Multiple Myeloma

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Oral melphalan at diagnosis hampers adequate collection of peripheral blood progenitor cells in multiple myeloma

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Background and Objectives. Since optimal collection of peripheral blood progenitor cells (PBPC) remains crucial for high-dose therapy in patients with multiple myeloma (MM) in relapse phase or refractory to chemotherapy, we evaluated several variables that may influence mobilization.

Design and Methods. Eighty-nine patients who underwent a standard mobilization procedure with cyclophosphamide (3 g/m^2) and growth factors entered the study. A composite collection totalling at least 2×10^6 CD34$^+$/kg was defined as a sufficient yield: 59 patients achieved an adequate collection. A reliable factor to predict adequate yields was prior therapy: an adequate collection was obtained in 92% of patients treated with conventional non-alkylating therapy (VAD-based regimens), in 56% treated with oral melphalan and in 23% who had received intravenous melphalan.

Results. The three groups were similar for most clinical features. After adjustment for several potential confounders, the probability of an adequate PBPC collection remained higher in the group treated with non-alkylating agents, with an odds ratio (OR) of 6.14 (95% confidence interval, CI=1.34, 28.13) and lower in those treated with intravenous melphalan (OR=0.08; CI=0.01-0.61), when compared to the group treated with oral melphalan. Among the other prognostic factors (stage, percentage of bone marrow plasma cells, β2-microglobulin, labeling index, isotype, monoclonal component, Bence-Jones proteinuria) evaluated at diagnosis, there was no clear association with progenitor cell yield.

Interpretation and Conclusions. In conclusion, patients who are potential candidates for high-dose therapy with PBPC support should not receive conventional alkylating therapy, even orally. Alternatively, progenitor cells should be collected early in the course of MM.

Key words: myeloma, PBPC harvest, alkylating therapy.

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Multiple myeloma (MM) is a neoplastic disease characterized by a poor outcome. Cure is confined to a small fraction of young, good-prognosis patients treated with autologous bone marrow transplantation or allogeneic transplantation.[1,2]

Autologous bone marrow transplantation was first demonstrated to be a useful approach for patients with MM in relapse phase or refractory to conventional chemotherapy.[3-5] It has since been recognized to be superior to conventional chemotherapy in myeloma patients at diagnosis and under 60 years old.[6] The use of peripheral blood progenitor cell (PBPC) support has further reduced the morbidity of the procedure,[7] which is now applicable to selected patients up to 70 years old. An intensified approach with a reduced, and repeated, dose of melphalan (100 mg/m^2) and PBPC support is a useful and low toxicity approach for elderly patients.[8] Prognostic factors such as β2-microglobulin, C-reactive protein (CRP), chromosome 13 abnormalities and renal function remain excellent predictors of response and outcome even in the high-dose setting.[2,9]

A French group has shown a similar survival for patients who received high-dose treatment at diagnosis or at relapse.[10] Thus, it is still unclear whether the best timing for high-dose chemotherapy with PBPC support is early or late in the course of MM. Early transplantation has the theoretical advantage of introducing high-dose therapy before the induction of tumor resistance and at a time of low tumor burden. High-dose therapy could theoretically be more beneficial to patients with chemotherapy-sensitive disease when applied early.[11] Late transplantation has the advantage of deferring transplant-related morbidity and mortality, and applying high-dose therapy when the tumor burden is high and the patient will derive symptomatic benefit.[12]

Moreover, patients with favorable prognostic factors could be safely treated with conventional chemotherapy and rescued at relapse.

