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Brief report

The persistence of immunophenotypically normal residual bone marrow plasma cells at diagnosis identifies a good prognostic subgroup of symptomatic multiple myeloma patients

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Multiparameter flow cytometry immunophenotyping allows discrimination between normal (N-) and myelomatous (MM-) plasma cells (PCs) within the bone marrow plasma cell compartment (BMPCs). Here we report on the prognostic relevance of detecting more than 5% residual normal plasma cells from all bone marrow plasma cells (N-PCs/BMPCs) by multiparameter flow cytometry in a series of 594 newly diagnosed symptomatic MM patients, uniformly treated according to the Grupo Español de MM 2000 (GEM2000) protocol. Our results show that symptomatic MM patients with more than 5% N-PCs/BMPCs (n = 80 of 594; 14%) have a favorable baseline clinical prospect, together with a significantly lower frequency of high-risk cytogenetic abnormalities and higher response rates. Moreover, this group of patients had a significantly longer progression-free survival (median, 54 vs 42 months, *P* = .001) and overall survival (median, not reached vs 89 months, *P* = .04) than patients with less than or equal to 5% N-PCs/BMPCs. Our findings support the clinical value of detecting residual normal PCs in MM patients at diagnosis because this reveals a good prognostic category that could benefit from specific therapeutic approaches. This trial was registered at www.clinicaltrials.gov as NCT00560053. (Blood. 2009; 114:4369-4372)

Introduction

Multiparameter flow cytometry (MFC) immunophenotyping allows discrimination between myelomatous plasma cells (MM-PCs) and normal/reactive PC (N-PCs).[1-4] This has been used for the differential diagnosis between monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM),[3] the identification of high-risk MGUS and smoldering MM (SMM) patients,[4] minimal residual disease investigation,[5-8] definition of prognostic antigenic profiles,[1] and identification of new therapeutic targets.[2] Typically, in symptomatic MM patients at diagnosis, most bone marrow plasma cells (BMPCs) are clonal, and N-PCs from all BMPCs are only detected in a minority of MM cases.[3,9] By contrast, 82% of MGUS and 40% of SMM patients show more than 5% residual normal plasma cells from all bone marrow plasma cells (N-PCs/BMPCs), in association with a low risk of progression to symptomatic MM.[4] Here we report on the results of a prospective analysis of the prognostic impact of the presence of more than 5% N-PCs/BMPCs at diagnosis in a large series of uniformly treated symptomatic MM patients.

Methods

Patients

The study included 594 untreated, symptomatic MM patients diagnosed according to the International Myeloma Working Group criteria[10] and uniformly treated following the Spanish GEM2000 protocol (VBMCP/VBAD ×6 plus autologous stem cell transplantation [ASCT]).[1,6] Written informed consent was obtained in accordance with the Declaration of Helsinki, as well as Institutional Review Board approval from each participating hospital. Response was assessed using the European Group for Blood and Marrow Transplant criteria.[11,12] At the study endpoint, 380 patients (64%) relapsed/progressed and 188 (32%) died, with a median follow-up of 54 months; median progression-free survival (PFS) and overall survival (OS) were 44 and 97 months, respectively. It is the current standard of clinical practice in MM to report cytogenetic results on purified PCs,[13] but unfortunately this was not part of the initial workload of the GEM2000 protocol. Therefore, we used a complementary series of 501 patients with complete cytogenetic/fluorescence in situ hybridization data

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Table 1. Patient demographics and baseline characteristics of symptomatic MM patients

