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PROLIFERATIVE ACTIVITY OF PLASMA CELLS IS THE MOST RELEVANT PROGNOSTIC FACTOR IN ELDERLY MULTIPLE MYELOMA PATIENTS

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Although multiple myeloma (MM) is predominantly a disease of the elderly, few studies have focused on the identification of prognostic factors in this group of patients. Four hundred twenty five MM patients >65 years were uniformly treated with chemotherapy (MP or VCMP/VBAD). Multivariate analysis identified 4 factors with independent unfavorable prognostic influence: high percentage of S-phase bone marrow plasma cells (>2.5%); elevated β_2 microglobulin (B2M) (>4 mg/L); age >80 years old; and LDH serum levels (above normal limit). The S-phase value was the most powerful independent prognostic factor to discriminate subgroups of patients with different prognosis. Thus, 3 main risk categories could be identified according to S-phase values: ≤1%, 1–3% and >3%, with median survivals of 34, 22 and 12 months, respectively ($p < 0.0001$). Our study also proved the value for elderly patients of the recently developed International Score System (ISS) based on B2M and albumin. Furthermore, the number of S-phase cells helped to subdivide the ISS III Group identifying a subset of patients with very poor prognosis defined by an additional high S-phase, who displayed a median survival of only 8 months. These results demonstrate that elderly patients can be accurately classified according to prognosis, which may be particularly valuable when comparing the efficacy of new treatment strategies. Moreover, our results underline the high prognostic value of proliferative activity of PC, a parameter that should be considered in routine laboratory investigations of MM.

Key words: *multiple myeloma; elderly; S-phase PC; ECOG; β_2 microglobulin; flow cytometry*

Multiple myeloma (MM) is predominantly a disease of elderly people, with a median age at diagnosis >65 years.[1,2] In addition, although some authors have shown no effect of age on either response to treatment or survival,[3–5] advanced age is usually considered to be a negative prognostic factor.[6–13] Management of elderly patients with MM is a relevant problem because it involves a high number of patients who may require an adapted treatment approach to both their physical condition and disease prognosis. Currently, several therapeutic possibilities can be provided for these patients (conventional melphalan and prednisone, polychemotherapy, thalidomide alone or in combinations, -MPT, TAD, ThaCyDex-, or Bortezomib). No data is available on which therapy would be better for elderly patients, however, or on the potential value of risk-adapted protocols. As such, a good stratification based on prognosis could be very useful for comparing different treatment strategies.

Many clinical and biological prognostic factors have been reported in MM patients,[6,14–16] There are, however, no specific studies designed for identifying independent prognostic factors in elderly patients. Moreover, up to now, the influence of the proliferative activity of the PC on disease outcome in elderly MM patients remains unexplored.

Our study was designed to identify the prognostic value of clinical and biological disease characteristics, including the number of PC in S-phase in a large population of elderly untreated MM patients.

PATIENTS AND METHODS

Patients

A total of 415 untreated symptomatic MM patients diagnosed according to the criteria of the Chronic-Leukemia-Myeloma Task Force[17,18] were included in the present study. The only selection criteria for entering the study were: (*i*) to be older than 65 years of age; and (*ii*) to have a cell-cycle analysis. All were consecutive cases diagnosed at hospitals from the Central-Western region of Spain (Castilla y León) and they were referred to our institution for immunophenotyping and cell cycle analysis. Patients were treated according to the protocols of the Spanish cooperative group (Programa Español para el Tratamiento de Hemopatias Malignas-PETHEMA),[19–23] which included either melphalan and prednisone (69% of cases) or alternating cycles of polychemotherapy (VCMP/VBAP) in 31% of cases. No significant differences in outcome were observed according to treatment protocols.[20]

Clinical and laboratory disease characteristics in elderly patients, documented at diagnosis included: age, gender, performance status, presence of plasmacytomas, blood hemoglobin level, white blood cell and platelet counts, serum levels of creatinine, urea, calcium, lactate dehydrogenase (LDH), C reactive protein (CRP) and β_2 microglobulin (B2M) levels, total proteins and albumin, type of monoclonal component (MC), presence of urine Ig light chains and percentage of bone marrow plasma cells (BMPC) counted either by morphology or flow cytometry (FCM). The performance status and bone lesions were scored according to criteria described previously (ECOG and Durie and Salmon scales, respectively).[19,24] Patients were grouped into clinical stages according to the Durie and Salmon criteria[24] and the International Staging System (ISS) developed recently.[25] Cytogenetics was not included in the analysis due to the low efficiency for obtaining clonal metaphases in this group of patients. The response was considered to be complete (CR), objective (OR), minor (mR) or failure according to the standard criteria of the PETHEMA group.[19] For objective response, a decrease of 50% in the serum M-component or 90% in the urine M-component was required, whereas for complete remission, a complete clearance of the paraprotein assessed by immunoelectrophoresis was required.

