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## Amyloidosis

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Amyloidosis is an uncommon plasma-cell dyscrasia with an incidence of eight patients per million per year. It is often difficult to recognize because of the myriad symptoms and vague nature of the clinical presentation. Symptoms include fatigue, dyspnea, edema, paresthesias, and weight loss. Clinical syndromes at presentation include nephrotic-range proteinuria with or without renal insufficiency, cardiomyopathy, hepatomegaly, symptomatic peripheral neuropathy, and autonomic failure. Recent advances have occurred in evaluation of patients by using the free light chain assay and new prognostic assessments with cardiac biomarkers. Newly developed therapeutic strategies, involving high-dose and intermediate-dose chemotherapy, have evolved in the last 3 years. This paper reviews a diagnostic pathway clinicians can use to diagnose the disorder, assess a patient's prognosis, and logically plan a therapeutic strategy.

**Key words:** amyloid; amyloidosis; cardiomyopathy; monoclonal gammopathy; multiple myeloma; nephrotic syndrome; stem-cell transplantation.

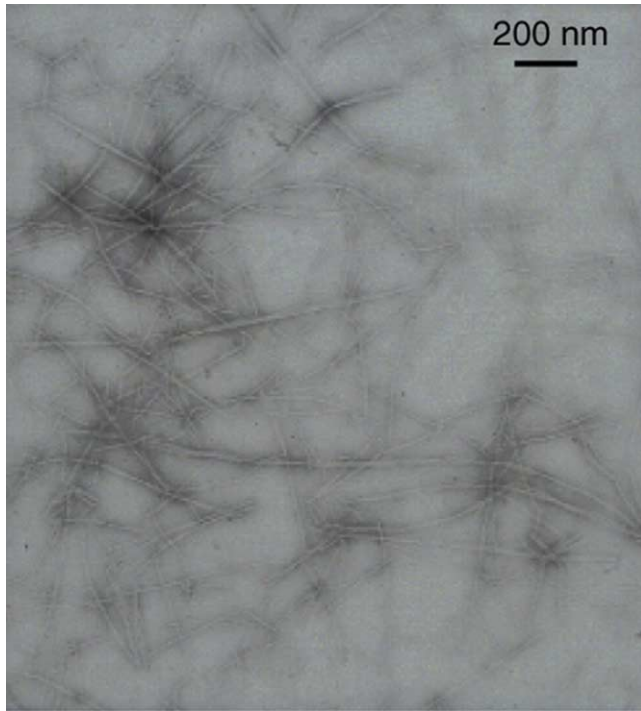
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Amyloidosis results from the extracellular deposition of fibrillar amyloid protein (Figure 1).<sup>1</sup> Amyloid is defined by the tinctorial properties of binding of Congo red dye and green birefringence under polarized light.<sup>2</sup> X-ray diffraction microscopy demonstrates that amyloid is a protein that configures as a  $\beta$ -pleated sheet rather than the normal  $\alpha$ -helical structure of physiologic proteins.<sup>3</sup> Amyloidosis is a generic term and includes all forms of systemic amyloidosis, those related to light chain deposits, amyloid A protein, and inherited forms, which include transthyretin, apolipoprotein, lysozyme, and fibrinogen. It also includes the forms of localized amyloidosis that are seen in the brain in Alzheimer disease, genitourinary tract,

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**Figure 1.** Electron micrograph demonstrating the fibrillar ultrastructure of amyloid.

tracheobronchial tree, and skin.<sup>4</sup> The form of amyloidosis that is associated with the shortest survival and poorest outcome is primary, also known as immunoglobulin light chain amyloidosis (AL). A structural subunit of this form of amyloid is the monoclonal (M) immunoglobulin light chain derived from plasma cells or lymphoplasmacytic cells, usually in the bone marrow.<sup>5</sup>

The symptoms of the disorder are vague. They include fatigue, edema, and weight loss, and are generally not helpful in the formulation of an appropriate differential diagnosis.<sup>6</sup> Occasional patients are recognized initially by the hematologist because they have an M protein with a small percentage of plasma cells in the bone marrow<sup>7</sup>, and the disease is mislabeled as atypical multiple myeloma with no clear-cut explanation for the fluid retention, weight loss, and fatigue. Because there is no diagnostic blood test, radiograph, or scan, a heightened awareness of this entity is essential to identify patients. Patients can present in several ways. Patients whose disease is ultimately diagnosed because of nephrotic syndrome frequently are treated empirically with high-dose corticosteroids for the possibility that they may have minimal change disease<sup>8</sup>, membranous nephropathy, or membranoproliferative glomerulonephropathy. Often, patients with nephrotic syndrome develop dramatic increases in lipid values and are treated for hypercholesterolemia without recognition that it is due to heavy proteinuria.

Patients with cardiac amyloid frequently fail to develop cardiomegaly or interstitial pulmonary edema and have no ischemic symptoms. Therefore, the normal chest radiograph offers no clues as to the etiology of fatigue and dyspnea, and these patients

are often mistakenly thought to have non-cardiac dyspnea. The patients are seen regularly by pulmonologists and occasionally by psychiatrists when the symptoms are not believed to be organic. Peripheral neuropathy often is treated empirically as chronic inflammatory demyelinating polyneuropathy or monoclonal gammopathy-associated neuropathy without a sufficient diagnostic investigation to exclude amyloid neuropathy.<sup>9</sup> In our experience, patients have symptoms for a median of 2 years before the correct diagnosis is established.<sup>10</sup> Patients with unexplained hepatomegaly are usually thought to have metastatic malignancy and undergo liver biopsy (without complication). AL is a surprise finding.<sup>11</sup>

Five important questions need to be addressed by each physician evaluating patients with amyloid.

