

Primary (AL) amyloidosis: A concise review

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The diagnosis of primary amyloidosis (AL) should be considered in non-diabetic patients with nephrotic range proteinuria, patients with cardiomyopathy without an ischemic history, patients with hepatomegaly, or patients with peripheral neuropathy in the absence of diabetes. The suspicion of AL should be confirmed by immunofixation of the serum and urine. All patients with AL require histologic confirmation; biopsy is simplest in the subcutaneous fat and the bone marrow. Echocardiography is a routine study in AL patients to assess prognosis. Treatment can include melphalan and prednisone, high-dose melphalan followed by autologous stem cell transplantation, and solid organ transplantation in patients with good control of their systemic diseases. Dose-intensive therapy with hematopoietic cell transplantation is effective at reversing AL but is not risk-free. Guidelines have been developed for patient selection in order to maximize benefit and minimize treatment-related mortality. This review is intended to help clinicians in managing their patients with AL amyloidosis. [Turk J Cancer 2001;31(1):5-14]

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AL amyloidosis (AL) is a clonal plasma cell proliferative disorder, in which immunoglobulin (Ig) light chains are deposited as amyloid in critical organs leading to organ dysfunction and death (1-4). The filaments in this variant of amyloidosis are predominantly comprised of Ig light chain variable regions, and form typical fibrils organized as antiparallel β -pleated sheets in extracellular depositions that resist proteolysis. The fibrils possess unique tinctorial properties (apple-green birefringence with Congo red staining when viewed in polarized light) as well as a distinctive morphology by electron microscopy (5).

At diagnosis, approximately 80% of patients with AL do not meet criteria for multiple myeloma (AL without myeloma) while the rest do (AL with myeloma), a distinction of limited significance (3). The diagnosis requires the identification of amyloid, usually found on biopsy of a symptomatic organ or aspirate of abdominal fat, in association with a clonal plasma cell proliferative disorder. The

latter is usually identified by immunofixation of urine and serum revealing a monoclonal protein (M-protein) and by bone marrow biopsy with immunohistochemical staining for light chain isotypes demonstrating the clonal dominance of λ (κ/λ ratio <1) or κ (κ/λ ratio >3) staining plasma cells (6). Immunofixation of urine and serum are specifically indicated after a tissue diagnosis of amyloidosis has been made because protein electrophoresis often fails to reveal the presence of an M-protein (7).

The insoluble AL depositions in the heart, kidneys, vascular walls, liver, spleen, bone marrow, and autonomic and peripheral nerves, as well as the constitutional symptoms of fatigue, weakness and weight loss associated with the deposition process, result in progressive debilitation with organ failure and death occurring in a median of 13 months from diagnosis (3). Paradoxically, the clonal plasma cell burden in this uncommon aggressive disease is low; sixty percent of patients with AL have less than 10 percent plasma cells on bone marrow biopsy. By comparison, patients with Stage I multiple myeloma or MGUS, whose clonal plasma cell burdens are roughly equivalent to those in AL, have median survivals 2- to many-fold longer (8).

Several large randomized prospective clinical trials have shown that treatment with oral melphalan and prednisone results in objective responses and prolonged survival up to median 18 months (9-11). Given the results of these trials, we considered it reasonable to investigate the use of dose-intensive therapy with intravenous melphalan to treat this disorder. We believed that the low plasma cell burden would favor significant responses to dose-intensive therapy. At Boston University Medical Center since January 1994, we have employed dose-intensive intravenous melphalan with autologous mobilized blood stem-cell support as therapy for AL (12-14). Our encouraging results are analogous to those obtained with dose-intensive therapy in multiple myeloma and support the position that AL patients meeting standard criteria for autologous transplantation should routinely be considered for dose-intensive therapy and hematopoietic cell transplantation (15-16). In this review, clinical course of AL and application of dose-intensive therapy are described.

CLINICAL COURSE OF AL AMYLOIDOSIS

Sixty percent of patients with AL are between fifty and seventy years of age at diagnosis; only 10% are younger than fifty. More than half of newly diagnosed patients give a recent history of fatigue and weight-loss, one fifth present with carpal tunnel syndrome and only one in eight with textbook macroglossia. At the same time, however, a significant fraction of asymptomatic patients are diagnosed with renal amyloidosis after proteinuria is discovered by urinalysis as part of a routine physical examination (3).