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TABLE I – UNIVARIATE AND MULTIVARIATE ANALYSIS OF PROGNOSTIC FACTORS IN THE WHOLE SERIES[1]

Variable	n	Median of survival (months)	*p* (univariate)	*p* (multivariate)
S-Phase BMPC				
≤2.5%	321	29	<0.0001	<0.001
>2.5%	94	9		
β_2Microglobulin				
≤4 mg/L	163	30	<0.0001	<0.001
>4 mg/L	252	13		
Age				
≤80 yr	361	25	<0.0001	0.002
>80 yr	54	9		
LDH				
≤460 U/L	352	25	<0.0001	0.006
>460 U/L	63	11		
BMPC by FCM				
≤10%	145	28	<0.0001	—
>10%	270	29		
BMPC (morphology)				
≤50%	274	27	<0.0001	—
>50%	141	17		
Clinical Stage				
I & II	175	30	<0.0001	—
III	240	18		
Creatinine				
≤2 mg/dL	316	27	<0.0001	—
>2 mg/dL	99	10		
Urea				
≤65 mg/dL	260	29	<0.0001	—
>65 mg/dL	125	15		
Serum calcium				
≤11.5 mg/dL	338	25	<0.0001	—
>11.5 mg/dL	77	12		
Hemoglobin				
≤8.5 g/dL	110	14	<0.0001	—
>8.5 g/dL	305	26		
PS (ECOG scale)				
<3	296	30	<0.0001	—
≥3	119	13		
Bone lesions scale				
0–2	302	27	0.0008	—
3	113	20		
Albumin				
≤3.5 g/dL	161	16	0.0039	—
>3.5 g/dL	254	26		
CRP				
≤6 mg/dL	171	25	0.0036	—
>6 mg/dL	55	12		
Monoclonal component				
Only light chain	60	17	0.0516	—
Others subtypes	355	23		
Response				
CR or OR	151	38	0.0000	Not included
mR or failure	250	15		

[1]S-phase BMPC, S phase bone marrow plasma cells using flow cytometry (FCM); β_2M, β_2 microglobulin serum levels; PS, performance status according to the ECOG scale; BMPC by FCM, bone marrow plasma cell infiltration percentage using FCM; Stage, Durie and Salmon clinical stage; CRP, C reactive protein levels; Hb, hemoglobin levels; PC DNA index, plasma cell DNA index using FCM; BMPC (morphology), bone marrow plasma cell infiltration percentage using morphology; NS, not significant ($p > 0.05$).

Immunophenotypic quantification of BMPC

The percentage of plasma cells present in the bone marrow (BM) at diagnosis was assessed using a immunophenotypic approach by flow-cytometry.[26,27] For that purpose, a total of 2×10^6 BM cells were incubated with the fluorochrome-conjugated monoclonal antibodies (MoAb) anti-CD38-phycoerytrine (PE) (10 ml, IgG1 mouse, MHCD3801 clone; BDB, San Jose, CA) and anti-CD138-fluoresceine-isothiocyanate (FITC) (10 ml, IgG1 mouse, BB4 clone; Cytognos, S.L, Salamanca, Spain), according to well-established methods.[26,27] Immunophenotypic information from 20,000 stained cells was recorded on a dual-laser FACSCalibur flow cytometer (BDB) equipped with a specific software (CellQuest, BDB) for acquisition. The Paint-A-Gate PRO software program was used for the data analysis (BDB).