Patient demographics and baseline characteristics	% of normal PC from all BMPC (N-PC/BMPC) ≤ 5% (n = 514)	> 5% (n = 80)	*P*
Male/female*	283 (55%)/231 (45%)	48 (60%)/32 (40%)	NS
Age, y	58 (32-70)	58 (35-70)	NS
Subtype of MM*			
IgG	304 (59%)	37 (46%)	.06
IgA	123 (24%)	17 (22%)	NS
Bence-Jones protein	82 (16%)	22 (27%)	.04
Nonsecretory	5 (1%)	4 (5%)	NS
ISS*			
Stage I	180 (35%)	38 (48%)	.03
Stage II	226 (44%)	30 (37%)	NS
Stage III	108 (21%)	12 (15%)	NS
β2-microglobulin, mg/L†	3.2 (0.09-41)	2.5 (0-24)	.009
Hemoglobin, g/L†	106 (10-167)	121 (60-168)	< .001
Albumin, g/dL†	3.6 (0.3-7)	3.8 (1.8-6.4)	.02
Platelet count, $\times 10^9$/L†	207 (6-990)	241 (58-486)	< .001
M-component, g/dL†	4 (0-47)	2 (0-22)	< .001
Immunoparesis, 1 or 2 Ig*	427 (83%)	34 (42%)	.003
Plasma cells by morphology, %†	40% (1-100%)	17 (1-95)	< .001
Plasma cells by MFC, %†	13 (0.1-90)	1.6 (0.05-22)	< .001
Plasma cells in S-phase, %†	1.4 (0-15)	2.0 (0-15)	NS
$CD117^+$ cases by MFC, n (%)*	175 (34)	39 (49)	.04

Patient demographics and baseline characteristics of symptomatic MM patients grouped according to the percentage of N-PC/BMPC.

MM indicates multiple myeloma; N-PC/BMPC, normal plasma cells from all bone marrow plasma cells; PC, plasma cells; BMPC, bone marrow plasma cells; NS, statistically not significant ($P > .05$); ISS, international staging system; and MFC, multiparameter flow cytometry.

*Results expressed as percentage of cases.

†Results expressed as median (range).

from immunomagnetic-enriched plasma cells, with a rather short follow-up, which precludes survival analysis. Information of serum-free light chain (sFLC) was also extracted from this series.

MFC immunophenotypic studies

Erythrocyte-lysed whole BM samples were stained using a 4-color direct immunofluorescence technique, previously described in detail.[1,6,8] The following monoclonal antibody combinations (fluorescein isothiocyanate/phycoerythrin/peridinin chlorophyll protein-Cy5.5/allophycocyanin) were used to identify aberrant antigen expression in PCs: CD38/CD56/CD19/CD45, CD138/CD28/CD33/CD38, and CD20/CD117/CD138/CD38.[1,6,8,14] Acquisition was performed in a FACSCalibur flow cytometer (BD Biosciences) using the CellQuest program (BD Biosciences).[1,2,4,6,8] Information was recorded for at least 3×10^3 BMPCs/tube. The Paint-A-Gate PRO program (BD Biosciences) was used for data analysis, following the recommendations of the European Myeloma Network.[14]

Statistical analyses

The χ^2 and Mann-Whitney U tests were used to estimate statistical significant differences. Survival curves were plotted using the Kaplan-Meier method, assessing differences with the log-rank test. For the multivariate analysis, the Cox regression proportional hazard model (stepwise regression) was used. Statistical analyses were carried out with SPSS (Version 15.0; SPSS Inc).

Results and discussion

Two groups have identified the presence on N-PCs in MM patients at diagnosis in a small series.[9,15] However, neither the number of these cells nor the clinical and prognostic value was analyzed. Here, 80 of 594 newly diagnosed MM patients (14%) had more than 5% N-PCs/BMPCs by MFC, all of them with CRAB features (increased calcium, renal insufficiency, anaemia, or bone lesions). Interestingly, the presence of more than 5% N-PCs/BMPCs was associated with characteristics related to a favorable prognosis[16-19] (Table 1): lower β_2 microglobulin $(\beta)_2$M ($P = .01$) and M-component serum levels ($P < .001$), lower BMPC infiltration by both morphology ($P < .001$) and MFC ($P < .001$), together with

Table 2. Cytogenetics

Cytogenetics	% of normal PC from all BMPC (N-PC/BMPC) ≤ 5% (n = 439)	> 5% (n = 62)	*P*
IgH translocations	187 (43%)	8 (13%)	< .001
t(4;14)	66 (15%)	0 (0%)	.002
t(11;14)	79 (18%)	8 (13%)	NS
t(14;16)	18 (4%)	0 (0%)	NS
Others	35 (8%)	0 (0%)	.009
Del (13q)	180 (41%)	3 (5%)	< .001
Del (17p)	36 (8%)	2 (3%)	NS
High-risk: any t(4;14), t(14;16), or del(17p)−	114 (26%)	2 (3%)	.006

Information on patient cytogenetics corresponds to a parallel series of not uniformly treated MM patients, not to the GEM2000 series reported in the present paper.

