Effect of pre-transplant cumulative doses of chemotherapeutic drugs on early and long-term hematological recovery after autologous bone-marrow transplantation for lymphoma

Alejandro Martín
Josè Antonio Pérez-Simón
Maria Dolores Caballero
Natalia López-Holgado
Lourdes Vázquez
Maria Consuelo del Cañizo
Jesus F. San Miguel

Background and Objectives. It has recently been demonstrated that autologous bone-marrow transplantation (ABMT) is feasible in heavily pretreated patients who do not mobilize peripheral blood progenitor cells (PBPC), suggesting that bone marrow (BM) progenitor-cells are not as sensitive to chemotherapy as are PBPC. However, information regarding the impact of previous chemotherapy on the performance of BM grafts is scanty.

Design and Methods. We have retrospectively analyzed 40 consecutive lymphoma patients treated with the BEAM regimen and ABMT at our institution. The impact of the chemotherapeutic drugs (individual cumulative doses) received before transplant on stem-cell yield and hematologic recovery was investigated. Univariate analysis failed to identify any variable that significantly affected progenitor-cell content.

Results. Regarding the impact of pre-transplant chemotherapy on early engraftment, only cumulative doses of cytarabine (r=0.28, p=0.04) and cisplatin (r=0.32, p=0.02) had a negative influence on neutrophil recovery (to >0.5×10^9/L), but the significance of this was not maintained in multivariate analysis. We did not find any chemotherapeutic drug that negatively affected platelet recovery (to >20×10^9/L). By contrast, administration of several drugs, including doxorubicin, procarbazine, nitrogen mustard, cytarabine and cisplatin, significantly delayed complete trilineage reconstitution. In multivariate analysis, only previous doxorubicin retained statistical significance (p=0.014).

Interpretations and Conclusions. Our results show that pre-transplant chemotherapy has little or no influence on progenitor-cell yield and short-term engraftment after ABMT.In contrast, we found that cumulative doxorubicin doses have an independent influence on long-term engraftment. In heavily pretreated lymphoma patients in whom poor PBPC mobilization is expected, BMT may represent an attractive option.

Key words: autologous transplantation, bone marrow, hematologic recovery, chemotherapy.

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Frrom the Servicio de Hematología, Hospital Clínico Universitario de Salamanca, Salamanca, Spain.

Correspondence:
Alejandro Martín, Hematology Department, Hospital Universitario de Salamanca, Paseo de San Vicente 58-182, 37007 Salamanca, Spain.
E-mail: amartingar@aehh.org

High-dose chemotherapy followed by autologous stem-cell transplantation is a successful treatment for patients with relapsing or resistant non-Hodgkin's lymphoma or Hodgkin's disease.[1-6] Mobilized peripheral-blood progenitor cells (PBPC) have largely replaced autologous bone marrow (BM) as the source of stem cells, mainly due to the non-invasive collection technique and faster engraftment after PBPC transplantation, which significantly reduces morbidity and cost of the procedure.[7-9] However, PBPC transplantation has some limitations. In particular, it has become clear that the number of CD34$^+$ cells infused is crucial in order to ensure a safe procedure,[10,11] and there is a significant proportion of patients (ranging from 10% to 30%)[11,12] who do not mobilize enough progenitor cells to proceed to transplant.

Previous reports focusing on PBPC transplantation provide evidence that pretreatment with cytotoxic agents is the overriding factor adversely affecting yields and performance of PBPC grafts.[13-18] By contrast, Lemoli *et al.* recently showed that granulocyte colony-stimulating factor (G-CSF)-primed BM induces sustained and multilineage hematopoietic recovery after myeloablative chemotherapy in heavily pretreated patients with insufficient PBPC yields.[11] This suggests that marrow progenitor cells are not as sensitive to stem-cell toxic drugs as are PBPC. However, in contrast to the numerous studies devoted to investigating the factors influencing PBPC mobilization or engraftment after PBPC transplantation, there are few reports on the influence of previous chemotherapy on the performance of BM grafts. Altogether, the detrimental effects of cytotoxic agents

Table 1. Patients' characteristics and treatment before transplantation.

