Peripheral Blood Monoclonal Plasma Cells as a Predictor of Survival in Patients With Multiple Myeloma

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The purpose of this study was to quantitate the number and labeling index of monoclonal plasma cells in the blood of patients with newly diagnosed multiple myeloma (MM) to learn if these values were independent prognostic factors for survival. Patients were candidates for this study if they had untreated myeloma requiring therapy, were evaluated at our institution between 1984 and 1993, and had a sample of blood analyzed with a sensitive immunofluorescence technique for monoclonal plasma cells and the blood B-cell labeling index (BLI). The % blood monoclonal plasma cells (%BPC) and the BLI were analyzed along with stage, marrow plasma cell LI, % marrow plasma cells, calcium, creatinine, albumin, β-2-microglobulin, and C-reactive protein as univariate and multivariate factors for survival. Eighty percent of the 254 patients accrued to this study had monoclonal BPC detected. The median % BPC was 6% and 57% (144 of 254) of patients had a high number (≥4%). Patients with ≥4% BPC had a median survival of 2.4 years vs 4.4 years for those with <4% BPC ($P < .001$). The BLI was also prognostic ($P = .008$). In a multivariate analysis, the %BPC, age, albumin, stage, marrow plasma cell LI, and the BLI were independent factors for survival. The %BPC and the marrow plasma cell LI best separated the group into low, intermediate, and high risk myeloma with median survivals of 52, 35, and 26 months, respectively. Patients with high %BPC were less likely to have lytic bone disease from their MM ($P = .002$). The % BPC and the BLI are independent prognostic factors for survival and are useful in identifying patients as low, intermediate, and high risk. Clonal cells in the blood should be quantified in future clinical trials for myeloma.

MULTIPLE MYELOMA (MM) is a malignancy of plasma cells that results in their accumulation in the bone marrow (BM). These cells produce monoclonal immunoglobulins and cytokines that are responsible for the anemia, bone pain, hypercalcemia, renal insufficiency, and infections that occur in these patients. The diagnosis of MM is usually confirmed by documentation of >10% monoclonal plasma cells in the BM. Although the blood is usually not overtly contaminated with monoclonal plasma cells, many studies using a variety of methods have documented small numbers of circulating tumor cells.[1-15]

There are several reasons to study the blood of patients with MM for circulating tumor cells. The presence of increased numbers of blood tumor cells has been found to be a marker of active disease.[4,6,7,9,10,12,14-23] In addition, current therapeutic approaches to MM include high-dose chemotherapy with total body irradiation followed by stem cell rescue.[24-29] The source of stem cells is usually the blood, and tumor cells have been shown to contaminate apheresis samples from patients with MM undergoing blood stem cell harvests.[30-32]

Although the number of blood tumor cells correlates with disease activity, it has not yet been documented whether the number of circulating tumor cells at diagnosis is a prognostic factor for overall survival. This study measured the number and labeling index of monoclonal blood plasma cells in 254 patients with untreated MM to learn whether these measurements were independent factors for survival.

MATERIALS AND METHODS

Patient selection and characteristics. Newly diagnosed cases of untreated MM requiring chemotherapy were eligible if they had a study of the blood for circulating monoclonal plasma cells before beginning chemotherapy. Patients were required to have a serum or urine M-protein, >10% bone marrow monoclonal plasma cells, and clinical findings consistent with the diagnosis. Patients with nonsecretory myeloma requiring chemotherapy and those with a history of a solitary plasmacytoma who received radiation therapy and later developed overt MM requiring chemotherapy were also permitted on the study. Patients with concomitant primary amyloidosis were excluded. All patients were evaluated by a hematologist at Mayo Clinic, Rochester, MN, between 1984 and 1993. The evaluation included a complete blood count, serum chemistry, serum and urine protein electrophoresis and immunoelectrophoresis, and a skeletal radiograph survey that included views of the humeri and femurs. For the purposes of this study, a positive skeletal survey was the finding of fractures or lytic bone lesions; osteoporosis without fracture or lytic lesions was not considered a positive result. This study did not include every new case of MM seen at our institution during that time period; however, this group is representative of untreated MM in that the clinical characteristics (*vide infra*), and overall survival is similar to other studies. Twenty-one patients were included in a prior analysis of prognostic factors for MM.[33] The clinical and laboratory data from these patients were reviewed with the approval of the Institutional Review Board of the Mayo Clinic/Mayo Foundation.