AL is a multi-system disorder and can present as a rapidly progressive syndrome. As a rule, performance status, prognosis and survival are correlated with a patient's predominant organ involvement. Survival in patients with cardiac involvement is negatively influenced by diminished left ventricular ejection fraction and by increased mean left ventricular wall thickness (17). For patients with symptomatic peripheral and autonomic neuropathy in association with orthostasis, gastric atony, nausea and chronic diarrhea, performance status and survival are also severely compromised. The latter group of patients have a

median survival of just over two years (3). Patients with nephrotic syndrome are at high risk for developing renal failure and becoming dialysis dependent, a situation that occurs in nearly one-fifth of AL patients (18). It is believed that the majority of AL patients die of heart failure or sudden cardiac events; gastrointestinal bleeding and infection are also not uncommon causes of death.

EVALUATING THE PATIENT WITH AL AMYLOIDOSIS

Patients are evaluated clinically and with studies that include blood counts, coagulation tests with a Factor X level and thrombin time, renal and hepatic chemistries, 24 hour urinary protein and creatinine clearance, electrocardiogram, echocardiogram, and, if indicated by clinical findings, gastric emptying scan, CT scan or cardiac catheterization. Presence of a clonal plasma cell disorder is assessed by serum and urine immunofixation and bone marrow biopsy as described above.

Performance status

It is important to distinguish the reversible components of an individual patient's performance status at the time of consideration for dose-intensive therapy. Reversible components of performance status include side effects of medications such as antihypertensives (hydralazine, β -blockers), volume overload, orthostasis, chronic diarrhea, symptomatic anemia, pleural effusion which all could be treated with various interventions with subsequent relief and improve performance status. Usually, patients with SWOG performance status 0-2 should be considered for dose-intensive therapy. Because dose-intensive therapy in patients with predominant cardiac involvement is more risky, every effort should be made to optimize a patient's functional status prior to stem-cell mobilization.

Amyloid-related organ involvement

1) Cardiac: Amyloid cardiac involvement is diagnosed based on symptoms and physical findings (and endomyocardial biopsy, when available) with either echocardiographic findings of a mean left ventricular wall thickness greater than 11 mm in the absence of a history of hypertension or valvular heart disease, or otherwise unexplained low voltage on ECG (<0.5 mV) (6,18). Often there is also a pattern of pseudo-myocardial infarction on the electrocardiogram. The classic features of amyloid heart involvement are physical findings of right-sided heart failure. Although the major clinical trials of intermittent oral melphalan and prednisone have demonstrated only a modest increase in survival with treatment, patients with predominant cardiac involvement often cannot receive sufficient intermittent chemotherapy to modify their survival (14). It is possible that patients with conduction system abnormalities and syncope are at greater risk of death during dose-intensive therapy than patients with more common manifestations of cardiomyopathy such as restrictive disease or congestive heart failure.

2) Renal: Renal amyloidosis presents as proteinuria (>0.5 g/day) and often as nephrotic syndrome (>3 g/day). Indeed, nearly one third of patients with nephrotic syndrome spill ten or more grams of protein a day, and half have serum albumin levels less than 2 g/dl (3). Blood urea nitrogen and serum

creatinine are usually normal or minimally elevated. Nephrotic syndrome can improve in response to various chemotherapeutic regimens, including intermittent oral therapy. In the most recent major trial, 12 of 68 patients (18%) with nephrotic syndrome treated with an intermittent oral regimen including melphalan experienced a 50% decrease in daily proteinuria without progressive renal insufficiency (14). Renal transplantation has been performed in a limited number of patients with AL on dialysis, with surprisingly good long-term survival rates (19,20). Obviously, patient age, tempo of amyloid progression and the extent of amyloid involvement of other organ systems must be weighed carefully, particularly when the planned donor is a living relative.

3) Hepatic: One quarter of newly diagnosed AL patients have hepatomegaly on physical exam and elevation of the serum alkaline phosphatase (ALP), although only half of them have hepatomegaly more than 5 cm below the costal margin (3). Liver failure can occur as a result of disease progression. Splenic involvement with amyloid is common in the setting of hepatic amyloidosis and is associated with the presence of Howell Jolly bodies on the peripheral blood smear, a sign of splenic dysfunction. In the most recent major trial of oral therapy, 6 of 12 patients (50%) with palpable livers and ALP levels over 500 U/L who were treated with melphalan had reductions in liver size and at least 50% declines in ALP (14).