The availability of PBPC to reduce the toxicity of high-dose or intensified chemotherapy is the most important factor for successful treatment at both diagnosis and relapse.[7,13] Obviously, patients tend to release a higher number of PBPC at diagnosis than after chemotherapy. However, at relapse the correlation between several characteristics of patients and adequate mobilization has not been assessed in detail. To address this issue we evaluated a series of MM patients at first relapse or refractory to chemotherapy who underwent a standard mobilization procedure with cyclophosphamide (3 g/m^2) and growth factors.[14]

Design and Methods

Patients

Eighty-nine patients were retrospectively identified and entered the study. Inclusion criteria were: (a) diagnosis of MM at first relapse or resistant to chemotherapy (between 1993 and 2000), (b) age < 75 years, (c) serum creatinine < 3 mg/dL, (d) normal echocardiogram with ejection fraction > 40%, (e) normal liver function (AST and ALT < 3 × normal value), (f) normal pulmonary function on the basis of lung function tests. Relapse from complete remission (CR) or partial remission (PR) was defined as the reappearance of detectable monoclonal component and recurrence of bone marrow infiltration, or a 25% increase from minimal tumor mass. Refractory disease was defined as primary drug resistance for at least four months or progression (increase of monoclonal component or osteolytic lesions) during induction chemotherapy.

Patients were divided into three groups according to their previous treatment: 37 patients had received VAD-based conventional chemotherapy (vincristin, adriamycin, dexamethasone); 39 had received oral melphalan and prednisone (MP) therapy, and 13 had been treated with high-intermediate intravenous (I.V.) dose melphalan with autologous PBPC support.

Treatment schedule

All patients were treated according to the same mobilization protocol: cyclophosphamide 3 g/m^2 was administered on day 0 in 2 doses, and subsequently 4 g/m^2 of 2-mercaptoethansulphonic acid (MESNA) in 5 divided doses; granulocyte colony-stimulating factor (G-CSF) was administered at 10 μg/kg from day 3 to the last day of the leukapheresis. For leukapheresis, a Fresenius Cell Separator AS 104 (MTS, Schweinfurt, Germany) is currently employed. Blood samples from each patient were analyzed for the presence of PBPC daily from day 9 following cyclophosphamide administration until the last collection: leukapheresis was initiated when at least 10 PBPC/μL were detected.

The number of PBPC was determined by direct immunofluorescence on whole blood, using anti-CD34 monoclonal antibody and flow cytometry analysis[15] with a FACSCalibur analyzer (Becton Dickinson, San José, CA, USA) equipped with a filter set for FITC-PE dual-color fluorescence. Data acquisition and analysis were performed with CELL Quest Software and each measurement included at least 40,000 cells. The frequency of cells expressing CD34$^+$ was calculated as the percentage of all analyzed cells. Dead cells were excluded on the basis of forward- and side-scatter analysis. The number of circulating CD34$^+$ cells per μL of blood or the total number of CD34$^+$ in the PBPC collections was obtained by multiplying this percentage by the total number of the leukocytes. A composite collection totalling at least 2×10^6 CD34$^+$/kg was defined as a sufficient yield.

Statistical analysis

Patients were analyzed to assess the role of various clinical and laboratory parameters on yield of an adequate number of PBPC. We took into account time-related variables (year of diagnosis, year of collection, time from last treatment to mobilization) and other characteristics of the patients (age, gender, stage, percentage of bone marrow plasma cells, β_2-microglobulin, isotype, monoclonal component, Bence-Jones proteinuria).

Odds ratios (OR) of PBPC yield with corresponding 95% confidence intervals (CI) were estimated using unconditional logistic regression according to Breslow[16] and available in the SAS procedure LOGISTIC. A few variables (age, gender, year of diagnosis, time from last therapy, stage and isotype) were selected according to *a priori* biological relevance and introduced as confounders in the multivariable model with type of therapy.

Results

Eighty-nine patients in relapse phase or refractory to chemotherapy underwent PBPC collection. Fifty-nine patients achieved an adequate collection of at least 2×10^6 CD34$^+$/kg.

A powerful predictor of adequate yields was the previous chemotherapy: an adequate collection was obtained in 92% (34/37) of patients treated with conventional non-alkylating therapy, in 56% (22/39) of patients treated with oral melphalan and

Table 1. Patients' characteristics according to prior chemotherapy.