1. When should the diagnosis of AL be considered?
2. If a diagnosis is under consideration, what is the appropriate diagnostic evaluation?
3. If there is a strong suspicion, how is the diagnosis confirmed?
4. What is the prognosis for patients with known disease?
5. What is the appropriate therapy?

Physical findings in AL include tongue enlargement, periorbital purpura, and periarticular amyloid infiltration (shoulder pad sign). When present, these are specific for amyloid but can be easily overlooked and occur in only approximately 15% of patients with AL. Symptoms and signs without physician awareness will result in misdiagnosis. The organs most often involved with amyloid include the kidney, liver, heart, and nerves. In any adult patient with nephrotic-range proteinuria, AL should be part of the differential diagnosis. Non-diabetic patients with nephrotic syndrome are found to have renal amyloid 10% of the time.<sup>12</sup> Nearly half of patients with AL have cardiomyopathy demonstrable by echocardiography.<sup>13</sup> The symptoms can range from none to easy fatigability to advanced heart failure. Any patient who has cardiac symptoms including easy fatigability, dyspnea, or edema and has no history of ischemic heart disease and no symptoms suggestive of exertional angina should be screened for amyloid, as outlined below.

The electrocardiogram can show low voltage, easily overlooked, or a pseudo-infarction pattern, which can be misinterpreted as silent ischemia.<sup>14</sup> Echocardiography shows wall infiltration, which most often is interpreted as hypertrophy.<sup>13</sup> Most patients with cardiac symptoms undergo echocardiography. If valvular disease is not present and there is wall thickening without hypertension, amyloid must be considered, particularly if there is no suggestion of ischemia. Patients with heart failure have undergone cardiac catheterization, and when no coronary artery disease was found, the evaluation was terminated although a right-sided endomyocardial biopsy would reveal amyloid. All patients with an unexplained cardiac disorder or unexplained proteinuria should be screened for the possibility of immunoglobulin light chain amyloid. Fifteen percent of patients with amyloid have hepatic involvement.<sup>11</sup> Most often this manifests as palpable hepatomegaly with increased serum alkaline phosphatase concentration. Radionuclide liver scan, computed tomographic scan, and magnetic resonance image usually show little or no abnormality. The primary symptoms of hepatic amyloidosis are unexplained weight loss, early satiety, and right upper quadrant fullness. In addition to the usual studies for hepatitis, biliary cirrhosis, and other infiltrative liver disorders, screening for immunoglobulin light chain amyloid is mandatory.

The neuropathy of AL can be axonal and demyelinating and is seen in 15% of AL patients.<sup>10</sup> Symptoms are primarily in the lower extremities. Sensory changes tend to

precede motor changes. Upper extremity involvement occurs late. Delays of 2 years are common between the onset of paresthesias and a diagnosis. An important clue is the presence of associated autonomic neuropathy. This can be gastrointestinal (GI) tract neuropathy manifested in the lower GI tract as diarrhea or constipation, in the upper GI tract as pseudo-obstruction and vomiting, and in the autonomic nervous system as orthostatic hypotension, syncope, and impotence. The peripheral neuropathy is painful and frequently requires analgesics. The use of amitriptyline and gabapentin is common but frequently provides inadequate symptom relief. Often, these patients are found to have an M protein and are misdiagnosed as having monoclonal-gammopathy-associated neuropathy or multiple-myeloma-associated neuropathy and, rarely, macroglobulinemia-associated neuropathy, without sufficient testing to exclude amyloid as a possibility.<sup>15</sup>

In conclusion, any patient with nephrotic-range proteinuria, unexplained non-ischemic cardiomyopathy, peripheral neuropathy, unexplained hepatomegaly, pseudo-obstruction, or atypical multiple myeloma should be screened for amyloid.<sup>16</sup>

### SCREENING FOR AMYLOID

By definition, immunoglobulin light chain amyloid is a plasma-cell dyscrasia, and a monoclonal population of plasma cells in the bone marrow should always be detected if sufficiently sensitive techniques are used.<sup>7</sup> The amyloid deposits are composed of the N-terminus of immunoglobulin light or heavy chains.<sup>17</sup> Most patients have an immunoglobulin abnormality detectable by immunofixation of serum or urine, or detectable abnormal free light chain level or free light chain ratio by the new nephelometric free light chain assay.<sup>18</sup> Screening serum protein electrophoresis is inadequate because more than 25% of patients do not have an intact immunoglobulin protein in the serum or have Bence Jones proteinemia, which rarely produces a spike on the electrophoretic pattern (Figure 2). The urine also must be screened by

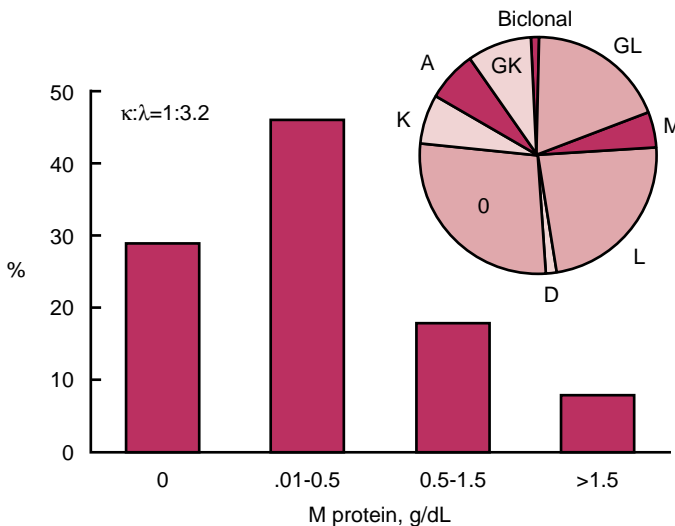
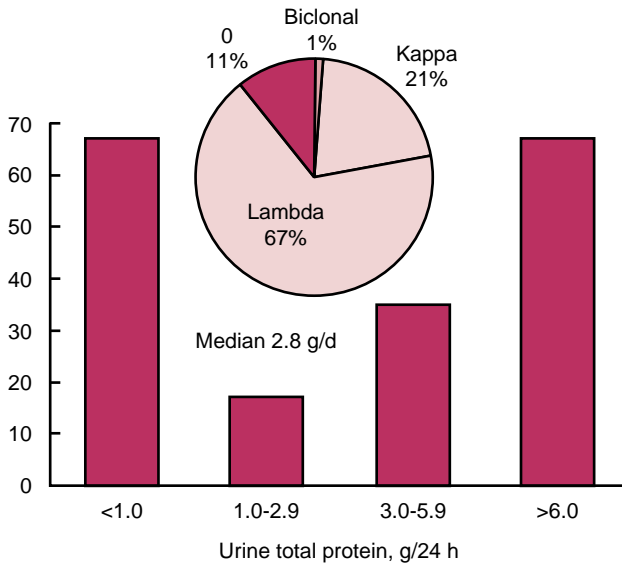