4) Neuropathic: Sensorimotor peripheral neuropathy is present in one sixth of patients at diagnosis. Since the fine sensory fibers are affected initially, dysesthesias painful enough to be incapacitating are a classic presentation. It is important to confirm the diagnosis of AL, even in the co-presence of amyloid cardiomyopathy, since variants of familial amyloidosis may present in this way. Additional manifestations of neuropathic involvement include orthostatic hypotension, blunted heart rate response to exercise, gastroparesis, chronic diarrhea and impotence. Treatment-associated improvements in AL polyneuropathy had never been reported until we described the rapid reversal of neuropathic symptoms after dose-intensive therapy (12-14,21). However, the possibility of the reversal of neurologic findings is inversely related with the severity of neuropathy at diagnosis.

5) Other organ systems: Patients with macroglossia, enlarged submandibular glands and dysphagia are at risk for potential airway compromise during the toxic period of dose-intensive therapy when mucositis and thrombocytopenic bleeding in the oropharynx may occur. Also, patients with pleural effusions due to pulmonary amyloid may require repeated thoracentesis to maintain adequate pulmonary function, and the timing of such interventions may be critical with respect to the course of stem-cell mobilization, collection and dose-intensive therapy. These potential problems should be considered with the risks and benefits of dose-intensive therapy.

6) Hematologic panorama in AL Amyloidosis: Ninety percent of patients have either a serum or urine M-protein identified by immunofixation. Seventy five percent have free monoclonal light chains in their urine and fifty percent have such light chains in their serum. The free light chains often accompany a whole Ig. If a patient lacks a serum or urine M-protein, careful examination of a marrow biopsy stained appropriately for light chain isotypes often demonstrates a clonal predominance of λ or κ staining plasma cells. Useful information is also obtained by staining bone marrow biopsy specimens with Congo red and

grading amyloid marrow depositions. Quantitative serum Ig levels and quantitative 24-hour Bence-Jones protein by isotype are also important objective measures of disease. With respect to the response to dose-intensive therapy, a complete hematologic response of the plasma cell proliferative disorder is defined as the absence of an M-protein on serum and urine immunofixation and a marrow biopsy showing less than 5% plasma cells with polyclonal λ and κ staining.

Anemia, changes in red blood cell morphology, thrombocytosis, functional hyposplenism, hypogammaglobulinemia, the immune impairment of nephrotic syndrome, the coagulopathies associated with AL, the multifactorial character of AL bleeding diatheses, and the frequent presence of significant marrow depositions of amyloid are features of AL that require consideration in the context of dose-intensive therapy (3,22,23).

STEM CELL MOBILIZATION AND COLLECTION

Complications of mobilization and collection can occur in patients with nephrotic syndrome who are hypoalbuminemic and salt-avid. Clinical management of their volume status is critical; therefore, we diurese judiciously and infuse albumin to maintain a level of 2 g/dl in symptomatic patients. In patients with renal and cardiac involvement, rapidly accumulating pleural effusions and flash pulmonary edema can occur with mobilization and leukapheresis. With these concerns in mind, during mobilization and pre- and post-leukapheresis, we routinely follow a patient's systolic blood pressure, heart and lung examination, weight, and room air oxygen saturation. We cease collecting stem cells in patients who experience decreases in their oxygen saturation to less than 95% and fail to improve promptly with diuretics, or demonstrate unexplained declines in their systolic blood pressure to less than 90 mm Hg. We obtain CD34+ cell estimates from the peripheral blood prior to stem-cell collection and dose stem-cell components based on CD34+ counts, using a target dose of 5×10^6 /kg and a minimum of 2×10^6 /kg.

Contamination of stem-cell product with clonotypic cells

As is the case in multiple myeloma, AL patients frequently have B-cells in their peripheral blood that are related to their plasma cell clone (24). We evaluated marrow specimens from 7 AL patients containing 4% to 10% plasma cells with a light chain isotype preponderance by RT-PCR employing PCR primers for Ig light chain subgroups as described by Welschof et al (25). A unique single overexpressed Ig V_L gene was identified in each case. Patient-specific primers were designed based upon the *CDR3*, or *CDR1* and *CDR3*, sequences of the cloned overexpressed Ig V_L genes. All patient-specific primers included the codons for amino acids 95 and 96 in the *CDR3* region (23).