Analysis of the blood for monoclonal plasma cells. The number of monoclonal plasma cells in the blood and the B-cell labeling index (BLI) were obtained by use of a slide-based immunofluorescence microscopy technique as previously described.[34,35] This technique identifies monoclonal plasma cells by their classic morphology and visual documentation, in situ, of cytoplasmic immunoglobulin (cIg) light chain restriction. Photomicrographs of typical cases have been previously published.[12,35] There is an excellent correlation of monoclonal plasma cells by this technique with the finding of cIg+ light chain restricted CD38+ CD45^{-dim} cells by three-color flow cytometry[36] and with the finding of clonal cells by molecular techniques (allele-specific oligonucleotide polymerase chain reaction) when they are performed on the same blood sample.[37] Plasma cells are usually not detectable in the blood from normal healthy controls. Blood from 14 patients who were either healthy or had a disorder

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Submitted January 25, 1996; accepted April 25, 1996.

Supported in part by Grant No. CA62242 from the National Cancer Institute (National Institutes of Health) and a Career Development Award from the American Cancer Society to T.E.W.

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0006-4971/96/8805-0018$3.00/0

other than a plasma cell proliferative disease were tested with the immunofluorescence microscopy technique, and monoclonal plasma cells were not detected in any of the cases. In another normal control study, we examined blood from 49 subjects by flow cytometry and found that in 98% (48 of 49) of cases no events were found in the $CD38^+$ $CD45^-$ quadrant; in one case there were five $CD38^+$ $CD45^-$ events (0.01% of the total cells).[36] In our experience, the immunofluorescence microscopy technique can detect one clonal plasma cell in 10,000 mononuclear cells. This is less sensitive than the allele-specific oligonucleotide technique, which can detect one malignant cell in 100,000 cells.[37]

By use of the immunofluorescence technique, three values were obtained that were subsequently examined in the analysis of survival: the % blood monoclonal plasma cells (%BPC), the absolute number of monoclonal BPC (absolute #BPC), and the BLI. To perform this test, mononuclear cells were isolated on Ficoll-Hypaque; depleted of T cells by use of an anti-CD2 monoclonal antibody conjugated to magnetic beads (DynaBeads; Inc, Great Neck, NY); 1.0×10^6 cells were incubated for 1 hour at 37°C in RPMI-1640 containing 10 μmol/L bromodeoxyuridine, 1 μmol/L fluorodeoxyuridine, 10% fetal calf serum, and antibiotics. The cells were washed once with phosphate-buffered saline (PBS) at pH 7.4; cytocentrifuge slides made; air-dried; and then fixed for 10 minutes in 95% ethanol. Twenty micrograms of BU-1 antibromodeoxyuridine monoclonal antibody was added to the cell spot, incubated for 30 minutes at room temperature, washed in PBS/0.5% Tween 80, and air-dried. Eight micrograms of goat antimouse IgG labeled with rhodamine isothiocyanate (RITC) was added to detect BU-1 antibody; simultaneously, lymphocytes and plasma cells were identified by the addition of monospecific anti-κ and anti-λ reagent labeled with fluorescein isothiocyantate (FITC) (Tago, Burlingame, CA). Before the study, the anti-κ and anti-λ reagents were tested for specificity and lack of cross-reactivity to cells of the opposite light chain type. After the fluoresceinated antilight chain reagents were added, the slides were incubated a second time for 30 minutes, washed in PBS/Tween, air-dried, and read in an epi-illumination fluorescence microscope (Zeiss, Thornwood, NY) with FITC and RITC filters and a HBO 100-watt light source.

The absolute #BPC was calculated by use of the following equation: absolute # monoclonal blood plasma cells $\times$ 10^6/L = white blood cells (WBC) $\times$ % mononuclear cells (MNC) $\times$ % cIg^+ cells $\times$ % plasma cells. The % cIg^+ cells was calculated by scanning the entire slide and enumerating, on representative sections of the slide, the number of B lymphocytes and plasma cells staining with the FITC antilight chain reagent corresponding to the patient's known M-protein. The % plasma cells was the # of cells with plasma cell morphology as a % of 500 cIg^+ cells. These plasma cells were confirmed to be light chain restricted by visual documentation, in situ, of the cIg light chain corresponding to the patient's monoclonal protein light chain isotype. In addition, the slide stained with the opposite light chain was examined to confirm that none of the cIg^+ cells on that slide had plasma cell morphology. The % MNC was the % lymphocytes + % monocytes determined on the WBC differential performed on the day of the blood studies; and the WBC was the white blood count $\times$ 10^9/L.[34] It should be noted that some of the small cIg^+ B lymphocytes without plasma cell morphology that are included in the % cIg^+ cells may be clonal. However, when the cIg^+ cells were evaluated to calculate the % plasma cells, we only included cells in the calculation of monoclonal BPC that had classic plasma cell morphology and were cIg light chain restricted. The total number of clonal cells in the blood may, therefore, be greater than the number of monoclonal plasma cells calculated by the immunofluorescence technique.