Figure 2. Serum protein electrophoresis and serum immunofixation in 185 patients with AL.

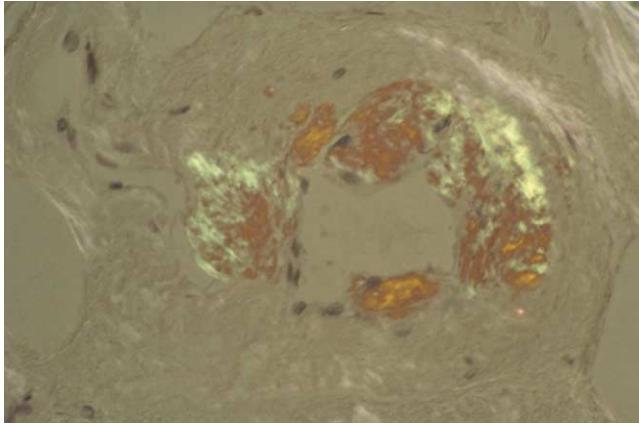


**Figure 3.** Urine immunofixation and 24-hour urine total protein in patients with AL.

immunofixation (Figure 3). If the serum and the urine are studied by immunofixation, nearly 90% of these patients have a detectable monoclonal light chain. The immunoglobulin free light chain nephelometric assay result is abnormal in three quarters of the remaining patients and is useful in raising one's suspicion of amyloidosis.<sup>18</sup> In patients who had a positive urine immunofixation result but a negative serum immunofixation result, an abnormal free light chain is seen in 85% with  $\kappa$  amyloid and 80% with  $\lambda$  amyloid. Overall, immunofixation of serum and urine and the free light chain assay constitute the best non-invasive screens for AL, and a positive finding can justify more invasive diagnostic studies to confirm the diagnosis. Radioscintigraphy with radiolabeled amyloid P component can also detect amyloid deposits<sup>19</sup> non-invasively, but this technique is not widely available.

## HOW IS THE DIAGNOSIS OF AMYLOIDOSIS CONFIRMED?

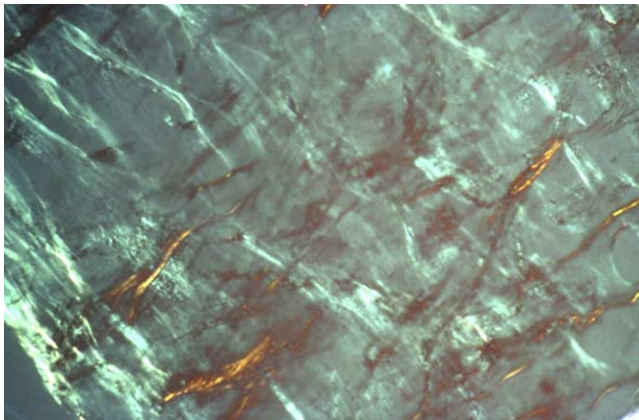
If a patient has a compatible clinical syndrome as outlined above and is confirmed to have an immunoglobulin light chain abnormality by immunofixation or nephelometry, it is likely that AL is present. Biopsy verification of the amyloid is required, but a visceral biopsy is not usually necessary. Although patients with amyloid nephrotic syndrome, cardiomyopathy, hepatomegaly, or neuropathy will have the diagnosis established by renal, cardiac, liver, or nerve biopsy, respectively, the majority of patients do not need to have this invasive, potentially risky procedure. Renal and hepatic biopsies carry a small risk of bleeding and often require overnight hospitalization. Rarely, severe bleeding has been reported after liver biopsy, as has hepatic rupture.<sup>20</sup> If the likelihood of amyloid is high, then the diagnosis can be established by doing Congo red stains on a bone-marrow biopsy specimen (Figure 4), which will detect amyloid deposits in 50–60% of patients. The subcutaneous fat aspirate is a convenient, non-invasive technique that



**Figure 4.** Congo red staining viewed under polarized light demonstrates a blood vessel found on a bone-marrow biopsy ( $\times 1000$ ).

demonstrates amyloid deposits in 70–80% of patients (Figure 5). Other centers perform biopsies of the minor salivary glands, gingiva, rectum, and skin, and all can demonstrate deposits at little risk to the patient. Because amyloid has a low prevalence in the general population, use of the fat aspirate as a screening tool in patients unlikely to have amyloid not only results in a low yield but a significant number of false positives due to overstaining of the specimen with Congo red.<sup>21</sup>