DNA plasma cell analysis

A double-staining procedure for nuclear DNA (with propidium iodide, IP) and surface PC antigens (with CD38 and CD138) was used for the specific analysis of DNA PC content (DNA ploidy) and the distribution of the PC into the different cell-cycle phases (G0G1, S and G2M).[27,28] Briefly, a total of 2×10^6 BM cells were incubated for 15 min at room temperature with the MoAbs anti-CD38 (10 μl; Cytognos) and anti-CD138 (10 μl; Cytognos), washed once in PBS and centrifuged 5 min at 500*g*. The cell pellet was resuspended and incubated for another 15 min in darkness (room temperature) with an FITC-conjugated anti-mouse immunoglobulin MoAb (F[ab′]2 fragments) (Dakopatts, Copenhagen, Denmark). Afterward, 2 mL of a red cell lysing solution (containing ammonium chloride) was added and cells were incubated in darkness for 10 min (room temperature). Cell lysate was then centrifuged 5 min at 500*g*. Afterward, 1.5 ml of a solution containing Ribonuclease A (R4875; Sigma, St. Louis, MO) was added and the cell pellet was incubated for another 10 min-period in darkness at room temperature. Finally, 1.5 mL of a solution containing PI (Calbiochem, San Diego, CA) was added and another incubation for at least 15 min in darkness (room temperature) was required. Measurements were carried out on a FACSCalibur flow cytometer (BDB) using the CellQuest software. At least 20,000 cells/sample, were acquired. After excluding cell doublets on FL2-Area/FL2-Width dot plot using the Paint-A-Gate PRO program (BDB), myelomatous plasma cells were clearly discriminated from normal residual BM cells according to their CD38-CD138 strong-positive intensity. The percentage of S-phase cells in the myelomatous fraction was calculated using the ModFit program (Verity Software House, Topsham, ME), according to criteria described previously.[5,30] For DNA ploidy analysis, a DNA index was calculated as the ratio obtained between the modal channel of the G0G1 plasma cells and G0G1 of the remaining cell populations present in the sample. Those cases with a DNA index equal to one were considered diploid. Aneuploid cases were hyperdiploid when DNA index was >1.08 or hypodiploid when DNA index was ≤0.9.

Statistical methods

To estimate the statistical significance of differences observed between means of continuous variables, the t-text was used with the SPSS statistical software (SPSS Inc, Chicago, IL). The χ^2 text (SPSS) was used for comparison of dichotomous variables between groups. Survival curves were plotted according to the method of Kaplan-Meier and compared using the log-rank text (survival SPSS). The variables considered for possible inclusion in a regression analysis (Cox regression, SPSS) were those displaying a significant association with survival in the univariate analysis ($p < 0.05$) or for which prior studies have suggested a possible prognostic value. The stepwise regression method was discontinued when *p*-value for entering an additional factor was >0.05. The model was tested both by including the variables in a continuous fashion (continuous model) and by grouping them into categories (dichotomous model).

RESULTS

The median survival of the whole series was 22 months (range = 1–117 months). Only 14% of patients survived >5 years. The median age of the cohort studied was 73 years (range = 66–88) with 51% males and 49% females. Patients were distributed in Durie and Salmon clinical stages as follows: Stage I, 7%; Stage II, 36% and Stage III, 57%. A serum creatinine >2 mg/dL was present in 23% (Stage B) and a serum calcium >11.5 mg/mL