For the calculation of the BLI, at least 500 B cells staining positively for the same light chain isotype as the patient's M-protein were counted to determine an immunofluorescence B-cell labeling index. The bone marrow plasma cell labeling index (BMLI) was performed as previously described.[38] Plasmablastic classification was performed by review of the bone marrow slides by one of us (P.R.G.) using previously established criteria for plasmablasts.[39]

The C-reactive protein (CRP) measurements were performed in 242 cases using immunonephelometry (Beckman Instruments, Inc, Brea, CA). The normal value is <0.8 mg/dL. In 74% (179 of 242) of the cases, the analysis was performed on serum retrieved from a frozen serum bank. The β2M values were obtained in 249 cases using a microparticle enzyme immunoassay (MEIA technology) on Abbott Diagnostics Instrumentation (Abbott Laboratories, Abbott Park, IL). The normal value is ≤2.7 μg/mL. In 20% (50 of 249) of the cases, the analysis was performed on serum retrieved from a frozen serum bank.

Statistical methods. Patients were followed until death or, for those still alive, until 1994. The major goal of the study was to assess the impact of the variables on overall survival (OS). The absolute #BPC, %BPC, BLI, CRP, β2M, BMLI, stage, age, albumin, creatinine, and calcium were examined for their ability to predict OS. Cut-offs or threshold values for the various parameters were defined on the basis of known normal ranges or published results. Where multiple thresholds were considered, the one yielding the minimum *P* value using the log-rank test was selected for multivariate modeling. Age and albumin were examined as continuous variables and by use of cut-off values. For age, the cut-off used was 70 years, because in current clinical practice, patients in this age group are candidates for protocol studies of the role of aggressive high-dose therapy with stem cell rescue.

The product-limit method of Kaplan and Meier[40] was used to estimate survival; the survival curves were compared by use of a log-rank test. For other parameters, percentages were compared using Fisher's Exact test and median values compared using rank sum tests. A two-tailed P value $< .05$ was considered significant. The Cox Proportional hazards model was used to assess the impact of each factor (univariate) and their joint impact (multivariate) on survival. A step-wise process was used to build the multivariate model. The results of the standard multivariate analyses were validated with bootstrap analysis as previously described.[41] In brief, this technique attempts to validate the results of the multivariate analysis by randomly selecting with replacement 254 patients from among the 254 under study and repeating the stepwise process to determine the independent factors for survival. In each analysis, some patients will appear more than once. This reanalysis of 254 patients was performed 500 times and if the particular variable was found significant in ≥350 of the samples, it was considered to have been validated.

The multivariate analysis was done with all variables and then repeated without the BLI because some clinical laboratories may prefer to delete the steps in the procedure that allow calculation of the BLI. The BMLI was not initially included in the multivariate analysis because it was done on only 227 of the patients. It was added back into the analysis after the multivariate analysis was complete to learn if it added additional prognostic information. The lactate dehydrogenase was also not evaluated in the multivariate analysis because data was available on only 73 patients and the test could not be performed on stored serum.

RESULTS

Patient characteristics. Two hundred fifty-four patients with untreated MM were included in the study. The clinical and laboratory results are summarized in Table 1. Clinical stage using the Durie-Salmon system was distributed as follows: 10% (26 of 254) IA; 1% (2 of 254) IB; 46% (118 of 254) IIA; 6% (15 of 254) IIB; 31% (78 of 254) IIIA; 6% (15 of 254) IIIB. The initial chemotherapy program was melphalan and prednisone in 60% (153 of 254), vincristine,

bis-chloroethyl nitrosourea (BCNU), melphalan, cyclophosphamide, and prednisone (VBMCP) in 29% (74 of 254), vincristine, doxorubicin and dexamethasone (VAD) in 7% (18 of 254), and 4% (9 of 254) had other regimens. Twenty-four patients underwent high-dose therapy with autologous stem cell rescue after initial chemotherapy; one patient had an allogeneic transplant.

