Diagnosis, prognosis and treatment of amyloidosis

G. Merlini, G. Palladini

Amyloidosis Center. Fondazione IRCCS Policlinico San Matteo.
Department of Biochemistry. University of Pavia. Italy

Amyloidosis is caused by the aggregation of normally soluble proteins into bundles of insoluble β-sheet fibrils that are deposited in target tissues causing progressive organ dysfunction[1,2]. The present classification identifies amyloidoses according to the nature of the main amyloid precursor protein and up to 27 different proteins have been recognized to date to be amyloidogenic in humans (Table 1)[3]. In the last 10 years, a large body of biochemical studies has begun to unveil the molecular mechanisms through which these soluble proteins, sharply differing in biochemical function and in three-dimensional structure, may become prone to undergo a irreversible transition from their native conformation into highly ordered aggregates sharing the unique structural features of amyloid fibrils. An adequate precursor protein pool is a common prerequisite in order to achieve the initial nucleation event that subsequently triggers additional deposition of polypeptide molecules, leading to amyloid fibril growth and tissue damage. In acquired systemic amyloidoses, such as AL and AA amyloidosis, the precursor is a circulating protein that becomes amyloidogenic when it reaches a substantial and persistent concentration in plasma. In the hereditary forms, amyloid results from the presence of mutated proteins that circulate from birth at a stable plasmatic concentration. The absolute amount of each of these proteins in plasma and the time required for the initial nidus of tissue aggregates to be formed is variable and may partially account for some differences in disease severity and rate of progression observed among systemic amyloidoses. Additionally, a significant heterogeneity in clinical presentation and in the pattern of amyloid-associated organ toxicity may be observed. Several clinical findings suggest that factors related to the protein itself are likely to be involved in the generation of local environmental conditions that may promote fibrillogenesis and mediate tissue damage. These factors may include binding to known or putative receptors possibly linked to the protein physiologic function, specific interactions with extracellular components and/or cellular mechanisms controlling protein secretion.

Diagnosis

Light-chain amyloidosis (AL) is the most common form of systemic amyloidosis in western countries, with an estimated incidence of 0.8 per 100,000 person years. Systemic AL amyloidosis is a plasma cell disorder characterized by the overproduction and tissue deposition of a monoclonal immunoglobulin light chain (LC), or fragments containing the LC variable region and a portion of the constant region. The deposits are composed of amyloid fibrils, presenting a cross beta supersecondary structure. The process of amyloid deposition produces tissue damage and eventually organ failure, leading to death in untreated patients.

The optimal management of patients with AL amyloidosis requires early diagnosis, correct amyloid typing, effective treatment, tight follow-up and careful supportive therapy[2]. One of the most important determinants of outcome is early diagnosis, as severe amyloid organ disease may preclude the use of potentially effective treatment regimens. In addition, the systemic involvement affecting vital organs such as heart, kidney and liver renders these patients particularly fragile and sensitive to the toxicity of chemotherapy.

Early diagnosis depends on the level of alertness of the physician: any patient with nephrotic-range proteinuria, unexplained right-sided heart failure, progressive peripheral neuropathy, unexplained hepatomegaly or functional hyposplenism, orthostatic hypotension and other manifestations of autonomic neuropathy with weight loss should be screened for amyloidosis. The diagnosis of AL is biopsy based and requires the demonstration of deposits with apple green birefringence after Congo red staining, or the prototypic, non-branching, 10-nm diameter fibrillar structures by electron microscopy. Fine-needle aspiration of abdominal fat is innocuous, fast, inexpensive, and sensitive (87%)[4]. The method is based on the almost constant involvement of subcutaneous adipose tissue in AA, AL and ATTR forms of systemic amyloidosis, and probably in other systemic amyloidoses as well. Amyloid is found both in the walls

Table I. Amyloid fibril proteins and their precursors in human (adapted from Westermark, _et al._[3])