When a diagnosis of amyloidosis is established histologically, one must confirm that the amyloid is the AL type. When patients have a light chain present by immunofixation or nephelometry, the likelihood of AL is high, but immunohistochemical verification of the diagnosis with  $\kappa$  and  $\lambda$  antisera is essential.<sup>22</sup> Three percent of older adults have monoclonal gammopathy of undetermined significance, and the possibility of a non-immunoglobulin form of amyloidosis associated with an incidental monoclonal gammopathy of undetermined significance must be considered.<sup>23</sup> Clinically, it is difficult to distinguish primary, secondary, familial, and senile systemic forms of amyloid from



**Figure 5.** Subcutaneous fat aspirate stained with Congo red and viewed under polarized light ( $\times 1000$ ).

each other. Half of the patients with inherited amyloidosis do not have a positive family history. Patients with secondary amyloidosis typically have renal, intestinal, and thyroid involvement histologically indistinguishable from AL.<sup>24</sup> Patients with hereditary amyloidosis can have renal involvement, hepatic involvement, and neuropathy. Patients with senile systemic amyloid most commonly present with amyloid cardiomyopathy in which echocardiographic features are difficult to distinguish from AL.<sup>25</sup>

Immunohistochemical staining of tissues with commercially available antisera helps confirm the nature of the amyloid. All forms of amyloid contain amyloid P component, and antisera to the P component provide a positive control for immunohistochemical studies.<sup>26</sup> Staining amyloid deposits for  $\kappa$  and  $\lambda$  immunoglobulin light chains, amyloid A, and transthyretin will detect most forms of amyloid. When positive,  $\kappa$  and  $\lambda$  immunostaining of amyloid deposits is quite specific but may not be sensitive because commercial antisera may not recognize immunoglobulin light chain-derived components in their amyloid conformation.<sup>27</sup> Electron and immunoelectron microscopy of specimens have been used successfully to characterize amyloid fibrils by using immunogold.

There is an inherited form of amyloid that causes cardiomyopathy in African Americans.<sup>28</sup> This mutation is carried by 3.9% of African Americans. Any African American older than 70 years with cardiac amyloidosis should be screened for a mutant transthyretin as the cause of the amyloid. Fibrinogen A $\alpha$  causes nephrotic syndrome and can be detected immunochemically. Micromethods have been developed that permit screening of small samples to determine the subunit protein of amyloid.<sup>29</sup> In one study, 10% of patients who were thought to have immunoglobulin light chain amyloid had amyloid of other types, including 5% with fibrinogen amyloid and 4% with unrecognized mutations of transthyretin, causing an unrecognized inherited amyloid syndrome.<sup>23</sup> Exclusion of inherited amyloidosis is important before therapy is initiated.

## PROGNOSIS

The most common cause of death in AL is progressive congestive cardiomyopathy or sudden death due to ventricular fibrillation.<sup>30</sup> The clinical outcome in patients—and even the likelihood of a response to therapy—are in large part determined by the extent of cardiac involvement at diagnosis. Echocardiography with Doppler studies to assess diastolic function is important in the assessment of all patients with newly diagnosed AL, whether or not cardiac symptoms are present. Echocardiography is performed routinely on all patients every 6 months after diagnosis.<sup>31</sup> The presence of overt heart failure is associated with a median survival of only 6 months and is the most important predictor of survival.<sup>32</sup> Echocardiography allows measurement of ejection fraction and interventricular septal thickness, both of which are important in predicting outcomes in patients with amyloid. Doppler echocardiography measures diastolic performance and the extent of relaxation abnormalities of the ventricle during diastole. Measurement of the deceleration time provides important information that correlates well with survival.<sup>31</sup>

The serum  $\beta_2$ -microglobulin value has repeatedly been shown in single- and multi-institutional studies to be important prognostically in AL.<sup>33</sup> In multiple myeloma,  $\beta_2$ -microglobulin is important but is thought to reflect the total tumor mass. In AL, the percentage of bone-marrow plasma cells is generally low. The increased  $\beta_2$ -microglobulin concentration is clearly not related to the plasma-cell

burden but predicts survival. A combination of the  $\beta_2$ -microglobulin value and degree of cardiac involvement can reliably separate patients into groups with distinct survivals.<sup>34</sup>

New measures of myocardial injury that are more reproducible and less subject to interobserver interpretation have been developed recently.<sup>35</sup> These biomarkers include serum troponin T. Troponin is a known sensitive marker for ischemic cardiac injury but has been shown to be a powerful predictor of survival in AL for those treated with conventional chemotherapy and those who receive high-dose therapy followed by stem-cell transplantation. The three break points for serum troponin include  $<0.03$ ,  $0.03\text{--}0.1$ , and  $>0.1$ . This allows stratification of patients into three groups with markedly different survivals.

The N-terminal fragment of pro-brain natriuretic peptide (NT-pro-BNP) is produced when the atria are dilated. Increased concentration of NT-pro-BNP has been shown to predict survival after the diagnosis of amyloid, and a normal NT-pro-BNP virtually excludes cardiac amyloidosis.<sup>36</sup> The NT-pro-BNP value is also a powerful prognostic factor. A combination of the serum troponin value with the NT-pro-BNP (or BNP) value can be used as a new staging system for AL.<sup>37</sup> Troponin and BNP or NT-pro-BNP should be measured routinely in all patients with AL, as should the serum  $\beta_2$ -microglobulin concentration.