Laboratory studies. A monoclonal serum or urine monoclonal protein was found in 98% (248 of 254) of patients; six cases were nonsecretory. In 81% (206 of 254) a monoclonal heavy chain was found: 70% (144 of 206) were IgG, 28% (57 of 206) IgA, 2% (4 of 206) IgD, and 0.5% (1 of 206) IgM. In 98% (248 of 254) a monoclonal light chain was found in the serum or urine; 66% (163 of 248) were monoclonal κ and 34% (85 of 248) were λ. Table 1 summarizes the laboratory studies performed in these patients. The serum creatinine was $\geq$2 mg/dL in 13% (32 of 254), and the serum calcium was $\geq$11 mg/dL in 10% (25 of 254) of patients. Four patients had a lactate dehydrogenase $\geq$300 U/L.

The number of monoclonal blood plasma cells detected at various cut-off values are summarized in Table 2. The BLI showed proliferating cells (BLI $>$ 0) in 61% (154 of 254) of the patients.

Univariate analysis for prognostic factors. The median overall survival of the entire group was 36.9 months. Table 3 summarizes the results of the analysis of survival for the variables at different cut-off values. Age as a continuous variable was significant for survival, but when age was dichotomized using a cut-off of 70 years, it was not significant (P = .08). Albumin was significant both as a continuous variable ($P < .001$) and when dichotomized at 3.5 g/dL (P = .006). In the Cox model analysis of univariate factors (Table 4), all variables were significant predictors of overall survival except gender and the serum calcium.

Table 1. Summary of Variables Used in Analysis of Survival

Variable	No.	Mean	Median	Range
Age (yr)	254	62.0	63.6	20.0-92.9
Sex				
Male	152	—	—	—
Female	102	—	—	—
Stage				
<IIB	146	—	—	—
≥IIB	108	—	—	—
% BMPC	244	39.3	36	0-97
Plasmablastic*—No.	211	—	—	—
Plasmablastic*—Yes	22	—	—	—
BMLI (%)	227	1.2	0.6	0-12.6
Hb (g/dL)	254	11.0	11.1	4.5-16.6
Albumin (g/dL)	247	3.52	3.6	1.64-4.7
Creatinine (mg/dL)	254	1.6	1.1	0.5-18.2
Lactic dehydrogenase	73	158	152	53-373
Calcium (mg/dL)	254	9.7	9.5	7.5-17.2
β2M (μg/mL)	249	4.7	3.3	0.8-26.5
CRP (mg/mL)	242	1.3	0.4	0.05-29.7
Absolute PC × 10^6/L	254	130.1	4.4	0-12,925
%BPC	254	19.5	6	0-100
BLI (%)	254	0.5	0	0-13.6

Abbreviations: PC, plasma cell; %BPC; percentage plasma cells of clg+ cells in blood; Hb, hemoglobin; BM, bone marrow; BLI, blood labeling index; BMLI, bone marrow plasma cell labeling index; β2M, β-2-microglobulin; CRP, C-reactive protein.

* Plasmablastic refers to the morphology of the bone marrow plasma cells.

Table 2. Number of Patients With Blood Monoclonal Plasma Cells at Various Cut-off Values

Parameter	No. (%) ≥Cut-Off
% Blood plasma cells*	
>0	204 (80)
≥1	193 (76)
≥2	170 (67)
≥3	157 (62)
≥4	144 (57)
Absolute no. blood plasma cells	
≥1 × 10^6/L	174 (69)
≥2 × 10^6/L	156 (61)
≥3 × 10^6/L	144 (57)

* % Blood plasma cells; the percentage of light chain restricted cytoplasmic immunoglobulin+ cells with plasma cell morphology.

Figure 1A-D shows the separation of the survival curves by the respective prognostic factor. Although the P values for the CRP and β2M are statistically significant ($P < .05$), they only identify 65 and 55 patients, respectively, in the adverse survival group. This is in contrast to the %BPC, which identifies 144 patients in the adverse survival group with a median survival of 2.4 years.

Multivariate analysis of prognostic factors. In the multivariate analysis of survival, the age and albumin were considered as continuous variables. From the three parameters determined by the immunofluorescence microscopy technique, the %BPC and the BLI, but not the absolute #BPC, were considered in the multivariate analyses. The absolute #BPC is based on the %BPC and, therefore, these two variables measure the same abnormality and would have competed with each other.