Amyloid protein	Precursor	Systemic (S) or Localized (L)	Syndrome or Involved Tissues
AL	Immunoglobulin light chain	S, L	Primary Myeloma-associated
AH	Immunoglobulin heavy chain	S, L	Primary Myeloma-associated
Aβ2M	β2-microglobulin	S	Hemodialysis-associated
ATTR	Transthyretin	S	Familial Senile systemic
AA	(Apo)serum AA	S	Secondary, reactive
AApoAI	Apolipoprotein AI	S / L	Familial Aorta, meniscus
AApoAII	Apolipoprotein AII	S	Familial
AApoAIV	Apolipoprotein AIV	S	Sporadic, associated with aging
AGel	Gelsolin	S	Familial (Finnish)
ALys	Lysozyme	S	Familial
AFib	Fibrinogen α-chain	S	Familial
ACys	Cystatin C	S	Familial
ABri	ABriPP	S	Familial dementia, British
ADan	ADanPP	L	Familial dementia, Danish
Aβ	Aβ protein precursor (AβPP)	L	Alzheimer's disease, aging
APrP	Prion protein	L	Spongiform encephalopathies
ACal	(Pro)calcitonin	L	C-cell thyroid tumors
AIAPP	Islet amyloid polypeptide	L	Islets of Langerhans Insulinomas
AANF	Atrial natriuretic factor	L	Cardiac atria
APro	Prolactin	L	Aging pituitary Prolactinomas
AIns	Insulin	L	Iatrogenic
AMed	Lactadherin	L	Senile aortic, media
AKer	Kerato-epithelin	L	Cornea, familial
ALac	Lactoferrin	L	Cornea
AOaap	Odontogenic ameloblast-associated protein	L	Odontogenic tumors
ASemI	Semenogelin I	L	Vesicula seminalis
ATau	Tau protein	L	Alzheimer's disease, fronto-temporal dementia, aging, other cerebral conditions

of small vessels and around the individual fat cells. False-positive Congo red-stained biopsies may occur, but are the result of overstaining and inexperienced review. If the abdominal fat is negative, the second choice biopsy site at the Pavia Amyloid Center is the minor labial salivary glands. Renal and hepatic biopsies carry a small risk of bleeding. Once the diagnosis of amyloidosis has been established histologically, the type must be determined, because the prognosis and treatment depend on the biochemical amyloid forms. There are specific treatments available for some systemic amyloidoses, this means that exact and safe determination of the type of protein constituting the amyloid deposit in the individual patient is critical. This can be accomplished using immuno-histochemistry, immunoelectron microscopy[4] or by biochemical methods which are applicable also to formalin-fixed tissue samples. Immunohistochemistry is usually reliable for identifying or ruling out AA amyloidosis, but is frequently not diagnostic with respect to AL amyloidosis. Immunoelectron microscopy and biochemical methods provide definitive results; however, they are labor-intensive and require expertise. If these techniques are not available, the DNA analysis should be performed upfront in order to exclude the hereditary amyloidoses whose clinical presentation is consistent with the patient's manifestations. In order to characterize AL type amyloidosis, a plasma cell clone should be documented. The demonstration of the clone requires sensitive techniques.
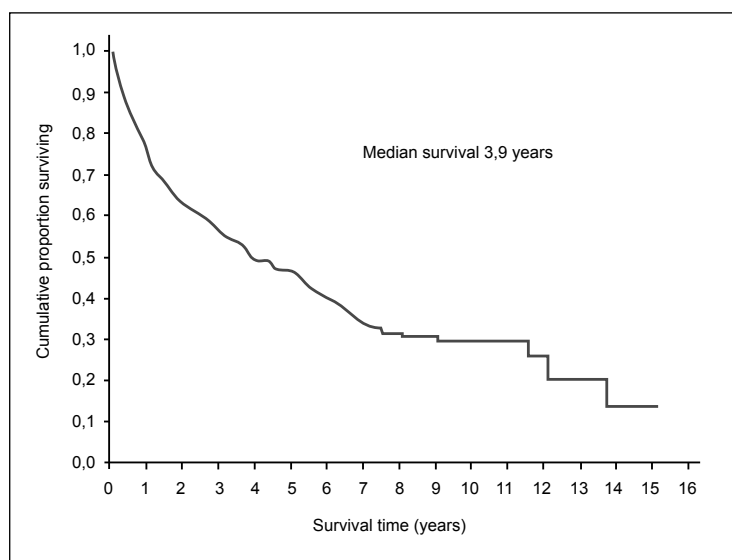
Figure.1. Overall survival of 822 patients with AL amyloidosis.