Results expressed as percentage of cases.

NS indicates not statistically significant.

Table 3. Response to therapy

	≤ 5% N = PC/BMPC		> 5% N = PC/BMPC		*P*	
EBMT criteria	**After induction**	**After ASCT**	**After induction**	**After ASCT**	**After induction**	**After ASCT**
CR	56 (11%)	168 (33%)	17 (21%)	51 (64%)	.01	< .001
nCR	61 (12%)	99 (19%)	19 (24%)	8 (10%)	.005	< .001
PR or less	397 (77%)	247 (48%)	44 (55%)	21 (26%)	< .001	< .001

Results are expressed as number (percentage) of cases. N = PC indicates normal plasma cells; BMPC, bone marrow plasma cells; and ASCT, autologus stem cell transplantation.

higher hemoglobin levels ($P < .001$), among other features (Table 1). In addition, immunoparesis (reduction below the lower normal limit in the levels of one or 2 uninvolved Ig) was significantly less frequent among patients with more than 5% N-PCs/BMPCs than in those with less than or equal to 5% N-PCs/BMPCs (42% vs 83%, respectively; $P = .003$), probably reflecting higher levels of residual N-PCs. Concerning the MM-PC immunophenotypic profile, the only antigen showing significant differences was CD117, a phenotypic marker associated with a favorable outcome,[1,20,21] with cases holding more than 5% N-PCs/BMPCs showing a higher incidence of positive CD117 expression (49% vs 34%, respectively; $P = .04$). In the complementary series of patients with complete cytogenetic/fluorescence in situ hybridization information (Table 2), the frequency of cases with more than 5% N-PCs/BMPCs (12%; Table 1) was almost identical, which excludes a bias in patient selection. Interestingly, patients with more than 5% N-PCs/BMPCs had a lower incidence of del (13q) (5% vs 41%; $P < .001$) and IgH translocations (13% vs 43%; $P < .001$) than cases with less than or equal to 5% N-PCs/BMPCs. Moreover, whereas the incidence of t(11;14), which has little influence on survival, was similar in both group of patients (13% vs 18%; not significant), t(4;14) was not detected in cases with more than 5% N-PCs/BMPCs, although it represented 15% of cases with less than or equal to 5% N-PCs/BMPCs ($P = .002$). In addition, when high-risk cytogenetic cases [t(4;14), t(4;16), or del (17p)] were grouped, we founded a significantly lower frequency in cases with more than 5% N-PCs/BMPCs (3% vs 26%, respectively; $P = .006$). Moreover, considering patients with sFLC data available (N = 261) at diagnosis, cases with more than 5% N-PCs/BMPCs presented a higher frequency of normal sFLC ratio than those with less than or equal to 5% N-PCs/BMPCs (10 of 33, 30%; vs 16 of 228, 7%; $P < .001$).

Concerning response to therapy (Table 3), patients with more than 5% compared with those with less than or equal to 5% N-PCs/BMPCs showed higher rates of complete remission after induction (21% vs 11%, respectively; $P < .001$), and at day 100 after ASCT (64% vs 33%, respectively; $P < .001$). Accordingly, cases with more than 5% N-PCs/BMPCs were more frequently minimal residual disease negative at day 100 after ASCT (68% vs 37%; $P < .001$) and also showed a faster and more complete recovery of the polyclonal PC population at day 100 after ASCT, compared with less than or equal to 5% N-PCs/BMPCs cases (mean ratio of N-PCs/BMPCs of 87 vs 64%, respectively; $P < .001$). Differences in response rates translated into distinct survival, as cases with more than 5% N-PCs/BMPCs showed a better outcome than patients with less than or equal to 5% N-PCs/BMPCs with significant differences in both PFS (median, 54 vs 42 months; $P = .001$) and OS (median, not reached vs 89 months, $P = .04$)

