The clinical utility and prognostic value of multiparameter flow cytometry immunophenotyping in light-chain amyloidosis

Bruno Paiva,1,2 Marí-Belén Vidriales,1,2 José J. Pérez,1,2 María-Consuelo López-Berges,1 Ramón García-Sanz,1,2 Enrique M. Ocío,1,2 Natalia de las Heras,3 Rebeca Cuello,4 Alfonso García de Coca,4 Emilia Pardal,5 José Alonso,6 Magdalena Sierra,7 Abaeldo Bárez,8 José Hernández,9 Lissbett Suárez,10 Josefina Ganelde,11 María-Victoria Mateos,1,2 and Jesús F. San Miguel1,2

1Hospital Universitario de Salamanca, Salamanca, Spain; 2Centro de Investigación del Cáncer, Instituto de Biología Molecular y Celular del Cáncer (CIC IBMC), Universidad de Salamanca—Consejo Superior de Investigaciones Científicas (USAL-CSIC), Salamanca, Spain; 3Complejo Hospitalario de León, León, Spain; 4Hospital Clínico Universitario de Valladolid, Valladolid, Spain; 5Hospital Virgen del Puerto, Plasencia, Spain; 6Hospital Rio Carrión, Palencia, Spain; 7Hospital Virgen de la Concha, Zamora, Spain; 8Hospital Nuestra Señora de Sonsoles, Ávila, Spain; 9Hospital General de Segovia, Segovia, Spain; 10Hospital Santos Reyes, Aranda de Duero, Spain; and 11Hospital Comarcal del Bierzo, Ponferrada, Spain

The clinical value of multiparameter flow cytometry (MFC) immunophenotyping in primary or light chain amyloidosis (AL) remains unknown. We studied 44 consecutive bone marrow samples from newly diagnosed patients with amyloidosis; 35 patients with AL and 9 with other forms of amyloidosis. Monoclonal plasma cells (PCs) were identifiable by MFC immunophenotyping in 34 of 35 (97%) patients with AL, whereas it was absent from all but 1 of the 9 (11%) patients with other forms of amyloidosis. Quantification of bone marrow plasma cells (BMPCs) by MFC immunophenotyping was a significant prognostic factor for overall survival (OS) (< 1% vs > 1% BMPC cutoff; 2-year OS rates of 90% vs 44%, P = .02). Moreover, detecting persistent normal PCs at diagnosis identifies a subgroup of patients with AL with prolonged OS (> 5% vs ≤ 5% normal PC within all BMPC cutoff, 2-year rates of 88% vs 37%, P = .01). MFC immunophenotyping could be clinically useful for the demonstration of PC clonality in AL and for the prognostication of patients with AL. (Blood. 2011;117(13):3613-3616)

Introduction

Primary or light chain amyloidosis (AL) is the most frequent type of systemic amyloidosis and is characterized by the accumulation of monoclonal light chain fragments, leading to end-organ damage and short survival.1-3 Other forms of amyloidosis, such as localized, inherited, or secondary, are associated with different clinical courses and therapies.4,5 In contrast to these other forms of amyloidosis, in AL bone marrow (BM), plasma cells (PCs) are responsible for fibril deposition;6 however, the bone marrow plasma cell (BMPC) infiltration is usually low,7 and clonality is not always apparent.8,9 Multiparameter flow cytometry (MFC) immunophenotyping has shown to be of clinical value in multiple myeloma (MM),10-13 but its use in other PC disorders such as AL remains largely unexplored.13 In this study, we investigate the clinical utility and prognostic value of MFC immunophenotyping in patients with AL.

Methods

Patients, material, and methods

We evaluated 44 consecutive BM samples from patients with newly diagnosed amyloidosis referred to our institution for immunophenotypic investigations. Samples were collected after informed consent was given, in accordance with Hospital Universitario Salamanca ethical committee guidelines and the Declaration of Helsinki. From the 44 cases, 35 had a confirmed diagnosis of AL based on the presence of an amyloid-related systemic syndrome, positive amyloid tissue staining with Congo red, and evidence of a monoclonal PC (M-PC) proliferative disorder at the cellular or serologic level. The remaining 9 cases were diagnosed with localized (n = 6), secondary (n = 2), or familial (n = 1) forms of amyloidosis. Table 1 shows the demographics and disease characteristics of the 35 patients with AL; 20 were treated with chemotherapy (melphalan-prednisone or melphalan-dexamethasone), whereas 10 received an autologous stem cell transplant; 5 patients were deemed too ill to tolerate any therapy.