The results of the multivariate analysis showed that age, albumin, stage, %BPC, and the BLI were independent prognostic variables for survival (Table 5). Bootstrap analysis validated all factors, except stage, to be independent factors. When the BMLI was added after the age, albumin, BLI and %BPC were already included in the model it was found to add significant (P = .05) prognostic information.

Although the BLI was a significant factor in the multivariate analysis (Table 5), it only identified 30 patients with high values. In addition, the BLI requires that the cells be pulsed with BrdUrd and the calculation can be time consuming. For these reasons, we removed the BLI from consideration in the analysis and repeated the multivariate analysis. In this analysis, age, albumin, stage, and %BPC were determined to be independent prognostic factors for survival. Bootstrap analysis again confirmed all factors except stage.

Correlations with other disease factors. We examined the results of the metastatic bone radiographic survey in patients with and without an increased number of BPC. Interestingly, we found that 62% (88 of 143) of patients with increased BPC had bone lesions on radiographic skeletal survey compared with 80% (87 of 109) with low or no BPC (P = .002). The correlation between the BLI and the BMLI

Table 3. Median Survivals for Variables at Different Cut-off Values

Variable	Cut-off Value	No.	Median Survival (mo)	Cut-off Value	No.	Median Survival (mo)	P*
Absolute PC ($\times 10^6$/L)	<1	80	54.2	≥1	174	29.7	<.001
	<3	110	51.4	≥3	144	28.5	<.001
	<5	129	48.1	≥5	125	28.5	<.001
% Blood plasma cells	<1	61	52.3	≥1	193	33.8	.033
	<4	110	52.3	≥4	144	28.4	<.001
	<10	153	45.9	≥10	101	27.8	<.001
Blood LI (%)	<0.5	204	37.7	≥0.5	50	26.1	.008
	<1	224	38.1	≥1	30	19.9	<.001
% Bone marrow PC	<25	83	45.9	≥25	161	30.5	.028
	<50	159	37.3	≥50	85	29.9	.139
	<75	216	37.5	≥75	28	29.7	.116
Bone marrow LI (%)	<1	151	50.9	≥1	76	25.2	<.001
	<2	191	38.7	≥2	36	19.5	<.001
	<3	208	37.7	≥3	19	18.5	<.001
β2M (μg/mL)	<2.7	81	38.1	≥2.7	168	34.1	.101
	<4	151	44.6	≥4	98	29.7	.014
	<6	194	39.9	≥6	55	24.7	<.001
CRP (mg/mL)	<0.8	177	45.9	≥0.8	65	26.1	.001
	<4	225	38.1	≥4	17	19.9	.004
	<6	230	37.7	≥6	12	23.1	.050
Plasmablastic morph.	No	211	37.5	Yes	22	29.4	.004
Albumin (g/dL)	<3.5	97	28.5	≥3.5	157	45.9	.006
Stage	<IIB	146	45.9	≥IIB	108	27.6	.016
Age (yrs)	<70	190	37.3	≥70	64	27.8	.083

Abbreviations: Mo, months; PC, plasma cell; LI, labeling index; β2M, β-2-microglobulin; CRP, C-reactive protein.

• Log-rank test comparing those above and below the cut-off value.

was $r = .43$ ($P < .01$). There was a weak, but significant, correlation between the %BPC and the percentage of monoclonal BM plasma cells ($r = .39$; $P < .01$). Twenty-eight of the 30 patients with a high BLI (≥1%) also had a BMLI performed and in 75% the BMLI was also high (≥1%).

Bataille et al[42] have found that using the serum β2M and CRP together is useful in predicting survival and we previously found that a combination of the β2M and BMLI was useful.[33] Therefore, we analyzed survival using these and similar combinations of prognostic factors in all patients and also on those patients <70 years of age (Table 6). The combination of the percentage of blood plasma cells and the