## ASSESSING THE THERAPEUTIC EFFECT

Most experienced centers that see large numbers of patients with amyloid define responses by the suppression of the precursor immunoglobulin light chain. This is done in a fashion similar to the assessment of response in multiple myeloma: 50% reduction in serum and urine M protein.<sup>38</sup> Unlike multiple myeloma, however, most patients with AL do not have a quantifiable immunoglobulin protein in the serum, and the measurement of the size of the urine M spike can be difficult, particularly because so many patients have high levels of urinary albumin excretion due to kidney involvement with amyloid. The nephelometric immunoglobulin free light chain assay helps quantify M proteins and assess therapeutic response.<sup>39</sup> A hematologic response to therapy correlates well with subsequent organ response.<sup>40</sup> In other words, a  $\geq 50\%$  reduction in the serum and urine M protein generally leads to reduction in urinary protein loss, stabilization or improvement in cardiac function measured by echocardiography, and reduction in the serum alkaline phosphatase concentration in patients who have hepatic amyloid. We measure the immunoglobulin free light chain and light chain ratio in all patients who are on therapy and consider a 50% reduction to reflect a hematologic response and a normalization of the value and ratio to reflect a complete hematologic response. An attempt to define organ response criteria via a consensus panel of amyloidosis experts is under way.

The median survival of patients with AL is approximately 2 years but varies from center to center, in part related to the nature of referral patterns. In patients with significant cardiac disease, the median survival is less than 6 months. Table 1 provides an algorithm for the evaluation of patients suspected of having amyloid and should include testing to help distinguish light chain amyloid from non-light chain amyloid. Patients should not begin therapy until it is certain that the amyloid is of light chain origin.

**Table 1.** Diagnostic pathway for AL.

1. Consider AL in differential diagnosis if:
  - non-diabetic nephrotic syndrome
  - cardiomyopathy non-ischemic: echocardiogram shows left ventricular hypertrophy
  - hepatomegaly with no scan defects
  - chronic inflammatory demyelinating polyneuropathy
  - 'atypical myeloma,' urine light-chain-positive, and marrow <10% plasma cells
2. Perform immunofixation of serum and urine and immunoglobulin free light chain assay. If positive, AL becomes a likely explanation
3. Biopsy bone marrow and subcutaneous fat. Do Congo red stains. Biopsy of kidney or liver is usually not required
4. Assess prognosis. Echocardiography with Doppler. Measure serum troponin, NT-pro BNP or BNP, and  $\beta_2$ -microglobulin
5. Initiate therapy

## NON-SYSTEMIC AMYLOID

Occasionally, patients present with localized forms of amyloid. These patients do not require systemic therapy, and management can be either local or supportive. Typically, the location of the amyloid deposit is a clue to the localized nature. Amyloidosis is localized when confined to the ureter, bladder, urethra, or prostate.<sup>41,42</sup> Most forms of cutaneous amyloid are also localized.<sup>43</sup> Tracheobronchial amyloid<sup>44</sup> and pulmonary nodules<sup>45</sup> associated with amyloid are also typically localized. Soft tissue deposits of amyloid may or may not be associated with systemic disease.<sup>46</sup> Patients with localized forms of amyloid do not have a demonstrable systemic plasma-cell dyscrasia.

Ideally, modalities to dissolve the amyloid deposits or prevent the accumulation of toxic intermediates would be the best strategy to manage amyloid. If amyloid deposits could be rendered soluble, then patients would effectively have monoclonal gammopathy of undetermined significance. Unfortunately, this therapy does not exist, and the current approach is to attempt to prevent precursor protein production by directing therapy at the clonal population of plasma cells that is responsible for light chain synthesis.

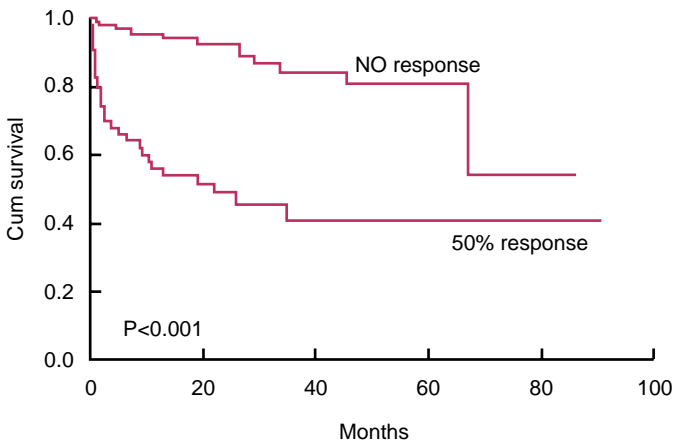
## THERAPY

Stem-cell transplantation is being used increasingly to treat patients with AL. However, most patients evaluated at Mayo Clinic are not eligible for the technique, and the treatment carries unique problems resulting in a mortality rate of 12%.<sup>47</sup> Patients selected for stem-cell transplantation by definition have better cardiac function and do not have advanced hepatic or renal failure.<sup>48</sup> The presence of significant visceral organ dysfunction places these patients at a higher risk for complications, including sudden cardiac death and profound GI tract bleeding.<sup>49</sup>

We have completed transplantation in 188 patients and have up-to-date follow-up information on 171. The amyloid was  $\kappa$  in 24% and  $\lambda$  in 76% of patients. The kidney was involved in 67%, the heart in 49%, the nerves in 15%, and the liver in 18%.

Our conditioning is risk-adjusted in that patients with advanced cardiac amyloid received reduced-dose melphalan, as did patients with increased creatinine values and multiorgan involvement. Twenty percent of our patients had an echocardiographic ejection fraction of less than 60% at transplantation. The histologic diagnosis of AL was established by rectal biopsy in 14 of 17, renal biopsy in 84, heart biopsy in 30, and liver biopsy in 11 of 12. A fat aspirate was positive in 111 of 153 patients (73%) and a bone-marrow biopsy was positive in 125 of 166 patients (75%). A previously published algorithm is used to stratify patients, either those eligible for full-dose conditioning with melphalan at 200 mg/m<sup>2</sup> or those who received 140 mg/m<sup>2</sup>.<sup>50</sup> The presence of a response has a profound effect on outcome. Our response rate was 68% overall (117 of 171). Non-responders had a median survival of 12.6 months, and responders will have a median survival that will exceed 6 years (Figure 6). There is no survival difference among responders as a function of the conditioning dose, although the proportion of responders appears to be higher in patients who receive higher doses. Treatment-related mortality was associated with the number of organs involved and the serum creatinine value before transplantation. Median survival has not been reached for patients with one or two organs involved at the time of transplantation and was 21.5 months for patients with more than two organs involved at the time of transplantation. Treatment-related mortality was primarily due to sudden cardiac death, although two patients died of massive hepatic failure and three of post-chemotherapy renal failure and dialysis-related complications.