Erythrocyte-lysed whole BM samples were stained by 4-color direct immunofluorescence, as described elsewhere.10-12,19 The monoclonal antibody combination (fluorescein isothiocyanate/phycocerythrin/peridinin chlorophyll protein-cyanin 5.5/allophycocyanin) CD38/CD56/CD19/CD45 allowed the identification, quantification, and discrimination between M-PCs and normal PCs (N-PCs) in more than 90% of patients with a sensitivity of 4.19,20 For the remaining patients, additional staining with cytoplasmatic immunoglobulin (Ig)κ/Igλ and surface CD38 plus CD19, CD45, or CD56 was performed. Cell cycle was analyzed as previously described13 in 10 of the 35 patients with AL. Cells were acquired in a FACSCalibur flow cytometer (BD Biosciences) using the CellQuest program (BD Biosciences), and information was recorded for ≥ 3 x 10^4 BMPCs/tube. Data were analyzed using the Paint-A-Gate-PRO 3.0 program (BD Biosciences).11,19

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 USC section 1734.

© 2011 by The American Society of Hematology
Next, we wanted to examine further the immunophenotypic characteristics of M-PCs in patients with AL. Phenotypically aberrant M-PCs were detected mainly on the basis of simultaneous intra-expression of CD19 and CD45, with or without overexpression of CD56 (44% and 32% of cases, respectively). The remaining cases (24%) featured less commonly detected aberrant phenotypes (supplemental Table 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article). Overall, these results are consistent with those of Matsuda et al\textsuperscript{15,16} from a shorter series (≤ 10 patients with AL). Interestingly, AL M-PCs showed an immunophenotypic profile that largely overlaps with MM M-PCs,\textsuperscript{10,11} although these entities have a clearly different clinical picture. We also explored the proliferative capacity of M-PCs in a subgroup of patients with AL. The median percentage of M-PCs in S-phase was 0.7% (range 0%-1.6%), which is lower than that observed in MM (1.6%-3.6%)\textsuperscript{12,13} and closer to that in smoldering MM (≈ 1%, our unpublished data).

BMPC infiltration is typically low in AL,\textsuperscript{6,7} and probably because of this characteristic its percentage, assessed by morphologic and immunohistochemistry techniques, is not usually considered when predicting the overall survival (OS) of patients with AL.\textsuperscript{8} We have recently found that in MM, although MFC generally yields lower PC counts than conventional morphology, the former is an independent prognostic factor.\textsuperscript{12} In the present AL series, the median BMPC percentage measured by morphology was also higher (P < .001) than that obtained by MFC immunophenotyping (Table 1). However, whereas the number of PC cases estimated by morphology did not influence OS (data not shown), patients with more than 1% BMPC measured by MFC immunophenotyping had a significantly shorter OS (2-year rates of 44% vs 90%, respectively; P = .02) than patients with 1% or less BMPC (Figure 1A). With respect to the potential prognostic value derived by the degree of clonality in AL, Kumar et al\textsuperscript{13} using the sFLC test have found that patients with normal baseline ratios have a longer OS than those with abnormal sFLC ratios.\textsuperscript{23} We have previously found that MFC immunophenotyping detection of more than 5% N-PC within the BMPC compartment (N-PC/BMPC) was useful for the differential diagnosis of MGUS and MM,\textsuperscript{10} and identification of patients with low-risk MGUS, smoldering MM, and symptomatic MM.\textsuperscript{11,19}