Table 4. Univariate Relationship of Each of Variables to Death

Variable	No.	Cut-Off	Coef.	SE	P	Hazards Ratio (95% CI)
BLI	254	≥1%	0.757	0.218	<.001	2.13 (1.39, 3.27)
%BPC	254	≥4%	0.720	0.178	<.001	2.05 (1.45, 2.91)
Absolute PC	254	≥3 × 10^6/L	0.648	0.175	<.001	1.91 (1.36, 2.70)
BPC Profile*	254	Normal/Abnormal	0.727	0.187	<.001	2.07 (1.43, 2.99)
%BMPC	244	≥25%	0.409	0.188	.029	1.51 (1.04, 2.17)
BMLI	227	≥1%	0.661	0.176	<.001	1.94 (1.37, 2.73)
Plasmablastic†	211	No/Yes	0.702	0.250	.005	2.02 (1.24, 3.29)
Stage	254	≥IIB	0.518	0.167	.002	1.68 (1.21, 2.33)
β2M	249	6 μg/mL	0.731	0.191	<.001	2.08 (1.43, 3.02)
CRP	242	0.8 mg/dL	0.583	0.184	.002	1.79 (1.25, 2.57)
Albumin	254	3.5 mg/dL	−0.455	0.167	.006	0.63 (0.46, 0.88)
Creatinine	254	mg/dL‡	0.172	0.054	.001	1.19 (1.07, 1.32)
LDH	73	mg/dL‡	0.004	0.002	.048	1.04§ (1.00, 1.09)
Age	254	≥70 yr	0.311	0.183	.089	1.36 (0.95, 1.95)
Sex (male)	254	—	0.030	0.171	.860	1.03 (0.74, 1.44)
Calcium	254	mg/dL‡	0.101	0.079	.197	1.11 (0.95, 1.29)

Abbreviations: Coef, coefficient; SE, standard error; PC, plasma cell; %BPC; percentage plasma cells of clg+ cells in blood; BM, bone marrow; BLI, blood labeling index; BMLI, bone marrow plasma cell labeling index; β2M, β-2-microglobulin; CRP, C-reactive protein; LDH, lactate dehydrogenase.

• BPC profile: Unfavorable BPC was defined as ≥3 × 10^6/L absolute BPC, ≥4% BPC, or a BLI ≥1%.

† Plasmablastic refers to the morphology of the bone marrow plasma cells.

‡ Used as a continuous variable.

§ For a 10 mg/dL change.