We generally do not use cytoreductive chemotherapy before stem-cell transplantation. It has been demonstrated that two cycles of melphalan and prednisone before transplantation actually complicate the mobilization procedure because of the effect of alkylating agents on the stem-cell compartment.<sup>51</sup> Because patients with AL have only 5% plasma cells on average at initial diagnosis, there are no data as yet to suggest that up-front cytoreduction contributes to improved subsequent outcome. Investigators at Boston University reported on 312 patients among 701 seen in the same time period who proceeded with stem-cell transplantation (45%). Median survival was 4.6 years.<sup>52</sup> A complete hematologic response was achieved in 40% and predicted prolonged



**Figure 6.** Survival after stem-cell transplantation in amyloidosis compares hematologic responders to non-responders.

survival. The treatment-related mortality rate was 13%. Patients with cardiomyopathy had the highest mortality rate. Criteria for melphalan at  $200 \text{ mg/m}^2$  were age  $< 65$  years, ejection fraction  $> 45\%$ , and stem-cell yield  $> 2.5 \times 10^6$ . The median survival for patients with cardiac involvement was 1.6 years. Our current goal for CD34 cell yield is  $4 \times 10^6$  CD34 cells/kg, but we will perform stem-cell transplantation in patients if the yield is only 2 million, anticipating a longer time to engraftment.

The optimal conditioning regimen has not been determined. Most centers use melphalan alone, but a feasibility study using total body irradiation alone has been reported recently, although its transplant-related complication rate did not appear to be lower than that for melphalan.<sup>53</sup> Attempts at tandem transplantation have been reported, but only a small proportion of patients eligible before the first transplantation will actually survive to the completion of the second transplantation, although an incremental complete response rate has been reported.<sup>54</sup> Post-conditioning renal insufficiency is one of the most serious complications seen in patients with AL receiving a stem-cell transplant. Among the 80 patients we reviewed, an increase in the serum creatinine value of at least  $0.5 \text{ mg/dL}$  was seen in 19% of patients. Age, hypoalbuminemia, heavy proteinuria, diuretic use, and urine sediment score were risk factors. The only independent risk factors for post-conditioning renal insufficiency were age and urine sediment score. These patients were more often dialyzed and had an inferior 1-year survival. We thought that melphalan was the causative agent of post-conditioning renal insufficiency, although ongoing tubular injury may be a prerequisite for post-conditioning renal insufficiency.

Most centers report clonal responses after stem-cell transplantation of 50–60%, which is approximately twice that reported with conventional melphalan and prednisone therapy.<sup>55,56</sup> Note that these patients are highly selected before transplantation, and this may influence outcomes. In fact, eligibility for stem-cell transplantation is a clear predictor of an improved survival compared with patients who are not eligible for transplantation but are treated similarly.<sup>48</sup> Renal response is the most common organ response, although we have seen a high proportion of liver and heart responses. Responses of peripheral neuropathy remain uncommon. A case-matched control study comparing survival of amyloid patients undergoing stem-cell transplantation with those not undergoing transplantation did suggest a survival advantage for patients receiving high-dose therapy.<sup>57</sup> There is currently a prospective, randomized French trial of stem-cell transplantation versus melphalan and dexamethasone.

To improve the outcome of patients with amyloidosis and declining renal function, we have performed living donor kidney transplantation before stem-cell transplantation. This strategy has been used in eight of our patients, five of whom had progressed to end-stage renal disease by the time of kidney transplantation. Two patients did not undergo stem-cell transplantation. One died shortly after kidney transplantation from causes unrelated to the procedure, and another chose to delay stem-cell transplantation. At 4 years, his renal allograft function remains stable despite documented recurrence of amyloid in the allograft. Of the six who had received stem-cell transplants, one died after stem-cell transplantation. The other five are alive, with three having achieved a complete hematologic response. A new monoclonal gammopathy developed in one, and one has yet to be determined because of short follow-up. Immunosuppression did not interfere with engraftment or increase infectious complications. Renal allograft function was not altered by stem-cell transplantation.

