 2). It is mandatory that urine be screened in a patient with a compatible syndrome (Figure 3). When the serum and the urine are studied by immunofixation, nearly 90% will have a detectable monoclonal light chain. The immunoglobulin-free light chain nephelometric assay will be abnormal in three-quarters of the remaining patients in support of a tentative diagnosis of amyloidosis. When screening for amyloid, immunofixation has a higher sensitivity (90%) than amyloid stains performed on routine biopsy specimens such as fat (73%) or the bone marrow (72%).

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**Figure 2. Serum M proteins in amyloidosis.**
The pie chart gives the immunoglobulin heavy (A, G, D, M) and light chain seen at diagnosis (K = kappa, L = Lambda, 0 = none).

**Figure 3. Urine M proteins in amyloidosis (g/24 hr).**
How Is the Diagnosis of Amyloidosis Confirmed?
When a patient is seen with one of the clinical syndromes in Figure 1 and is confirmed to have an immunoglobulin light chain abnormality by immunofixation or nephelometry, the index of suspicion for amyloid is high. As in all hematologic malignancies, biopsy verification of the diagnosis is required. All patients with amyloid nephrotic syndrome, amyloid cardiomyopathy, amyloid liver disease, or amyloid neuropathy can be confirmed with biopsy of the kidney, heart, liver, or sural nerve. Biopsies of the kidney and liver carry a risk of bleeding and often necessitate overnight hospitalization for the patient. Case reports exist of severe bleeding following liver biopsy and, rarely, hepatic rupture. These small risks can be avoided if the clinician is aware that amyloid is a likely diagnosis. Techniques exist that are easier and less expensive and that result in minimal risk to the patient. Congo red staining of a bone marrow biopsy will demonstrate amyloid in at least 60% of patients. A marrow biopsy is required since amyloid deposits are rarely seen in the marrow aspirate. The subcutaneous fat aspirate demonstrates amyloid deposits in 70%–80% of patients (Table 2). Other centers do minor salivary gland biopsies and gingival biopsies. The rectal biopsy still remains a sensitive diagnostic technique. Only 13% of amyloidosis patients have a negative bone marrow and fat aspirate. Because of the very low prevalence of amyloid, use of the fat aspirate as a screening tool in patients presenting with peripheral neuropathy in the absence of a monoclonal protein disorder has an extremely low yield.

Once amyloidosis is proven by tissue biopsy, one must be certain that the amyloidosis is of the AL type. When patients have a free light chain in the serum or urine, the likelihood of AL is high, but immunohistochemical staining of the biopsied amyloid deposits with kappa and lambda antisera to re-affirm the diagnosis is appropriate. It should also be kept in mind that nearly 3% of adults have MGUS. In patients who have intact immunoglobulin proteins in the serum with no detectable free light chain (FLC), the possibility of an incidental MGUS with a non-immunoglobulin form of amyloid must be kept in mind. Inherited forms of renal amyloidosis due to a mutant fibrinogen A-alpha chain have been described. This presentation is easily confused with nephrotic syndrome associated with light chain amyloid. Immunostains of available tissue for deposits of fibrinogen or transthyretin, which cause inherited amyloidosis, are important to reliably exclude a non-immunoglobulin form of amyloid.

Amyloid cardiomyopathy occurs with high frequency in men over the age of 80, so-called senile cardiac (systemic) amyloid. There is an inherited form of amyloid specific to African-Americans. A mutation of transthyretin is carried by 3.9% of African-Americans, which would translate to 1.3 million adults in the United States. African-American men over the age of 70 with cardiac amyloidosis should be screened for mutant transthyretin (Ile 122). Immunohistochemical characterization of amyloid deposits is helpful in confirming the subunit protein comprising the amyloid. All amyloid deposits contain P (pentagonal) component. P component is a glycoprotein comprising 20% of the amyloid fibril by weight. We routinely do amyloid typing with P component, as a positive control, as well as kappa and lambda to confirm the diagnosis. If the kappa and lambda results are negative, testing for transthyretin, fibrinogen, and occasionally lysozyme and apolipoprotein A is warranted. Micromethods have been developed that permit mass spectroscopic screening of small samples to determine the subunit protein of amyloid.