In this series, 17 of 35 (49%) patients with AL had more than 5% N-PC/BMPC at diagnosis and, interestingly, these patients had a significantly longer OS (2-year rates of 88% vs 37%, respectively, P = .01) than patients with 5% or less N-PC/BMPC (Figure 1B). On excluding the 5 patients who did not receive any treatment, the prognostic influence of having more than 5% versus 5% or less N-PC/BMPC remained significant (P = .02); moreover, patients with more than 1% BMPC measured by MFC immunophenotyping also showed a trend for an adverse outcome (P = .07). Therefore, BMPC enumeration and particularly the detection of more than 5% N-PC/BMPC by MFC immunophenotyping could be of prognostic value in AL, similar to what was previously found in MGUS and MM.\textsuperscript{11,12,19} We have also compared the baseline characteristics of patients with more than 5% vs 5% or less N-PC/BMPC. No significant differences were found for conventional parameters, including the number and type of organs involved, except for the percentage of BMPCs quantified by MFC immunophenotyping (0.8% vs 2.8%, respectively, P < .001), whereas differences by morphology were borderline (4% vs 7%, P = .1). The median difference between involved and uninvolved free light chain (dFLC) was higher in cases with 5% or less N-PC/BMPC (627 mg/L) compared with patients with more than 5% N-PC/BMPC (150 mg/L), but differences failed to reach statistical significance.
significance ($P = .2$); regarding the frequency of patients in stage 2 and stage 3 AL, defined by the N-terminal prohormone of brain natriuretic peptide and cardiac troponin T (Mayo Clinic staging system), no significant differences were observed on comparing patients with 5% or less versus more than 5% N-PC/BMPC (88% vs 60%; $P = .7$). The limited number of cases precluded a multivariate analysis to determine whether BMPC enumeration and detection of more than 5% N-PC/BMPC by MFC immunophenotyping are independent prognostic markers in AL.

In summary, our results raise the possibility that in patients with suspected amyloidosis, MFC immunophenotyping could be clinically useful for demonstrating PC clonality in AL versus other forms of amyloidosis and may also offer additional prognostication in AL; these results requiring validation in larger and prospective series.

Acknowledgments

This work was supported by the Cooperative Research Thematic Cancer Network (RTIC) grants RD06/0020/0006 and G03/136, MM Jevitt, SL firm, Instituto de Salud Carlos III/Subdirección General de Investigación Sanitaria (FIS) grants PI060339, 02/0905, 01/0089/01-02, PS09/01897, and Consejería de Sanidad, Junta de Castilla y León, Valladolid, Spain grant 557/A/10.

Authorship

Contribution: B.P. and M.-B.V. designed the study protocol, collected and assembled data, analyzed and interpreted data, analyzed the flow cytometric data, performed statistical analysis, and wrote the manuscript; J.J.P., and M.-B.V. analyzed and interpreted the flow cytometric data; R.G.-S, E.M.O., N.H., R.C., A.G.C., E.P., J.A., M.S., A.B., J.H., L.S., J.G., and M.-V.M. contributed with provision of study material or patients; and J.F.S.-M. conceived the idea, designed the study protocol, analyzed and interpreted data, and wrote the manuscript. All authors reviewed and approved the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

Correspondence: Jesús F. San-Miguel, Hospital Universitario de Salamanca, Paseo de San Vicente 58-182, 37007 Salamanca, Spain; e-mail: sanmigiz@usal.es.

References


The clinical utility and prognostic value of multiparameter flow cytometry immunophenotyping in light-chain amyloidosis

Bruno Paiva, María-Belén Vídriales, José J. Pérez, María-Consuelo López-Berges, Ramón García-Sanz, Enrique M. Ocío, Natalia de las Heras, Rebeca Cuello, Alfonso García de Coca, Emilia Pardo, José Alonso, Magdalena Sierra, Abelardo Bárez, José Hernández, Lissbett Suárez, Josefina García-Sanz, Enrique M. Ocio, Natalia de las Heras, Rebeca Cuello, Alfonso García de Coca, Emilia Bruno Paiva, María-Belén Vídriales, José J. Pérez, María-Consuelo López-Berges, Ramón García-Sanz, Enrique M. Ocío, Natalia de las Heras, Rebeca Cuello, Alfonso García de Coca, Emilia Pardo, José Alonso, Magdalena Sierra, Abelardo Bárez, José Hernández, Lissbett Suárez, Josefina García-Sanz, Enrique M. Ocio, Natalia de las Heras, Rebeca Cuello, Alfonso García de Coca, Emilia