Patients' characteristics	Prior chemotherapy		
	Non-alkylating agents	*Melphalan (oral)*	*Melphalan (I.V.)*
N. of patients[1]	37	39	13
Age (years)			
Median (range)	57 (34-72)	61 (35-70)	59 (49-79)
Gender			
Male	19 (51%)	21 (54%)	6 (46%)
Female	18 (49%)	18 (46%)	7 (54%)
Year of diagnosis			
< 1993	4 (11%)	19 (48%)	3 (23%)
1993-1995	13 (35%)	10 (26%)	8 (62%)
> 1995	20 (54%)	10 (26%)	2 (15%)
Year of collection			
< 1996	12 (32%)	13 (33%)	1 (8%)
1996+	25 (68%)	26 (67%)	12 (92%)
Time since last therapy (months)			
Median (range)	2 (0.5-28)	8 (0.5-64)	31 (6-86)
Isotype			
G	18 (49%)	26 (66%)	8 (62%)
A	7 (19%)	8 (21%)	2 (15%)
BJ	12 (32%)	5 (13%)	3 (23%)
Stage			
II	9 (24%)	13 (38%)	8 (62%)
III	28 (76%)	21 (62%)	5 (38%)
Bone marrow plasma cells (%)			
<20	9 (27%)	9 (31%)	4 (33%)
20+	24 (72%)	20 (69%)	8 (67%)
β_2 microglobulin (mg/L)			
<3	14 (45%)	15 (60%)	4 (57%)
3+	17 (55%)	10 (40%)	3 (43%)
Monoclonal component (mg/dL)			
<1000	10 (29%)	5 (16%)	3 (23%)
1000+	24 (71%)	27 (84%)	10 (77%)
Bence Jones proteinuria (g/day)			
<1	23 (64%)	19 (63%)	8 (62%)
1+	13 (36%)	11 (37%)	5 (38%)

[1]The number of subjects for some variables sums less than the total because of missing values.

Table 2. Effect of prior chemotherapy on probability of an adequate collection of PBPC. Odds ratios (OR) and 95% confidence intervals (95% CI) adjusted for all variables listed.

	Adequacy of collection[1]				
	YES (N=59)	*(%)*	*NO (N=30)*	*OR*	*95%CI*
Prior chemotherapy					
Melphalan (oral)	22	(56.4)	17	1.00	
Melphalan (I.V.)	3	(23.1)	10	0.08	0.01-0.61
Non-alkylating agents	34	(91.9)	3	6.14	1.34-28.13
Age (years)					
< 56	22	(75.9)	7	1.00	
56-60	13	(68.4)	6	1.40	0.25-7.77
> 60	24	(58.5)	17	0.60	0.16-2.26
Gender					
Male	32	(69.6)	14	1.00	
Female	27	(62.8)	16	0.76	0.24-2.42
Years of diagnosis					
< 1993	13	(50.0)	13	1.00	
1993-1995	20	(64.5)	11	2.85	0.66-12.28
> 1995	26	(81.3)	6	4.71	0.85-26.14
Time since last therapy (months)					
<6	36	(75.0)	12	1.00	
6+	23	(56.1)	18	2.59	0.63-10.58
Stage[2]					
II	16	(53.3)	14	1.00	
III	40	(74.1)	14	0.98	0.26-3.65
Isotype					
G	32	(61.5)	20	1.00	
A	10	(58.8)	7	0.61	0.14-2.73
BJ	17	(85.0)	3	4.20	0.76-23.34

[1] YES: if the collection achieved at least 2×10^6 $CD34^+$/Kg; NO: otherwise; [2]the number of subjects for the stage sums less than the total because of 5 missing values. A missing indicator category was introduced in the model.

in 23% (3/13) of patients who had received I.V. melphalan. These differences were statistically significant (p<0.05). Table 1 shows the distribution of other prognostic factors among the three groups of patients.

By univariate analysis, percentage of bone marrow plasma cells, β_2-microglobulin, monoclonal component, Bence-Jones proteinuria, and maintenance therapy evaluated at diagnosis and at relapse were not associated with PBPC cell yield, while a time-related variable significantly influenced the probability of adequate PBPC collection. A recent year of diagnosis was associated with an improved ability to collect an adequate yield (OR 4.3, 95% CI, 1.3 to 1.4). A higher proportion of patients had been treated with non-alkylating agents after 1995 and we may assume that we improved the ability to collect PBPC over time by using newer technical devices.