All seven patients had blood stem cells mobilized with G-CSF and routinely collected, with specimens available from the first stem cell collection for cDNA preparation. Four of 7 (57%) had evidence of clonotypic cells present in their first day's stem cell preparation, with appropriately sized restricted bands appreciated by PCR using patient-specific primers. These seven patients included one CD34-selected patient whose CD34-selected cell DNA also contained evidence of clonotypic contamination (23).

MAJOR CLINICAL TRIALS WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL TRANSPLANTATION IN AL-AMYLOIDOSIS

High-Dose Melphalan Trial (13)

Between January 1, 1994 and September 31, 1996, twenty-five AL patients, 18 to 61 years of age, were enrolled to receive dose-intensive intravenous melphalan (200 mg/m^2) with mobilized blood stem-cell support provided they had adequate cardiac (left ventricular ejection fraction $>50\%$), pulmonary (diffusion capacity $>50\%$ predicted), hepatic (bilirubin $<2.0 \text{ g/dl}$) and renal function (normal creatinine). All patients were mobilized with G-CSF, usually 16 ug/kg SQ daily for 4 to 5 days, with stem cell collections beginning on day 4. Fifteen patients were enrolled to receive unselected blood stem cells while ten were enrolled to receive CD34-selected blood stem cells (Isolex 300, Baxter Biotech, and Deer Park, IL Park). Dose-intensive melphalan was administered intravenously over 2 days, $100 \text{ mg/m}^2/\text{day}$, and stem cells were infused 72 hours later. Patients received antibiotic prophylaxis with an oral quinolone and Acyclovir beginning on day 3 after stem-cell infusion.

Patient Characteristics

The majority of patients had performance status of 1 or 2, within one year of the diagnosis of AL and minimally pre-treated with oral melphalan and prednisone. Fifteen patients had 1 or 2 organ systems involved, while ten had 3 or more major organ systems involved. Fifteen (60%) were hypogammaglobulinemic, seventeen (68%) had amyloid marrow depositions (eight with grade 2 or 3), and seven (28%) had hyposplenism due to amyloid (Howell-Jolly bodies). Fourteen patients (56%) had M-proteins present in serum and urine by immunofixation, nine (36%) had Bence-Jones proteins without a serum M-protein, and two (8%) had a clonal bone marrow plasmacytosis without serum or urine M-proteins. At enrollment and at follow-up three months after therapy and annually thereafter, each patient was evaluated for performance status, for response of amyloid-related organ/system involvement, and for response of the clonal plasma cell proliferative disorder.

Survival

Two patients had rapidly progressive amyloidosis and died after stem-cell collection prior to being treated. Twenty-three patients were treated. There were 3 deaths within 100 days of transplant giving a 100 day post-transplant mortality of 13%, and an early mortality based on intention-to-treat of 20%.

With a median follow-up of 24 months (12-38), sixty-eight percent of patients (17/25) are alive and the median survival has not been reached. Eighty-seven percent of patients (13/15) with <2 major organ systems involved survived, as opposed to only 40% (4/10) surviving among those with more than 2 involved systems ($p < 0.05$, one-tailed Fisher's exact test). Eighty-two percent of patients without predominant cardiac involvement at baseline (14/17) were alive in follow-up, as compared to 38% of patients with predominant cardiac involvement (3/8) ($p < 0.05$). Overall survival of patients with a baseline performance status of 1 was 80% (8/10), while that of patients with baseline performance status of 2 or 3 was 60% (9/15) ($p=0.402$). With respect to the seventeen patients surviving a

year or more in follow-up, the difference between their median performance status prior to therapy [2 (1-3)] and at follow-up [0 (0-3)] was statistically significant ($p < 0.05$, Wilcoxon rank sum). Currently, two thirds of the surviving patients (11/17) have experienced improvements of amyloid-related organ involvement in all systems, while 4/17 have stable disease.

