Haematologica 1999; 84:218-221 original paper

The degrees of plasma cell clonality and marrow infiltration adversely influence the prognosis of AL amyloidosis patients

VITTORIO PERFETTI,* MAURIZIO COLLI VIGNARELLI,# ERNESTO ANESI,° PIETRO GARINI,° SILVANA QUAGLINI,§ EDOARDO ASCARI,° GIAMPAOLO MERLINI#

#Biotechnology and Organ Transplantation Research Laboratories, *Clinical Immunology Unit, Department of Internal Medicine °Section of Internal Medicine and Medical Oncology; §Department of Computer Science and Systems, University of Pavia, IRCCS Policlinico S. Matteo, Pavia, Italy

ABSTRACT

Background and Objective. **Primary amyloidosis is a lethal form of plasma cell (PC) dyscrasia characterized by deposits of monoclonal immunoglobulin light chains that cause organ dysfunction. In contrast to multiple myeloma, the amyloid clone is typically indolent and of small size, and marrow PC clonality is not always apparent. This is generally investigated by analyzing the light chain isotype ratio in bone marrow PC. We investigated whether the degree of PC infiltration (PC%) and clonality (PC isotype ratio) affected survival in 56 consecutive patients with primary amyloidosis.**

Design and Methods. **PC% was determined by morphologic examination. Immunofluorescence microscopy was used to determine the PC light chain isotype ratio. Statistical analysis was carried out using Cox regression models.**

Results. **The degrees of PC clonality and infiltration were inversely correlated with survival (PC isotype ratio, p = 0.001; PC%, p = 0.008). The two variables were weakly correlated (p = 0.02; r = 0.3). Bone marrow PC isotype ratio demonstrated a powerful independent prognostic value at multivariate analysis when analyzed together with congestive heart failure (the major known negative prognostic factor) and PC%. κ/λ ratio cut-off values of 0.2 (λ patients, p = 0.022) and 16 (κ patients, p = 0.03) discriminated two groups with a similar number of patients and significantly different survivals.**

Interpretation and Conclusions. **PC clonality and marrow infiltration are important parameters that influence prognosis, presumably because they reflect the amount of pathogenic light chain synthesis.**

Key words: plasma cell, AL amyloidosis, prognosis

Correspondence: Giampaolo Merlini, M.D., Biotechnology Research Laboratory, IRCCS University Hospital San Matteo, p.le Golgi 2, 27100 Pavia, Italy.
Tel: international +39-0382-502947 – Fax: international +39-0382-526223 – E-mail: gmerlini@mbox.medit.it

In primary (AL) amyloidosis, small numbers of bone marrow plasma cells (PC) secrete monoclonal light chains that form extracellular fibrils, amyloid, which display a typical apple green color when observed under polarized light after Congo red staining.[1] In contrast to the situation in multiple myeloma, variable numbers of normal PC coexist[2] and clonality is not always apparent. This is generally investigated by analyzing the light chain isotype ratio through immunofluorescence microscopy, a technique that is routinely employed for diagnosis[3,4] and patient follow-up.[3]

AL amyloid causes progressive organ dysfunction, leading invariably to death. The survival range is wide, depending primarily on the extent of damage to vital organs, particularly the heart.[5,6] In a series of 474 cases collected over 12 years at the Mayo Clinic, the median survival of patients with congestive heart failure (CHF) at diagnosis was only 4 months – in sharp contrast to the 26-month life expectancy of patients with exclusive involvement of the peripheral nervous system.[5]

In addition to the degree of damage to major organs, factors related to the PC clone, such as high titer of β_2 microglobulin,[7] may have a negative impact on prognosis. In this report, the possible impact on survival of the degrees of marrow infiltration (PC%) and of PC clonality (PC light chain isotype ratio) were investigated in 56 consecutive unselected patients with primary amyloidosis.