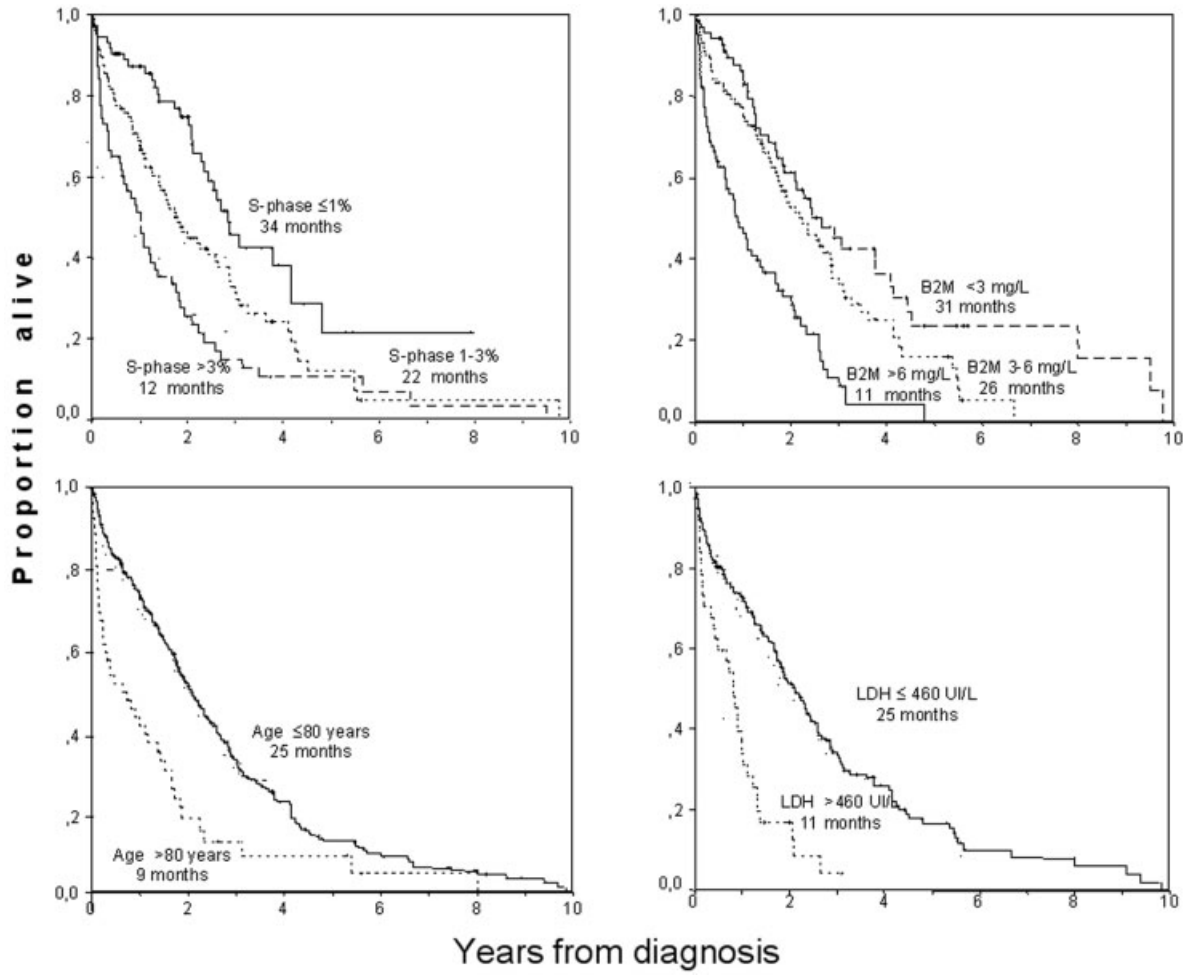

FIGURE 1 – Survival curves according to the four parameters with independent prognostic influence on multivariate analysis. Survival times indicated in months refer to median survivals.

TABLE II – COX MODEL FOR PRE-TREATMENT CHARACTERISTICS ACCORDING TO OVERALL SURVIVAL IN THE WHOLE SERIES[1]

Variable	Coefficient	SE	Wald Statistic	*p*
S-Phase BMPC > 2.5%	0.945	0.225	17.684	0.000
β_2 microglobulin > 6 mg/L	0.879	0.229	14.717	0.000
Age > 82	0.856	0.281	9.300	0.002
LDH > 460 UL/L	0.797	0.290	7.536	0.006

[1]Regression model: Ln[L(t)/l(lo)] = (1 · High S-phase PC) + (0.9 · High β_2M) + (0.9 · Older Age) + (0.8 · High · LDH).

in 17% of patients. The monoclonal serum component was IgG in 50% of cases, IgA in 33%, and IgD in 1%. In 15% of cases only a Bence Jones protein could be detected, whereas no MC was detected in 1% of cases. Urinary monoclonal light chain secretion was detected in 55% of the cases.

The univariate analysis identified 18 characteristics associated with a significant ($p < 0.05$) shorter survival (Table I): S-phase PC >2.5%, β_2 microglobulin >4 mg/L, age ≥80 years, PC infiltration detected by both morphology (>50%) or FCM (>10%), serum LDH above normal level, albumin ≤3.5 g/dL, Durie and Salmon Stage III, creatinine >2 mg/dL, urea >65 mg/dL, serum calcium ≥11.5 mg/dL, hemoglobin ≤8.5 g/dL, bone scale ≤3, CRP >6 mg/dL, Bence Jones subtype, plasma cell DNA index ≤1, and failure to achieve a favorable response to treatment (Table I). For the multivariate analysis, response to therapy was excluded because this parameter is not available at diagnosis. In addition, performance status was also excluded due to its subjectivity, which led to difficulties of reproducibility. Only 4 variables had an independent prognostic impact on overall survival at the multivariate analysis: S-phase PC >2.5%, serum B2M >4 mg/L, age higher than 80 years and serum LDH above the normal limit (Fig, 1, Tables I,II).