## Conventional therapy

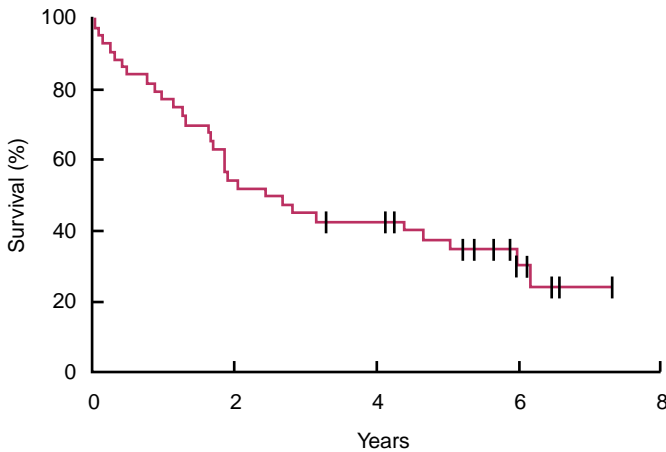
The first reports of the use of cytotoxic chemotherapy to treat AL were about 30 years ago.<sup>58</sup> Standard melphalan and prednisone therapy is effective for the treatment of AL.<sup>59,60</sup> Its disadvantages include the fact that it is often difficult to distinguish patients for whom therapy is destined to fail from those for whom an adequate trial has not been completed because it takes nearly a year to detect a response. Second, melphalan can cause myelodysplasia, and of all patients with amyloid initially exposed to melphalan for at least 1 year, myelodysplasia will develop in 7%.<sup>61,62</sup> Melphalan-based therapy fails in most patients with AL. No more than 30% of patients show evidence of a response, although this includes virtually all patients with amyloidosis without selection. The best responses occur in patients with nephrotic syndrome and a normal creatinine value. When carefully defined criteria are used, the overall response rate for all patients is only 18%.<sup>38</sup> A creatinine concentration > 3 mg/dL and an alkaline phosphatase concentration more than 4 times normal are rarely associated with an organ response. Responses occur, however, even in advanced amyloid cardiomyopathy. Eight of 153 patients with proven cardiac amyloidosis survived more than 5 years after the initiation of melphalan and prednisone therapy, and all showed evidence of a clear-cut hematologic response. The survival among responders to melphalan and prednisone is 78% at 5 years.<sup>38</sup> Among 810 patients with amyloid, all of whom received melphalan and prednisone, the 10-year survival rate was 4.7%.<sup>63</sup> Fourteen of the 30 10-year survivors had a complete hematologic response.

Two important prospective randomized studies of melphalan and prednisone compared with colchicine have been published; one, a three-arm study with 219 patients, showed a significantly superior survival in melphalan-treated patients (17 versus 8.5 months)<sup>64</sup>, and a second study of 100 patients comparing melphalan, prednisone, and colchicine with colchicine alone showed superior survival in the melphalan group (12.2 versus 6.7 months).<sup>65</sup> Colchicine was inferior therapy. Objective responses were seen in melphalan-treated patients. Continuous oral melphalan has been used as a single agent for patients with cardiac amyloidosis. Of 30 patients, seven of 13 who were evaluable achieved a partial hematologic response and three a complete hematologic response.<sup>66</sup>

## Dexamethasone-based regimens

Four patients were reported who received vincristine, doxorubicin (adriamycin), and dexamethasone (VAD). Two of the patients had a 50% reduction in the serum M protein, although the use of vincristine is not feasible in patients with neuropathy, and doxorubicin needs to be avoided in patients with significant cardiomyopathy. Three of four patients with amyloid nephrotic syndrome who were treated with VAD attained a partial response and were alive and in remission at 4.1, 6.5, and 9.3 years.<sup>67</sup> VAD has also been used as an induction scheme in an attempt to reduce patients' risk of transplant-related complications.<sup>68</sup> It has been suggested that clinical improvement after VAD reduces transplant-related mortality.<sup>69</sup> In 92 patients treated at the National Amyloidosis Center in London, four cycles of VAD were used. An organ response was seen in 39 patients (42%). The treatment-related mortality was 7%.<sup>39</sup>

Dexamethasone has been used as a single agent and combined with melphalan. In the first report of dexamethasone use, nine consecutive patients were treated with dexamethasone at 40 mg on days 1–4, 9–12, and 17–20 every 5 weeks. Improvement in



**Figure 7.** Survival in amyloidosis patients treated at Mayo Clinic with dexamethasone.

AL organ involvement was seen in eight of nine patients. Six of seven patients with nephrotic syndrome had a 50% reduction in proteinuria.<sup>70</sup> We have treated 19 patients, and only three had an objective organ response. This may have been related to patient selection.<sup>71</sup> Dexamethasone in previously treated patients also results in objective responses (Figure 7).<sup>72</sup> The toxicity of dexamethasone is substantial. Fluid retention occurs in patients with nephrotic syndrome and heart failure. Patients frequently require dose reduction to 20 mg per day.

The Southwest Oncology Group recently reported a multicenter study enrolling 92 patients with AL. Hematologic complete remissions were seen in 24% and improvement in amyloid-related organ dysfunction in 45%. The median survival of the entire group was 31 months. The 2-year overall survival was 60%. Heart failure and an increased  $\beta_2$ -microglobulin value predicted an adverse outcome. Patients with cardiomyopathy had a low response rate, as did patients with peripheral neuropathy. The median time to a hematologic response was 103 days. The median time to a complete response was 173 days, shorter than that seen in melphalan-treated patients. Patients with an organ response had significantly superior overall and progression-free survival. Toxic responses were greater in patients with more than two organ systems involved and GI tract involvement. In multivariate analysis, only heart failure predicted for excessive toxicity. These patients also received maintenance interferon. The role of interferon could not be separated from the overall benefit of the regimen.

Palladini et al<sup>40</sup> reported on a regimen of dexamethasone combined with melphalan. The hematologic response was 67% at a median of 4.5 months, with 33% complete responses and 48% organ responses. Treatment-related mortality was only 4% and translated into improved organ function in almost half of the patients. Heart failure resolved in 19%. Melphalan and dexamethasone therapy is a viable alternative to stem-cell transplantation, pending the results of the French phase 3 trial.

## Thalidomide

Sixteen patients were enrolled in a thalidomide study. The median maximum tolerated dose was 300 mg. Fifty percent of patients experienced grade 3–4 toxic responses.