Design and Methods

Patients

The patient population consisted of 56 consecutive unselected cases of primary amyloidosis, including one that met the criteria for smoldering myeloma [the current definition of primary amyloidosis includes patients with more than 20% marrow PC but no signs of active myeloma (renal failure, hypercalcemia, bone lesions), i.e. patients with smoldering myeloma]. Amyloid deposits were identified by Congo red staining on tissue biopsies and/or abdominal fat aspirates. Patients showed a monoclonal component at immu-

nofixation of serum or urine and/or an altered PC light chain isotype ratio or monotypic light chain deposits at immunohistochemistry. Clinical pictures were suggestive of primary amyloidosis and family histories were negative for hereditary forms. An association with clinically overt multiple myeloma was excluded by the absence of osteolytic bone lesions, generalized osteoporosis, or hypercalcemia, and by the fact that the BMPC <20%.

Bone marrow studies

Patients gave informed consent to BM aspiration. PC percentages were evaluated on May-Grünwald Giemsa stained slides. Marrow PC clonality was assessed by double-staining immunofluorescence on Ficoll-separated cells using fluorochrome-conjugated anti-light chain isotype antisera (Dako, Glostrup, Denmark).[3] Five hundred PC were counted for each patient. Clonality is indicated by a κ/λ isotype ratio <1.1 (λ PC clone), or >2.6 (κ clone).[3]

Statistics

Standard univariate and multivariate statistical methods were employed. The Cox proportional hazard model was applied to estimate the influence of the variables on survival and to compare their potency. For uni- and multivariate analyses, the isotype ratios were expressed as κ/λ in the κ group, and λ/κ in the λ patients. Logarithmic transformation served to improve the normality of the PC% and the isotype ratio distribution. Survival was estimated by the Kaplan-Meier method.

Results

Patient population

The characteristics of the population of patients analyzed in this study are summarized in Table 1. Patients were treated with melphalan and prednisone. Fourteen (25%) were responsive (reduction of the monoclonal component by more than 25% and amelioration of organ dysfunction), 22 (39%) were nonresponders, and 20 (36%) were not available for evaluation of response because of early death (≤ 6 months from diagnosis, 8 patients) or because of insufficient clinical and/or laboratory assessment of organ function. Since the aim of the study was to analyze the impact on survival of marrow PC clonality and infiltration, and not of therapy, all patients were included in the study. Twenty-four of the 56 patients (43%) were alive at the time of statistical analysis, the others having died from amyloidosis-related causes.

Serum/urine immunofixation and bone marrow studies

A monoclonal component was present at immunofixation of serum/urine in 52 patients (93%, Table 1). Bone marrow PC clonality was analyzed by the light chain isotype ratio and was observed in 44 cases (79%, Table 1). Similar proportions of positive cases have been reported in smaller series.[3,4] The size of the amyloidogenic clone was insufficient to generate abormal isotype ratios in 6 κ and 6 λ cases (values were in the reference ranges of ≥ 1.1 and ≤ 2.6, respectively). The median bone marrow PC κ/λ ratio for κ patients was 8.26 (range 1.1-124), whereas for λ patients it was 0.17 (range 0.004-1.87). Three of the four patients without a detectable monoclonal component at immunofixation clearly showed an expansion of κ (κ/λ ratio= 9.87 and 8.26) and one of λ PC (κ/λ ratio= 0.07) in the bone marrow. Monoclonal immunoglobulins are often present in very low concentrations in amyloidosis because of catabolism, tissue deposition or reduced synthesis; patients can therefore be negative at immunofixation, but evidence of marrow PC clonality permits a diagnosis of light-chain derived amyloidosis.[3,4] The tissue biopsy of the single patient negative at both analyses showed monotypic κ light chains. Overall, 35 patients had λ (62.5%) and 21 κ (37.5%) AL amyloidosis (Table 1).

Table 1. Characteristics of the AL amyloidosis patients studied.