The best single parameter for predicting overall survival in elderly MM patients was S-phase PC, with the best cut-off point being 2.5%. In addition, patients could be divided into three groups using the cut-off points of 1 and 3% S phase PC (Fig. 1*a*). Thus, the median survival of patients with more than 3% S-phase PC was only 12 months, compared to 22 months for the patients displaying S-phase PCs between 1–3% and 34 months in patients with S-phase PC ≤1%. The group of patients with a very high S-phase showed significantly higher incidence of advanced stages, bone lytic lesions, hypercalcemia; increased values of B2M, creatinine and urea, higher percentage of BMPC infiltration by FCM, and poor response to treatment (Table III). B2M was the second most powerful parameter for risk group discrimination with significantly different survival for patients with B2M ≤3 mg/dL, between 3–6 mg/dL and B2M >6 mg/dL (median survivals of 32, 27 and 11 months, respectively) (Fig. 1*b*).

The third variable with prognostic influence was the age; thus, those patients >80 years of age did worse than those between 65–80. The effect of age in this old population was not significant until the age of 78 years old, and then its impact was continuously

TABLE III – COMPARATIVE RESULTS OF CLINICAL AND BIOLOGICAL CHARACTERISTICS BETWEEN RISK GROUPS DEFINED BY THE CUT-OFF POINTS OF S-PHASE PC[1]

Variable	S-phase PC ≤ 2.5% n = 321	S-phase PC > 2.5% n = 94	p
Age > 80 years	15%	13%	NS
PS (ECOG scale) ≥ 3	19%	45%	<0.001
Stage = III	53%	82%	<0.001
Bone lesions scale = 3	21%	50%	<0.001
Calcium > 11.5 mg/dL	9%	31%	<0.001
Creatinine > 2 mg/dL	25%	75%	<0.001
Urea > 65 mg/dL	18%	63%	<0.001
LDH > 460 UI/L	13%	18%	NS
β_2 microglobulin > 6 mg/L	50%	72%	0.001
Albumin ≤ 3.5 g/dL	49%	73%	<0.001
Hemoglobin ≤ 8.5 g/dL	27%	40%	0.028
Platelets ≤ 100 × 10^9/L	8%	13%	NS
BM PC by morphology > 20%	35%	37%	NS
BM PC by FCM > 10%	65%	72%	NS
CRP > 6 mg/dL	19%	37%	0.036
Response (CR, OR, PR)	69%	40%	<0.001

[1]S-phase PC, S phase using flow cytometry (FCM); PS, performance status according to the ECOG scale; β_2M, β_2 microglobulin serum levels; BM PC by FCM, bone marrow plasma cell infiltration percentage using FCM; Stage, Durie and Salmon clinical stage; CRP, C reactive protein levels (not available in all cases); Hb, hemoglobin levels; BM PC (morphology), bone marrow plasma cell infiltration percentage using morphology; NS, not significant (p > 0.05).