Twenty-five percent had to discontinue the drug. Twenty-five percent of patients had a reduction in light chain proteinuria but not in total urinary protein loss.<sup>73</sup> We treated 12 patients; edema, cognitive difficulties and constipation were seen in 75%, and dyspnea, dizziness and rash in 50%. Progressive renal insufficiency developed in five, deep vein thrombosis and syncope in two. Median time on therapy was 72 days. Patients were intolerant of thalidomide.<sup>74</sup> When thalidomide was combined with dexamethasone to treat 31 patients with AL, only 11 tolerated 400 mg per day for a median of 5.7 months. Overall, 20 patients experienced severe grade 3 or greater treatment-related toxic responses (Merlini G et al, read at Tenth International Symposium on Amyloid and Amyloidosis, Tours, France, April 18–22, 2004). The National Amyloidosis Center reported using thalidomide at a median dose of 100 mg, but cessation of the drug was required in 31% because of toxicity. The hematologic response in patients treated with thalidomide alone was 55%. It is unclear why the response rate to thalidomide alone in AL was nearly double that for thalidomide used in patients with multiple myeloma. Serum amyloid P scintigraphy showed regression of amyloid in 18% of patients (Goodman HJB et al, read at Tenth International Symposium on Amyloid and Amyloidosis, Tours, France, April 18–22, 2004). Thalidomide and dexamethasone warrant further investigation in patients with AL. However, the dose that would be tolerated for the long term is unlikely to exceed 100 mg per day.

### Investigational therapies

Etanercept was used to treat patients with advanced AL for whom other therapies had failed and who were ineligible for other treatment regimens.<sup>75</sup> Sixteen patients with AL, including patients with severe cardiac or multiple organ involvement, were treated with etanercept and evaluated every 4–6 weeks for toxic and hematologic responses. Median treatment duration was 42 weeks. Fifty percent experienced objective improvement and 88% had subjective improvement in symptoms. Only one patient experienced an adverse event. Improvement in performance status for the group was statistically significant. Estimated median survival was 24.2 months. The survival of the eight patients with cardiac involvement was 13.2 months. Etanercept may provide a new therapeutic option for the management of AL.

Lacy et al<sup>76</sup> reported a clinical trial of dendritic cell-based idotype vaccination for AL. After apheresis, dendritic cell precursors were purified from peripheral-blood mononuclear cells and incubated *ex vivo* with patients' serum containing amyloid protein precursors diluted to 10 µg/mL. After 2 days of *ex vivo* expansion of dendritic cells, antigen-sensitized dendritic cells were infused into the patients at 2, 4, and 16 weeks. Ten patients were treated for cardiac, pulmonary, renal, liver, nerve, and GI tract amyloid in two, one, six, two, three, and two, respectively. Five had multiorgan involvement. Eight had been treated previously. There was no toxicity. One patient had a reduction in proteinuria from 1400 to 60 mg per day. One patient had improvement of painful peripheral neuropathy. Two patients have died. The patients who had a clinical response had a specific T-cell proliferative response to idotype. Improvement of the baseline immune function of AL patients would be essential to enhance response to vaccine strategies.

NC-531 is a sulfated glycosaminoglycan mimetic that can inhibit amyloid plaque formation and is in development by Neurochem for potential treatment of Alzheimer disease. Phase I clinical trials of NC-531 were ongoing in November 1999, and phase 2 trials were under way in October 2002. Because all forms of amyloid have a high

content of glycosaminoglycans, particularly heparan sulfate proteoglycan, this agent could have potential value in all forms of amyloidosis, including AL.<sup>77</sup>

Solomon et al<sup>78</sup> previously reported that murine antihuman light chain monoclonal antibodies recognized an epitope common to all types of AL and other types of amyloid fibrils. One such antibody, 11-1F4, was given to mice bearing AL amyloidomas induced by subcutaneous injection of human AL extracts. The monoclonal antibody bound to the amyloid had initiated an Fc-mediated cellular inflammatory response that led to rapid reduction in the tumor masses. A chimera of this antibody is now being produced, and its activity is being compared to that of unmodified antibody. The chimera antibody is able to interact with amyloid *in vitro*, and administration of the chimera reagent in the mice bearing human AL tumors resulted in marked reductions in amyloid burden with no evidence of toxicity, which has led to the decision to produce Good Manufacturing Practices grade 11-1F4 chimera for a phase I clinical trial in patients with AL. The use of amyloid-reactive antibodies is a new approach to the treatment of this disorder.

Pepys et al<sup>79</sup> have tested a competitive inhibitor of serum amyloid P that binds to amyloid fibrils. This compound cross-links and dimerizes serum amyloid P molecules, leading to their clearance by the liver. This produces a marked depletion of circulating amyloid P, and by removing it from the serum it is hoped that human amyloid deposits and fibrils will be destabilized and subsequently catabolized.

## SUMMARY

Amyloidosis should be considered in any patient with nephrotic syndrome, cardiomyopathy, unexplained hepatomegaly, or peripheral neuropathy. Initial evaluation includes immunofixation of serum and urine and an immunoglobulin free light chain nephelometric assay. Biopsies of the subcutaneous fat and bone marrow will lead to the diagnosis in more than 80% of patients, rendering invasive biopsy unnecessary. The prognosis of patients with AL can be assessed by using the biomarkers troponin T and NT-pro-BNP. Echocardiography and serum  $\beta_2$ -microglobulin measurement have prognostic value as well.

The optimal treatment of AL remains unknown, but most patients receive some form of cytotoxic chemotherapy either in the form of stem-cell transplantation or dexamethasone combined with oral or intravenous melphalan. Results of a phase 3 trial are required to determine the optimal initial induction therapy for this disorder.

### Research agenda

- a phase III trial comparing stem-cell transplantation to conventional therapy is underway
- the role of germ line gene usage in determining which patients will develop amyloid and what organs will be involved is promising
- the possibility that echocardiography will be supplanted by cardiac biomarkers is under study
- drugs that act by inhibiting fibril formation are a fruitful avenue of research

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