Demographics (n=56)	
Age	55 y (median, range 29-80)
Men/women	38/18
Interval from diagnosis to therapy	2 m (median, range 0-36)
Follow-up*	20 m (median, range 7-83)
Survival from diagnosis	34 m (median, range 1-84+)
λ/κ patients	35/21
Serum/urine immunofixation monoclonal component	93% of patients
Bone marrow aspirate	
clonal PC isotype ratio	79% of patients
PC%	7% (median, range 1-26)°
Organ predominantly involved	n. of patients
heart	21
kidney	20
liver	7
peripheral nervous system	3
others	5
N. of organs involved	n. of patients
1	12
2	23
3	13
4	8

**Follow-up of patients surviving longer than 6 months; °One patient met criteria for smoldering myeloma. Abbreviations: y, years; m, months; PC, plasma cells.*

Statistical analysis for prognostic factors

A previous analysis for prognostic factors in a series of 125 AL amyloidosis cases showed that CHF was the most important independent variable affecting survival, followed by hepatomegaly and the number of organs involved.[8] CHF and hepatomegaly were also identified in a series of 168 patients seen at the Mayo Clinic.[9] Table 2 shows the results of univariate

Table 2. Univariate survival analysis (Cox models) for prognostic factors in the 56 patients studied.

Variables	*Coefficient*	*p value*
CHF	1.13	0.004
Hepatomegaly	0.65	0.09
N. of organs involved	– 0.13	0.46
BMPC isotype ratio	0.40	0.001
PC%	0.70	0.008

Abbreviations: CHF, congestive heart failure; BMPC, bone marrow plasma cells; PC, plasma cells.

Table 3. Comparison of different Cox survival models involving bone marrow PC isotype ratio, PC% and congestive heart failure in the series of 56 patients studied.

Cox models	*Coefficient*	*p value*
Model 1		
CHF	1.13	0.005
BMPC isotype ratio	0.30	0.020
PC%	0.55	0.060
Model 2		
CHF	1.22	0.002
PC%	0.78	0.005
Model 3		
CHF	1.06	0.007
BMPC isotype ratio	0.38	0.002

Abbreviations: CHF, congestive heart failure; BMPC, bone marrow plasma cells; PC, plasma cells.

statistical analysis in the current series of 56 patients: CHF, PC% and bone marrow PC light chain isotype ratio significantly affected survival (Table 2). Hepatomegaly and the number of organs involved were not significantly correlated in this series of patients.

Cox survival models were constructed for possible combinations of a maximum of three variables (Table 2) at the same time, given the sample size. The most powerful models are reported in Table 3. CHF and PC isotype ratio showed independent prognostic value at multivariate analysis (Table 3, model 1), whereas PC% was significant only if analyzed separately with CHF (Table 3, model 2). This suggested that PC isotype ratios and PC% were related; indeed, a direct correlation between the two parameters was observed ($p = 0.02$, $r = 0.3$).

The search for a cut-off value indicated that the influence of clonality values on survival was continuous, i.e. there was more than one value that discriminated groups with significantly different survivals. Figure 1 shows Kaplan-Meier estimates of the survival curves for κ/λ ratio cut-offs of 0.2 (Figure 1A, λ patients, p=0.022) or 16 (Figure 1B, κ patients, p=0.03); these values identified groups composed of a similar number of patients. At 24 months of follow-up, only 45% of the λ and 20% of the κ patients in the poor prognosis group were still alive, as compared to about 75% survival among the others (Figure 1). For a κ/λ ratio cut-off value of 0.1 (Figure 2), none of the 8 λ patients with a ratio <0.1 was alive 20 months from diagnosis, whereas about 65% of the other 27 λ patients survived (p=0.002).

Discussion

Knowledge of the factors that may influence the clinical course of AL amyloidosis is useful for estimating prognosis and for deciding the most appropriate therapeutic approach. This is important, especially if one considers that high-dose chemotherapy with peripheral blood stem cell rescue[10] and heart or kidney transplantation[6,11] are now being proposed, and these demanding therapeutic procedures cannot be undertaken without information about the life expectancy of individual patients.