The bone marrow should always be examined, bearing in mind that, typically, the amyloid plasma cell clone infiltrates the bone marrow to a modest extent (median bone marrow plasma cell percentage 7%), often requiring anti-light chain immunohistochemistry/immunofluorescence for κ and λ light chain to be identified. Accordingly, also the circulating monoclonal protein, usually present at a low concentration, is missed by screening serum electrophoresis in approximately 50% of patients. Therefore, all patients with a clinical suspicion of AL amyloidosis should undergo sensitive immunofixation electrophoresis of serum and urine that is able to detect a monoclonal protein in up to 97% of patients[4]. The quantification of serum-free light chains (FLC) may complement immunofixation and now represents an irreplaceable tool for monitoring response to therapy. Evidence of progression or regression of amyloid deposits can be obtained from serum amyloid P (SAP) component scintigraphy.

Due to the relatively high prevalence of a monoclonal protein in the adult population, the possibility of a chance coexistence of a monoclonal protein in a patient with hereditary amyloidosis should always be considered. Clinically, it is difficult to distinguish AL from reactive, familial, and senile systemic forms of amyloidosis, because of their overlapping clinical presentations and the lack of an informative family history in half of the patients with hereditary amyloidosis. Since mistyping of amyloidosis may have catastrophic therapeutic consequences, such as transplanting hematopoietic stem cells instead of liver, great care should be devoted to the diagnostic process. When two possible sources of amyloid have been identified, patients should be referred to centers specializing in amyloidosis for further evaluation.

Prognosis

The prognosis of AL amyloidosis has significantly improved in the last decade due to earlier diagnosis and more effective specific and supportive treatments. The median survival of patients with AL depends in part on the treatment center and the nature of the referral pattern. The median survival of 822 patients with AL amyloidosis followed in Pavia is 3.9 years (Figure 1). Most patients with AL amyloidosis die of cardiac complications (~75% in our patient population), either congestive heart failure or sudden death. Median survival of patients with heart involvement was significantly shorter than that of patients without cardiac amyloidosis (24 vs 81 months, $p < 0.001$), and patients who obtained a hematologic response to chemotherapy survived longer than other patients (median 96 vs 20 months, $p < 0.001$). Actually, the Cox multivariate analysis showed that the only two significant independent prognostic factors were response to therapy (protective), and cardiac involvement ($p < 0.001$ for both variables). Our recent data indicate that patients with cardiac AL amyloidosis who achieve hematologic response to chemotherapy have a better outcome than non-responsive patients (median survival 68 months vs 11 months, $p < 0.001$) irrespective of the severity of heart involvement at diagnosis. Elevated serum cardiac troponins are related to poor prognosis in AL patients[5] and our group reported that the serum N-terminal portion of natriuretic peptide type B (NT-proBNP) is a sensitive marker of myocardial dysfunction in AL and a powerful prognostic determinant[6]. These two cardiac biomarkers were used to develop a reliable staging system for AL patients that can be used to stratify patients in randomized clini-

cal trials and to compare outcomes between therapeutic interventions when randomized clinical trials are not available[7]. NT-proBNP clearance relies almost exclusively on glomerular filtration while natriuretic peptide type B (BNP) is eliminated from plasma through both glomerular filtration and clearance receptors that promote its degradation. Therefore, it is likely that BNP will prove to be a more reliable marker of cardiac dysfunction than NT-proBNP in AL patients with advanced renal disease. In most patients, the reduction of the circulating FLC concentration induced by chemotherapy translates into a rapid reduction of serum NT-proBNP level and improving of heart failure, often before any reduction in amyloid load can be demonstrated at echocardiography[8]. This observation indicates that serum NT-proBNP can be used as a marker of cardiac response to therapy. It has been reported that normalization of FLC levels after peripheral blood stem cell transplantation predicted both complete hematologic response and organ response[9]. The serial concurrent quantification of the FLC and NT-proBNP in patients with cardiac amyloidosis allows titration of the anticlone treatment according to the organ response, optimizing the toxicity-benefit ratio and allowing a prompt change of therapy in the case of an inadequate response.