The nephelometric analysis for serum immunoglobulin free light chains enhances one’s ability to confirm the type of amyloid as AL. These antisera recognize epitopes of FLCs but do not detect light chains associated with an intact immunoglobulin molecule. When we applied this technique to 100 AL patients, the patients who had negative serum immunofixation showed an abnormal FLC ratio in 85% of kappa and 80% of lambda patients. When there was no monoclonal protein in the serum or in the urine by immunofixation, the FLC technique detected a kappa protein in 86% of kappa amyloid and a lambda in 30% of lambda amyloid. The detection of FLCs by the nephelometric system is particularly important in those patients who do not have light chains by immunofixation. We routinely measure the light chain in the serum of all patients, both to confirm its immunoglobulin light chain origin as well as to monitor therapy. In one study, nearly 10% of patients who were thought to have immunoglobulin light chain amyloid had amyloid due to other types, including 5% with fibrinogen amyloid and 4% with transthyretin mutations. Inherited amyloidosis should be considered in all patients before therapy is initiated. The typing of the amyloid deposit is important because the different forms are clinically indistinguishable from each other. Renal amyloid due to long standing infection (AA) presents to the clinician identically to primary renal amy-
l oid. Amyloid neuropathy due to a mutation of TTR presents with all the same clinical features of neuropathy seen in primary systemic amyloidosis. The tissues all appear the same by light and electron microscopy.7,8

**Prognosis**

The most common cause of death in amyloid is cardiac, either due to progressive congestive cardiomyopathy or sudden death due to ventricular fibrillation or asystole. Clinical outcome in patients and their likelihood of responding to treatment is, in large part, determined by the extent of cardiac involvement at diagnosis.9 Previously, echocardiography with Doppler studies of diastolic function was critical in the assessment of patients newly diagnosed with AL. The recent introduction of strain echocardiography has added significant sensitivity in the assessment of cardiac function in AL.10 Echocardiography is routinely done in all newly diagnosed patients and every 6 months during therapy. The presence of heart failure is associated with a median survival of only 6 months and is the most important clinical predictor of survival. Echocardiography allows measurement of both the ejection fraction and the interventricular septal thickness, both of which are important in predicting outcomes in patients with amyloid. Doppler echocardiography is used to measure diastolic performance and relaxation of the ventricle during diastole. If the deceleration time is 150 ms or less by Doppler, the 1-year survival is 49%. New measures of myocardial injury that are more reproducible than the echo have recently been introduced. Measurement of serum troponin T, a sensitive marker for ischemic cardiac injury,11 has been shown to be a powerful predictor of survival in amyloidosis patients, both those treated conventionally12 as well as those who become candidates for stem cell transplantation.13 Serum troponin levels of less than 0.03, 0.03 to 0.1, and greater than 0.1 have permitted classification of AL patients into three groups of approximately equal size with differing survivals.

The N terminal fragment of pro-brain natriuretic peptide NT-Pro BNP is produced when the atria are dilated.14 Elevation of the NT-Pro BNP has been shown to be predictive of survival following a diagnosis of amyloid. Combining the troponin with the NT-Pro BNP level has resulted in a new staging system. These two tests should be measured in all newly diagnosed patients with amyloidosis. Although a weaker prognostic indicator, the serum level of β2-microglobulin is valuable. Levels greater than 2.7 µg/mL predict shorter survival.

In conclusion, echocardiography, serum β2-microglobulin, troponin T, and NT-Pro BNP are important in assessing the prognosis in patients with amyloidosis.

**Assessing the Response in Amyloidosis**

Most centers define responses in amyloidosis based on suppression of the precursor immunoglobulin light chain. Unlike those with multiple myeloma, AL patients frequently do not have a quantifiable immunoglobulin protein in the serum, and serial measurement of the urine M protein can be fraught with difficulty, particularly in those patients who have albuminuria from renal amyloidosis. The nephelometric assay for immunoglobulin FLCs is an adjunct to assess response to therapy. Organ response parallels changes in the serum FLC assay. We serially evaluate the immunoglobulin serum free light chain and consider a 50% reduction to indicate a hematologic response and a normalization of the level to reflect a complete hematologic response. This technique has been incorporated into evaluation of response at most amyloidosis treatment centers.15

An accurate diagnosis of amyloidosis and its subtype classification is essential prior to treatment.16 In AL, the median survival is approximately 2 years and is less than 6 months when there is significant cardiac disease. The early recognition of amyloidosis using the algorithm listed below and the careful distinction between immunoglobulin light chain amyloid and the non-immunoglobulin forms of amyloid is critical because systemic therapy17 and transplantation18,19 will not have any benefit in the other forms of amyloid (Table 3).20,21

**Table 3. Key points—diagnostic pathway for amyloidosis.**

1) Consider AL in differential if:
   - Nondiabetic nephrotic syndrome
   - Cardiomyopathy nonischemic: echo shows “left ventricular hypertrophy (LVH)”
   - Hepatomegaly with no scan defects
   - Chronic inflammatory demyelinating polyneuropathy
   - “Atypical myeloma” urine light chain + and marrow < 10% plasma cells

2) Perform immunofixation serum, urine, and immunoglobulin free light chain assay. If positive, amyloidosis becomes a likely explanation.

3) Biopsy bone marrow and subcutaneous fat. Do Congo red stains. Biopsy of kidney or liver are usually not required.


5) Initiate therapy.

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