This study shows that the degrees of marrow PC

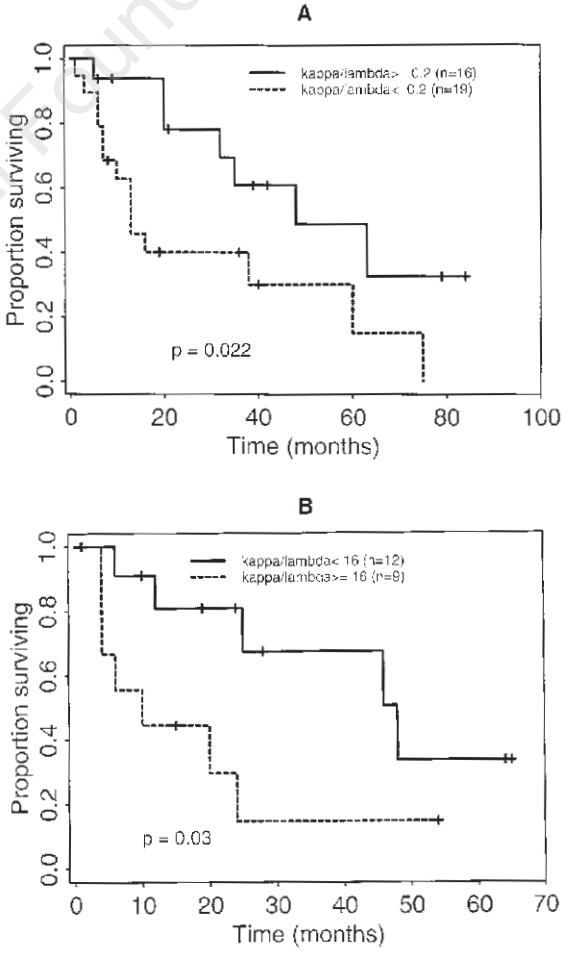

Figure 1. Kaplan-Meier estimates of the survival curves for patients with AL amyloidosis stratified according to bone marrow plasma cell κ/λ ratio values. A) λ patients (#35); B) κ patients (#21).

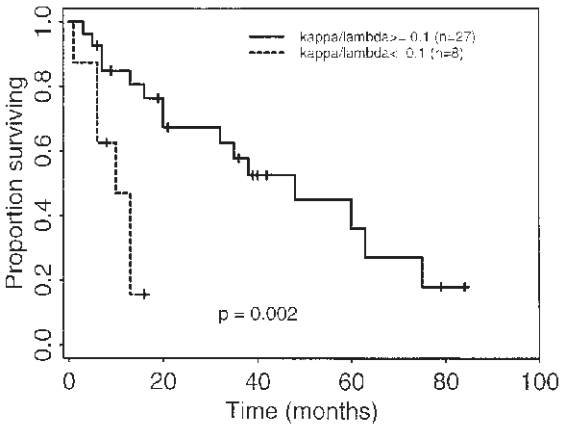

Figure 2. Kaplan-Meier estimate of the survival curves for patients with λ light chain AL amyloidosis (#35) stratified according to the bone marrow plasma cell κ/λ ratio cut-off of 0.1.

clonality and infiltration adversely influence the outcome of AL amyloidosis patients without multiple myeloma. Both the number of marrow PC and the degree of light chain isotype disequilibrium demonstrated strong negative predictive value (PC%, p = 0.008; PC isotype ratio, p = 0.001). Alteration of the PC isotype ratio was more powerful and showed independent prognostic relevance at multivariate analysis (Table 3). κ/λ ratio cut-off values of 0.2 and 16 in the λ and κ patients, respectively, discriminated groups with significantly different survivals (Figure 1).