Treatment

The treatment of systemic amyloidoses varies greatly according to the type of amyloidosis: therefore accurate diagnosis is of vital importance. At present, the best approach to treating amyloidosis remains in the realm of restricting the amount of protein substrate necessary for amyloid fibril formation. For instance, in ATTR amyloidosis the most effective approach is liver transplantation, and this is also true for other amyloidogenic protein variants synthesized mostly by the liver, such as fibrinogen, or by liver and intestine such as apolipoprotein-AI. For AL amyloidosis, the current therapeutic approach is based on the observation that organ function can be restored if the synthesis of the amyloidogenic precursor is shut down. The aim of therapy is to rapidly reduce the supply of the amyloidogenic monoclonal light chain by suppressing the underlying plasma cell clone, while using supportive measures to sustain the function of the organs involved[2]. A consensus panel from the International Society for Amyloidosis established the criteria for hematological (Table 2) and organ response (Table 3)[10]. It has been demonstrated that achievement of a hematologic response was an important predictor of prolonged survival after stem cell transplantation for patients with AL. The degree

Table 2. Criteria for hematologic response in AL amyloidosis[10]

Complete response	Serum and urine negative for a monoclonal protein by immunofixation Free light chain ratio normal Marrow < 5% plasma cells
Partial response	If serum M component > 0.5 g/dL, a 50% reduction If light chain in the urine with a visible peak and >100 mg/day and 50% reduction If free light chain >10 mg/dL (100 mg/L) and 50% reduction
Progression	From CR, any detectable monoclonal protein or abnormal free light chain ratio (light chain must double) From PR or stable response, 50% increase in serum M protein to > 0.5 g/dL or 50% increase in urine M protein to > 200 mg/day; a visible peak must be present Free light chain increase of 50% to >10 mg/dL (100 mg/L)
Stable	No CR, no PR, no progression

Table 3. Criteria for organ response in AL amyloidosis[10]

Organ involved	Response Criteria
Heart	Mean interventricular septal thickness decreased by 2 mm, 20% improvement in ejection fraction, improvement by 2 New York Heart Association classes without an increase in diuretic use, and no increase in wall thickness
Kidney	50% decrease (at least 0.5 g/day) of 24-hr urine protein (urine protein must be > 0.5 g/day pretreatment) Creatinine and creatinine clearance must not worsen by 25% over baseline
Liver	50% decrease in abnormal alkaline phosphatase value Decrease in liver size radiographically at least 2 cm
Nerve	Improvement in electromyogram nerve conduction velocity (rare)

of response is relevant because patients who achieved a complete response survived longer than those who achieved a partial response, thus indicating that hematologic response may be used as an endpoint in trials assessing AL amyloidosis therapies[11-13]. Table 4 summarizes the outcome in terms of response rate, on intention-to-treat basis, and toxicity of the main regimens used in the treatment of AL amyloidosis. Clearly, important progress has been achieved since the mid nineties when melphalan and prednisone

Table 4. Outcomes of the main regimens used in the care of AL amyloidosis (intention-to-treat analysis)

Regimen	Reported patients	Hematol. response (PR+CR)	CR	TRM	Reference
ASCT (MEL 200) (MEL 140-100)	258 173	76% 53%	33% 18%	10-12% 16%	11, 13
Melphalan-Dex.	46	67%	33%	4%	15
High-dose Dex.	55	53%	24%	7%	14
Thalidomide-Dex.	31	48%	19%	0% (SAE 65%)	16
Lenalidomide ± Dex.	55	41-50%	22%	3-18% (SAE up to 86%, 9%TE)	18, 19
Cyclophosphamide-Thalidomide-Dex.	65	74%	15%	4%	17
Melphalan-pred.	–	~30%	rare	0%	–

PR: partial response; CR: complete response; TRM: treatment-related-mortality; ASCT: autologous stem cell transplantation; MEL: melphalan; dex.: dexamethasone; pred.: prednisone; SAE: severe adverse events; TE: thromboembolism.