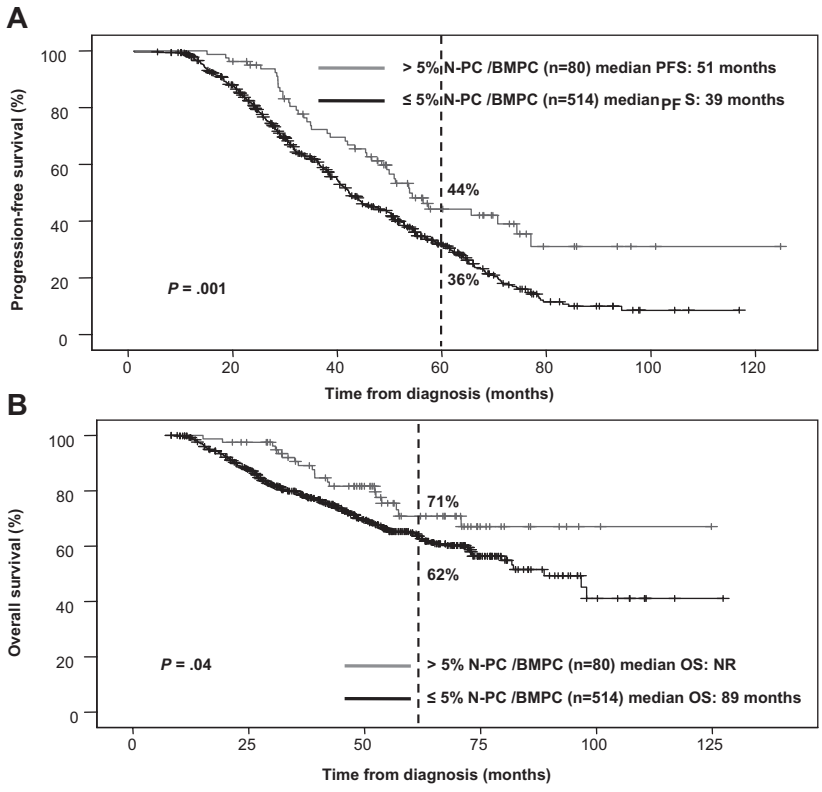

Figure 1. PFS and OS of symptomatic MM patients grouped according to the presence (N = 80) or absence (N = 514) of more than 5% N-PCs/BMPCs at diagnosis. (A) PFS. (B) OS.

(Figure 1). Therefore, the presence of more than 5% N-PCs/BMPCs emerges as a new prognostic factor for symptomatic MM patients, similar to what has previously been found in both MGUS and SMM.[4] Other baseline significant factors for survival in the univariate analysis were: low hemoglobin ($\leq$ 100 g/L; $P \leq .008$), higher calcium ($>$ 10 mg/dL; $P \leq .04$), increased creatinine ($>$ 2 mg/dL; $P = .002$) levels, higher percentages of BMPCs by microscopy ($>$ 30% BMPCs; $P \leq .006$) and MFC ($>$ 15%BMPCs; $P \leq .007$), advanced disease (International Staging System [ISS] stage III; $P \leq .004$), and high-risk cytogenetics ($P < .001$). Despite the association found between the presence of more than 5% N-PCs/BMPCs and immunoparesis, it should be emphasized that the latter variable did not appear to have a significant impact on survival, suggesting that it cannot be used as a surrogate marker to replace immunophenotyping. Multivariate analysis performed in cases with cytogenetic information (N = 176) showed only cytogenetics as an independent prognostic factor for PFS ($P < .001$) and OS ($P = .002$). Because cytogenetic information was only available for a subset of patients, a new multivariate analysis was performed for the whole series. With respect to PFS, both the ISS disease stage (relative risk of progression [RR] = 1.4; $P = .03$) and the percentage of N-PCs/BMPCs (RR = 1.6; $P = .008$) were selected as independent prognostic factors, whereas for OS hemoglobin levels (RR = 1.6; $P = .01$) together with the ISS disease stage (RR = 2.1; $P = .003$) fitted the model.

In conclusion, our results show that in symptomatic MM patients the identification of a significant number of residual normal PCs at diagnosis ($>$ 5% N-PCs/BMPCs) reveals a specific subgroup of patients with a unique biologic signature and prolonged survival who could benefit from specific therapeutic approaches.

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Authorship

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

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

A complete list of GEM Cooperative Study Group participants appears in the online "Appendix" (available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

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