In a previous study on 62 cases of AL amyloidosis without multiple myeloma, Wu *et al.*[12] suggested that the degree of plasmacytosis found at bone marrow biopsy was inversely related to patient survival (p = 0.033), but that the presence of clonality was not (p = 0.13). As suggested by these authors, the latter finding might be explained by the low sensitivity of their method; Wu *et al.* assigned clonality when all marrow PC stained with just one isotype, a feature that was found in less than two-thirds of patients (40 of 62 cases, 64%). We measured the marrow PC isotype ratio and found that abnormal values were common (79% of patients) and strongly influenced clinical outcome.

Though it remains true that, in some instances, a few PC may synthesize small quantities of light chains with high pathogenic potential that can rapidly lead the patient to death, this study indicates that the abundance of clonal PC, which relates both to PC number and their isotype ratio, favors the progression of the disease. Clones of increased size generally produce larger amounts of amyloidogenic light chains, thus leading to accelerated organ damage.

Contributions and Acknowledgments

VP developed the clonality assay, was responsible for the conception and design of the study, and drafted the manuscript; MCV performed most of the clonality assays; GM, EAn and PG were involved in the clinical assessment of patients; SQ performed the statistical analysis; EAs critically revised the manuscript; GM made a fundamental contribution to the analysis and interpretation of the data, critically revised the manuscript, and gave final approval of the version to be published.

Funding

Supported by AIRC, Italian Ministry of Health (project n. 261RFM92/02), CNR target projects ACRO (projects n°94.01322.PF39, 96.000626.PF39), the Ferrata-Storti Foundation, and the University Hospital-IRCCS Policlinico S. Matteo, Pavia, Italy.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received July 1, 1998; accepted November 11, 1998.

References

1. Solomon A, Weiss DT. Protein and host factors implicated in the pathogenesis of light chain amyloidosis (AL amyloidosis). Amyloid Int J Exp Clin Invest 1995; 2:269-79.
2. Perfetti V, Bellotti V, Garini P, et al. AL amyloidosis: characterization of amyloidogenic cells by anti-idiotypic antibodies. Lab Invest 1994; 71:853-61.
3. Perfetti V, Garini P, Colli Vignarelli M, Marinone MG, Zorzoli I, Merlini G. Diagnostic approach to and follow-up of difficult cases of AL amyloidosis. Haematologica 1995; 80:409-15.
4. Gertz MA, Greipp PR, Kyle RA. Classification of amyloidosis by detection of clonal excess of plasma cells in the bone marrow. J Lab Clin Med 1991; 118:33-9.
5. Kyle RA, Gertz MA. Primary systemic amyloidosis: Clinical and laboratory features in 474 cases. Semin Hematol 1995; 32:45-59.
6. Merlini G. Treatment of primary amyloidosis. Semin Hematol 1995; 32:60-79.
7. Gertz MA, Kyle RA, Greipp PR. Beta 2-microglobulin predicts survival in primary systemic amyloidosis. Am J Med 1990; 89:609-14.
8. Merlini G, Anesi E, Banfi G, et al. AL amyloidosis: Update of a multicenter study [abstract]. Haematologica 1996; 81(Suppl 1):52.
9. Kyle RA, Greipp PR, O'Fallon WM. Primary systemic amyloidosis: multivariate analysis for prognostic factors in 168 cases. Blood 1986; 68:220-4.
10. Comenzo RL, Vosburgh E, Falk RH, et al. Dose-intensive melphalan with blood stem-cell support for the treatment of AL (amyloid light-chain) amyloidosis: survival and responses in 25 patients. Blood 1998; 91: 3662-70.
11. Falk RH, Comenzo RL, Skinner M. The systemic amyloidoses. N Engl J Med 1997;337:898-909.
12. Wu S S-H, Brady K, Anderson JJ, et al. The predictive value of bone marrow morphologic characteristics and immunostaining in primary (AL) amyloidosis. Am J Clin Pathol 1991; 96:95-9