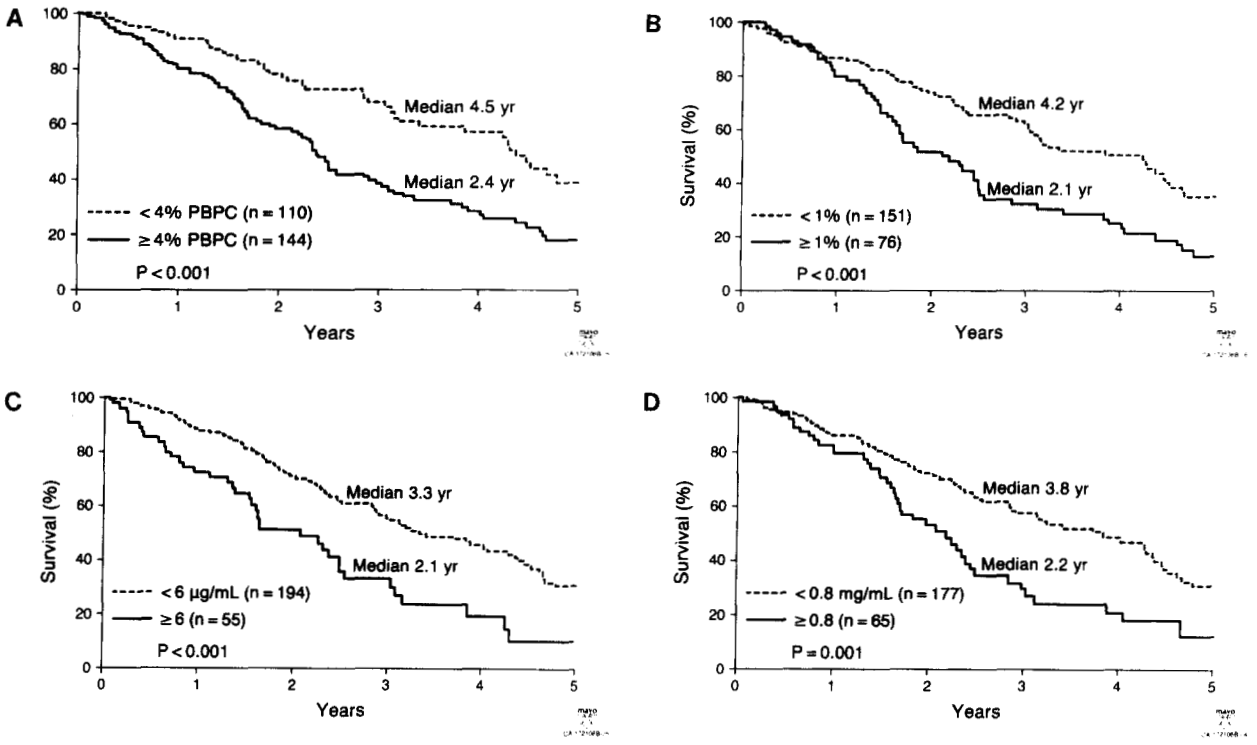

Fig 1. Kaplan-Meier survival curve for patients by (A) the % blood plasma cells; (B) the blood labeling index (C) the β-2-microglobulin; and (D) the C-reactive protein.

bone marrow labeling index best separated the patients into low, intermediate, and high risk groups with survivals of approximately 52, 35, and 26 months, respectively. Although other combinations of prognostic factors produced separations that were statistically significant, only a few patients were identified in the high-risk category.

DISCUSSION

This study of the blood of patients with untreated active myeloma indicates that the number and labeling index of the circulating monoclonal plasma cells are prognostic factors for survival. The number of blood plasma cells represents a new prognostic factor for MM and indicates the importance of circulating plasma cells and their proliferative rate in this disease at the time of diagnosis.

In addition to being a prognostic factor, this study of the blood compartment contributes to our understanding of the pathophysiology of the plasma cell proliferative disorders. If patients with early forms of plasma cell disease (such as monoclonal gammopathy of undetermined significance) are studied with molecular techniques, small numbers of clonal cells can be found in the blood; however, monoclonal plasma cells are not usually detectable using immunofluorescence microscopy.[37] Patients with smoldering multiple myeloma are more likely to have circulating plasma cells, and in a previous study, it was demonstrated that if these patients have increased numbers of BPC, they have a shorter time to the development of active myeloma.[34] A similar pattern is observed when the proliferative rate of the monoclonal plasma cells in these disease phases is measured. Patients with monoclonal gammopathy of undetermined significance and smoldering multiple myeloma have monoclonal marrow plasma cells that usually have a labeling index of 0%, whereas in active and relapsed myeloma, the cells are more likely to be actively proliferating.[43] Therefore, the plasma cell malignancies can be viewed as a spectrum of diseases from monoclonal gammopathy of undetermined significance to smoldering to active myeloma characterized by an increasing proliferation rate of the plasma cell clone and a propensity to involve the blood. Determination of the genetic

Table 5. Results of Multivariate Proportional Hazards Model

Variable	Coefficient	SE	P	Hazards Ratio (95% CI)
Original stepwise model				
%BPC ≥4	0.540	0.183	.003	1.72 (1.20, 2.45)
BLI ≥1%	0.595	0.223	.008	1.81 (1.17, 2.80)
Stage ≥IIB	0.437	0.171	.010	1.55 (1.11, 2.16)
Albumin	−0.456	0.182	.012	0.63* (0.44, 0.91)
Age	0.018	0.007	.015	1.02† (1.00, 1.03)
Bootstrap-verified model (without BLI)				
%BPC ≥4	0.661	0.179	<.001	1.94 (1.36, 2.75)
Albumin	−0.479	0.178	.007	0.62 (0.44, 0.88)
Age	0.015	0.007	.028	1.17 (1.02, 1.34)

Abbreviations: CI, confidence interval; %BPC, per cent blood plasma cells; BLI, blood labeling index.

*A 37% reduction in risk for each g/dL increase in serum albumin.

† A 2% increase in risk of death for each year increase in age.

Table 6. Survival of Patients by Various Combinations of Prognostic Factors

Variables	No.	All Favorable Median Survival (mo)	No.	Some High Some Low Median Survival (mo)	No.	All Unfavorable Median Survival (mo)	P
All patients							
%BPC and BMLI	85	52.3	80	34.6	62	26.1	<.001
β2M and BMLI	120	52.3	83	27.6	19	19.5	<.001
β2M and CRP	137	51.2	90	27.8	13	18.5	<.001
%BPC, β2M, and CRP	73	53.4	156	30.5	11	16.4	<.001
%BPC, β2M, CRP, BMLI	56	53.4	152	30.5	6	12.9	<.001
Patients <70 years old							
%BPC and BMLI	64	52.3	56	36.9	46	26.1	<.001
β2M and BMLI	94	52.3	56	26.9	13	29.7	.001
β2M and CRP	110	51.2	65	29.4	6	12.9	<.001
%BPC, β2M, and CRP	60	52.3	115	34.1	6	12.9	<.001
%BPC, β2M, CRP, BMLI	47	52.3	106	34.6	5	16.4	<.001