Stem cell product

In this study, a median of 8.9×10^6 CD34+ cells per kg were collected (range, 2.9-25.4) in 2 to 3 aphereses. The numbers of mobilized circulating baseline CD34+ cells in patients who had previously received greater than 200 mg of oral melphalan pulse therapy were significantly less than in patients who had previously received less or no melphalan (Mean \pm SE; 48 ± 7 versus $78 \pm 11 \times 10^6/L$; $p = 0.028$, two-tailed t-test). However, there were no significant differences between these groups with respect to CD34+ cells collected.

Engraftment

Neutrophil and platelet engraftment occurred within medians of 10 (8-17) and 14 days (8-65), respectively. There were no differences in neutrophil or platelet engraftment based on CD34+ cell dose or the use of unselected or CD34-selected cells, although differences in lymphocyte recovery were documented (23). Three patients had delayed platelet reconstitution (65, 52 and 26 days). All other patients engrafted for platelets within 17 days.

Toxicity

1) Infectious complications: There were no deaths due to sepsis. With respect to infectious complications, six patients had fever with positive blood cultures with various organisms.

2) Renal and hepatic toxicities: One of seven patients with predominant renal involvement experienced the only episode of significant treatment-related renal toxicity with an increase in creatinine from 0.9 to 4.9 mg/dl. One of five patients with predominant hepatic involvement experienced jaundice and a rise in bilirubin to 18 mg/dl, which was attributed to drug toxicities (opiates, trimethoprim-sulfamethoxazole) and veno-occlusive disease. The patient improved promptly and bilirubin returned to normal by the time of follow-up at 3 months.

3) Secondary leukemia: We observed secondary acute myelogenous leukemia with a complex karyotype including deletion of chromosome 7 in a 61 year-old woman eighteen months posttransplant. She died 2 months later, 20 months after treatment.

Intermediate Dose Melphalan Trial (26)

In this trial, AL amyloidosis patients ineligible for dose-intensive melphalan (200 mg/m^2) were treated with two cycles of intermediate-dose melphalan (100 mg/m^2) and mobilized blood stem cells. For mobilization patients were randomized to either GM-CSF $250 \text{ microgram/m}^2$ for 3 days followed by G-CSF 10 microgram/kg for 3 days (GM+G), or G-CSF 10 microgram/kg for 6 days (G-alone), with leukaphereses on days 5, 6 and 7. To minimize morbidity, we planned to support each cycle with at least 3×10^6 CD34+ cells/kg and had a collection target of 7×10^6 CD34+ cells/kg. Those who did not achieve the target

were treated with one cycle of IDM. Thirty patients, a median of 62 years old and 7 months from diagnosis, were enrolled. Both mobilization regimens were generally well tolerated, and similar in terms of CD34+ cells and CFU-GM collected, but only 6/28 patients achieved the collection target (GM+G four, G-alone two). Despite a 19% incidence of grade IV toxicities, IDM therapy was well tolerated. At a median follow-up of 24 months (19-36) 57% of patients had survived, 17% with durable complete hematological responses and 40% with improved or stable amyloid organ involvement, including 3/9 patients with predominant cardiac amyloid who are alive 2-3 years after treatment. The 100-day mortality was 20%. In conclusion, no definitive differences were identified between the mobilization regimens and IDM was found to be an active regimen in AL for selected patients.

Conclusions

In comparison to intermittent oral therapy, the hematologic and organ system responses documented in AL patients after dose-intensive therapy represent an unequivocal improvement, as others have suggested as well (27). The efficacy of this therapy is likely associated with obtaining a significant or complete response of the plasma cell proliferative disorder (15,28). However, prognosis and survival with dose-intensive therapy remain functions of predominant organ involvement (cardiac vs. other) and of number of major organ systems involved (1 or 2 vs. 3 or more). From these efforts we have learned that amyloid marrow depositions of all grades do not impair mobilization or engraftment, even of highly purified CD34-selected cells (29). We have also confirmed that intravenous melphalan is as effective in AL as in myeloma, and that its toxicity profile is of particular value given the visceral compromise many AL patients exhibit. Clearly the risk of secondary leukemia exists in association with prior oral melphalan therapy. Based on these results, dose-intensive intravenous melphalan should be standard therapy for contemporary patients who fit the eligibility criteria employed for our initial cohort. More liberal eligibility criteria are appropriately being used in subsequent trials in an attempt to extend this therapy. It is also reasonable to consider allogeneic transplant for young AL patients, perhaps with less toxic conditioning regimens that might include fludarabine and melphalan.

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