To estimate the effect of the type of treatment, adjusted for different potential confounders, a multivariable logistic model was used which included all major clinical and time-related prognostic factors (Table 2). The probability of an adequate PBPC collection was higher in the group treated with non-alkylating agents (OR=6.14; CI=1.34, 28.13) and lower in those treated with I.V. melphalan (OR=0.08; CI=0.01-0.61), when compared to those treated with oral melphalan. For patients treated with oral melphalan, the number

of prior cycles was not associated with a successful PBPC yield, with the median number of cycles for adequate or non-adequate collections being 6.

Furthermore, no other variables under study modified the results when they were added to the multivariate model (results not shown in detail).

Discussion

High-dose or intensified chemotherapy with PBPC support significantly improves the outcome of patients with MM.[6,8] PBPC collection is thus a key point in the therapeutic strategy. In this study we show that it is mainly influenced by the type of previous chemotherapy: the most active drug in the treatment of MM, namely melphalan, is a poison for progenitor cells. Even at the low doses used in standard oral MP chemotherapy, it reduces the possibility of collecting an adequate number of PBPC.

In a series of MM patients who underwent the same mobilizing regimen, the relationships between the amount of PBPC collected and several clinical and laboratory characteristics were evaluated to define factors predictive of adequate collection. Treatment with melphalan had the strongest influence on the ability to mobilize PBPC. The administration route was also very important: an adequate collection was obtained in 92% of patients treated with conventional non-alkylating therapy (VAD-based regimens), in 56% of patients treated with oral melphalan, but in only 23% of patients who had received I.V. melphalan. Univariate and multivariate analyses also showed that prior I.V. alkylating therapy had the greatest influence on subsequent PBPC collection. Thus, previous chemotherapy was the only factor that could enter the model.

Many studies have shown that the duration of previous melphalan therapy is highly predictive of impaired PBPC mobilization. Prince *et al.* found that the number of prior cycles of MP is proportional to the number of patients who mobilize PBPC. Goldschmidt *et al.*[16] and, more recently, Desikan *et al.*[17] also showed that duration of melphalan pretreatment was the main factor adversely correlated with a successful harvest. However, in our analysis, the number of prior MP courses was not a predictor of mobilization. Instead, the impact of the *type* of previous chemotherapy had the strongest influence in uni- and multivariate analyses. Studies comparing the mobilization of PBPC in patients with different malignancies have indicated that PBPC harvesting is more difficult in MM.[18-20]

However, the present study shows that in MM, after a stem cell-sparing chemotherapy, namely a VAD-based regimen, an adequate number of PBPC can be collected from most patients, even those who are refractory or in relapse.

MM patients who are potential candidates for high or intensified chemotherapy should not be treated at diagnosis with oral MP, whereas VAD-based regimens can be safely used. As an alternative, PBPC should be collected early from patients planned to be treated with oral or I.V. melphalan so that effective salvage treatment can be performed at a later stage.

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MB, AP APi: conception and design of the study and final approval of the version to be published. SB, CR, AB, LG, PO, PM: analysis and interpretation of data. FM, GC, LR: drafting the article and revising it critically.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Jean-Luc Harousseau, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Dr. Harousseau and the Editors. Manuscript received February 1, 2002; accepted May 6, 2002.

What is already known on this topic

The impact of previous treatment with melphalan on stem cell collection in multiple myeloma is already known. This paper is a useful confirmation.

What this study adds

This study shows that even orally administered melphalan has a detrimental effect on subsequent stem cell collection in multiple myeloma.

Potential implications for clinical practice

The clinical implications of this study are that when autologous transplantation is planned, patients should not receive melphalan or stem cells should be collected before melphalan treatment.

Jean-Luc Harousseau, Associate Editor