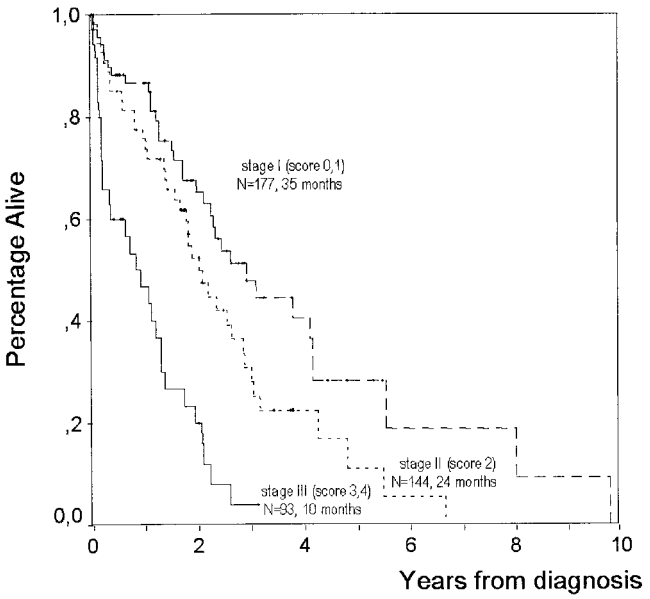

FIGURE 2 – Overall survival curves in the series according to the simplified prognostic model. Low risk patients: Stage I, one or no one adverse prognostic factors. Intermediate risk: Stage II, 2 adverse prognostic factors. High risk: Stage III, 3 or 4 adverse prognostic factors. Survival times indicated in months refer to median survivals.

increasing until a peak at 82, where its value started to decrease. Thus, only 30% of the 54 patients older than 80 survived more than 1 year, and only 8 were alive 2 years after diagnosis.

According to the regression model (Table II), a hazard rate could be established to divide patients into 3 risk groups. This model could be simplified by categorizing the risk groups according to the number of adverse prognostic factors present: low risk (patients with 0 or 1 risk factor), 42% cases; intermediate (2 risk factors), 34% of cases; and high risk (3 or 4 risk factors), 24% of cases, with median survivals of 35, 24 and 10 months, respectively (Fig. 2).

Upon applying the recently developed ISS (international scoring system)[25] to our series, significantly different median survivals of 34, 26 and 13 were obtained for Stage I (B2M ≤ 3.5 μg/L and albumin >3.5 g/dL), Sage II (B2M between 3.5–5.5 or B2M ≤ 3.5 and albumin <3.5) and Stage III (B2M > 5.5) patients. Interestingly, within the group of advanced stage (III), the proliferative activity of plasma cells (percentage of S-phase BMPC) was able to redefine 2 risk subgroups: patients with S-phase ≤2.5% had a median survival of 24 months, whereas patients with high S-phase (>2.5%) displayed a very poor outcome (median survival of 8 months).

DISCUSSION

Prognostic factors have been studied extensively in MM.[6,12,15,16,28–30] Very recently, a new international staging system (ISS) based on the combination of serum B2M and albumin values at diagnosis has been proposed.[25] Specific information on elderly patients, however, is very scanty.[31,32] Moreover, none of these studies have evaluated the prognostic impact of the proliferative activity of PC.

Our present study shows that 18 characteristics were associated with poor prognosis in elderly MM patients, but only 4 had independent value: S-phase PC, β_2 microglobulin, very advanced age and high LDH. PC proliferative activity had been reported previously to be one of the most powerful prognostic factors in MM patients.[5,16,30] We confirm its utility in elderly patients. Moreover, as a single variable it was able to stratify patients into 3 risk groups (those with ≤1% BMPC; between 1–3% and >3%), with median survivals of 34, 23 and 12 months, respectively. In addition, a high proliferative activity of BMPC was able to redefine the Stage III category of the ISS described recently. The combination of a high S-phase BMPC together with a high B2M proved to be the most useful parameter for identifying patients at high risk of early death, because 80% of cases displaying high values in these parameters died in the first year after diagnosis. The availability of well standardized flow cytometry protocols for cell cycle analysis reinforce the importance of including this parameter in routine clinical laboratory assessment of multiple myeloma patients. Because cytogenetic analysis is already recognized as one of the most relevant prognostic factors, our results suggest that the analysis of S-phase plasma cells could be a complementary tool to predict the outcome in MM patients.

The impact of very advanced age on survival is usually underestimated because these patients are frequently excluded from clinical trials due to limits in age inclusion criteria or due to the frequent presence of comorbid situations.[3–5,12] The relative short survival of the present series (22 months) was clearly influenced by age. This was particularly evident in patients >78 years. Nevertheless, it should be noted that none of the patients included in the present series had access to rescue treatment with new drugs such as thalidomide or bortezomib.

In summary we demonstrate that elderly patients can be accurately classified according to prognosis that may be particularly valuable when comparing the efficacy of new treatment strategies, such as thalidomide or bortezomib *vs.* conventional protocols (melphalan an prednisone), and to define risk-adapted treatment strategies. Moreover our results highlight the high prognostic value of the proliferative activity of PC, a parameter that should be adapted to routine laboratory investigations of multiple myeloma.

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