Abbreviations (cut-offs used): PC, plasma cell; %BPC; percentage plasma cells of clg+ cells in blood (≥4%); BM, bone marrow; BLI (≥1%), blood labeling index; BMLI, bone marrow plasma cell labeling index (≥1%); β2M, β-2-microglobulin (≥6 μg/mL); CRP, C-reactive protein (≥0.8 mg/dL).

changes, the alterations of cytokine secretion, and the response of the cells to cytokines in each phase of disease should greatly add to our understanding of why this progression occurs and provide leads to new therapeutic approaches.

An interesting finding in this study was that patients with low or no circulating BPC were more likely (80%) to have bone lesions than those with high numbers of BPC (62%). This association can also be inferred from comparisons of the frequency of bone lesions in studies of patients with overt plasma cell leukemia (defined as >2,000 plasma cells/mm^3 by routine WBC differential) with those of typical multiple myeloma. For example, Nöel and Kyle[44] studied 25 patients with primary plasma cell leukemia and found only 44% had lytic bone lesions. This is lower than the 73% of patients with lytic bone lesions or fractures found in a previous large study of patients with multiple myeloma.[45] Further studies of the adhesion receptor and cytokine profile of myeloma cells from patients with and without bone lesions may elucidate why they produce lytic bone lesions in some cases but not in others.

As with any new prognostic factor, it will be important to validate the usefulness of the number of BPC in large, prospective clinical trials. The fact that the blood can be easily sampled should facilitate these trials. There are several other methods to measure BPC than the immunofluorescence microscopy method. Flow cytometry has been used to detect CD38$^+$ CD45$^-$ CD45^{dim} cells in the blood and correlates well with the results of the slide-based test.[36] Billadeau et al[5] have used an allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) technique to quantify clonal cells in the blood. In studies on split samples of blood, we have shown that the ASO-PCR technique is clearly more sensitive at detecting circulating cells.[37] From a practical standpoint, of the three methods, the flow cytometric method would appear to be better suited for large clinical trials than ASO-PCR or the slide-based microscope method. One potential advantage of the immunofluorescence technique is that the labeling index can also be performed. However, only 12% of the patients in this study had circulating plasma cells with a high proliferative rate. In addition, the labeling index is easier to calculate on the marrow sample (because there are more plasma cells), and we have shown that the number of BPC by itself is an independent factor; therefore, it would appear that from a practical standpoint the labeling index on the blood plasma cells is not mandatory.

The detection of circulating monoclonal plasma cells has several implications for clinical practice. The presence of a high number of BPC indicates active disease and can predict the time to progression in patients who appear to have smoldering MM. Monitoring of the blood compartment can help choose the appropriate time to harvest blood stem cells[23,30] and can assist in choosing candidates for blood stem cell transplant.[46] It can also serve as another measurable disease marker in following patients on a new treatment regimen.

This study adds yet another factor that can be used to estimate the survival of patients with myeloma. By simply calculating the number of BPC and the labeling index of the marrow plasma cells, the patients can be stratified into low, intermediate, and high risk groups with median survivals of 52, 35, and 26 months, respectively (Table 6). When these two prognostic factors were applied to the group of patients <70 years old (ie, those typically eligible for clinical trials using high-dose therapy with stem cell rescue), they could be stratified into low, intermediate, and high risk groups with median survivals of 52, 37, and 26 months, respectively. Other combinations of prognostic factors do not separate the patients as well, and few patients are identified in the high risk group.

Although there would appear to be a long list of useful prognostic factors for myeloma, it is clear that no one factor can always correctly estimate survival in these patients.[47] It is helpful to have multiple factors that can be easily performed at diagnosis. These should include measurement of the serum markers β2M and CRP, the number of BPC, and the bone marrow labeling index. These simple tests can, along with the other factors that are available from routine blood and radiographic studies, assist the treating physician in assessing overall survival. These prognostic factors must

be used in clinical trials of new agents or more intensive treatment programs to properly stratify patients into multiple treatment arms and to assess whether the new treatment program can improve the survival of the patients at highest risk of short survival. It is only by proper application of these prognostic factors in these treatment studies that we will learn whether or not a new treatment modality is really having a positive impact on the survival of patients with this fatal disease.

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