regimen was considered standard therapy. This combination induced slow responses, which were rarely complete, in about 30% of patients. The advent of high-dose melphalan followed by rescue with autologous stem cells (ASCT) dramatically changed the perspective for AL amyloidosis care demonstrating that complete response can be achieved in a substantial proportion of eligible patients which translated into organ response and significant survival extension. However, treatment-related mortality (TRM) remains substantial, even in specialized centers, particularly in patients with heart failure and multi-organ involvement. Therefore the search for less toxic and rapidly acting regimens continued. A multicenter trial showed that dexamethasone alone achieves a 53% hematological response rate after a median time of 3.4 months, with 24% complete remissions and a TRM of 7%[14]. The addition of oral melphalan to dexamethasone (MDex) induces a hematological response in 67% (CR 33%) of AL patients ineligible for ASCT due to advanced disease, in a median time of 4.5 months, with a low TRM of 4%[15]. Although thalidomide is poorly tolerated in AL patients, its association with dexamethasone as second-line treatment induces a hematologic response in 48% of patients with 19% CR[16]. A risk-adapted oral regimen of cyclophosphamide, thalidomide, and dexamethasone (CTD) in patients with AL amyloidosis produced hematologic response in 74% of patients including complete responses in 15% with a TRM of 4%[17]. This regimen has the advantage of preserving stem cells for possible subsequent ASCT. New drugs have been recently adopted from the therapeutic armamentarium for multiple myeloma for the treatment of AL amyloidosis. The Mayo Clinic and the Boston University groups reported that the thalidomide analog, lenalidomide, particularly when used in combination with dexamethasone, in patients with AL amyloidosis, many of whom were previously treated, induced approximately 50% hematologic response with 22% complete responses. Fatigue and myelosuppression were the most common treatment-related adverse events, while thromboembolic complications were the most serious[18, 19]. These findings indicate that the combination of lenalidomide and dexamethasone represents a valid treatment option. The proteasome inhibitor, bortezomib, is undergoing an international phase I-II dose-escalating trial in previously treated patients, and preliminary results indicate that this drug can be rapidly effective in a significant proportion of patients with manageable toxicity.

The goal of chemotherapy is a rapid and effective suppression of the synthesis of the amyloidogenic light chain in order to induce a functional improvement of the organ involved, at the minimum cost in terms of toxicity. The only randomized trial comparing modern therapies is the French Multicentric Trial on ASCT and MDex[20]. The trial showed that ASCT was not superior to MDex in a multicenter setting and was associated with lower survival when patients were treated in centers without great experience. The results indicate that the MDex regimen is a viable alternative to stem cell transplantation. Optimal therapy for AL amyloidosis is undetermined

due to the lack of comparative randomized clinical trials to support the use of an agent over another. Nevertheless, some provisional therapeutical indications could be provided on the basis of the published trials. The results attained with ASCT in selected AL patients, namely high rate of CR, improvement of organ system dysfunction and quality of life, and long-term survival, is still unsurpassed. These brilliant results come at a price, since transplantation-related mortality, although progressively decreasing, is still substantial at experienced centers. Good risk patients (age < 65 years, normal NTproBNP and cardiac troponins, glomerular filtration rate > 50 mL/min) are candidates to ASCT, that should be performed with high-dose melphalan (200 mg/m^2). Patients who are fit enough to bear dexamethasone based therapy, but who are not eligible for ASCT, can be treated with melphalan-dexamethasone which produces high rate of durable responses with very low toxicity. However, the exposure to melphalan can jeopardize stem cell mobilization and patients who present with a potentially reversible contraindication for ASCT should have their stem cell harvested before MDex. Alternatively, the recently proposed combination of cyclophosphamide-thalidomide-dexamethasone, which produces also a high rate of response while preserving bone marrow stem cells, can be considered, although data on the durability of responses obtained with this regimen are lacking. Poor risk patients can be treated with MP or, preferably, included in investigational trials. Refractory and relapsed patients could be treated with lenalidomide-dexamethasone or thalidomide-dexamethasone. The association of bortezomib and dexamethasone is probably very effective and could be used once data on the safety of this regimen become available. It is likely that in the near future, early diagnosis and combination therapy with alkylating agents associated with dexamethasone, thalidomide and the new drugs lenalidomide and bortezomib, would lead to extended and better life for all AL patients.

Conclusions

Major advances have occurred in the last decade in the diagnosis and therapy of AL amyloidosis. Early and accurate diagnosis is the key to effective treatment. The skillful use of biomarkers for amyloid organ damage and clonal response allows the hematologist to tailor the treatment to the patient's specific needs optimizing the risk/benefit ratio. Although ASCT represents a breakthrough in the care of AL amyloidosis, its toxicity has limited its benefit to a minority of patients. New, effective and less toxic therapies are emerging that will allow effective treatment of most patients. The field is now mature for conducting controlled trials to assess the best therapy, and in order to reach this aim international collaboration is needed.

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