Characteristics	*No. of patients (N = 40)*	*% of patients*
At diagnosis		
Sex: male/female	28/12	70/30
Histology		
Indolent NHL[1]	6	15
Aggressive NHL[2]	17	42.5
Very aggressive NHL[3]	5	12.5
Hodgkin's disease[4]	12	30
Ann-Arbor stage III-IV	35	87.5
Extranodal disease	25	62.5
BM involvement	10	25
Previous treatment		
Initial treatment		
CHOP/CHOP-like	4	10
VECOP	8	20
PROMACE-MOPP	6	15
MOPPABV (hybrid)	6	15
MOPP/ABVD (alternating)	4	10
Others	12	30
Salvage treatment		
Mini-BEAM	18	45
ESHAP	10	25
Previous lines of chemotherapy		
1	9	22.5
2	16	40
≥3	15	37.5
Previous radiotherapy	7	17.5
At transplant		
Age (median and range)	42.5 (14 to 61)	
Ann-Arbor stage III-IV	9	22
Extranodal disease	9	22
BM involvement	0	0
Disease status		
First CR	11	27.5
Second or subsequent CR	12	30
Sensitive disease	11	27.5
Resistant disease	6	15

[1]Indolent NHL: 6 follicular grade 2; [2]aggressive NHL: 1 mantle-cell, 1 follicular grade 3, 11 diffuse large B-cell, 2 peripheral T-cell unspecified and 2 anaplastic large cell; [3]very aggressive NHL: 1 T-lymphoblastic, 1 B-lymphoblastic, and 3 Burkitt's; [4]Hodgkin's disease: 8 nodular sclerosis, 4 mixed cellularity. CHOP/CHOP-like: cyclophosphamide, doxorubicin or mitoxantrone, vincristine, prednisolone; VECOP: etoposide, epidoxorubicin, cyclophosphamide, vincristine, bleomycin, prednisolone; PROMACE-MOPP: cyclophosphamide, doxorubicin, etoposide, methotrexate, nitrogen mustard, vincristine, procarbazine, prednisolone; MOPP: nitrogen mustard, vincristine, procarbazine, prednisolone; ABVD: doxorubicin, bleomycin, vinblastine, dacarbazine; Mini-BEAM: carmustine, cytarabine, etoposide, melphalan; ESHAP: etoposide, solumedrol, methylprednisolone, cytarabine, cisplatinum.

on BM seem to be much weaker than those on PBPC,[13] but previous studies have been limited to estimations of the number of regimens or cycles of chemotherapy to which a patient had been exposed, or the effect of one specific regimen,[13,19-22] and, to our knowledge, there has been no analysis comparing the effects of cumulative doses of the different chemotherapeutic drugs on BM harvest and hematologic recovery after BM transplant. In this study we retrospectively analyzed 40 patients diagnosed with lymphoma who were homogeneously treated with the BEAM regimen and autologous BM transplantation at a single center. The primary objective of our study was to investigate the impact of the different chemotherapeutic drugs (individual cumulative doses) received before transplant on stem-cell yield and hematologic recovery.

Table 2. Chemotherapeutic drugs received before transplant.

N[1]		*Dose*		*Number of cycles*	
		Mean ± SD	*Median (range)*	*Mean ± SD*	*Median (range)*
Cyclophosphamide	29	3.240±2.580	3900 (0-7750)	4.7±3.9	5 (0-13)
Vincristine	40	17.2±9.4	15 (2-48)	6.8±3	6 (1-13)
Doxorubicin	32	222±159	210 (0-550)	4.9±3.4	6 (0-11)
Epidoxorubicin	8	75±163	0 (0-600)	1.5±3.3	0 (0-12)
Methotrexate	16	2190±3630	0 (0-12.000)	1.3±1.8	0 (0-5)
Etoposide	32	539±453	460 (0-1500)	2.7±2.5	2.5 (0-11)
Procarbazine	18	3176±4414	0 (0-16800)	2.9±3.8	0 (0-12)
Nitrogen mustard	17	25.7±37.7	0 (0-144)	2.8±3.8	0 (0-12)
Bleomycin	24	51±63	30 (0-220)	3.7±3.7	3 (0-11)
Dacarbazine	6	937±2394	0 (0-8250)	1.2±3.2	0 (0-11)
Carmustine	20	51±62	0 (0-180)	0.8±1	0 (0-3)
Cytarabine	25	2060±3150	1600 (0-16000)	1.3±1.4	1 (0-5)
Melphalan	19	25±31	0 (0-90)	0.8±1	0 (0-3)
Cisplatin	10	53±120	0 (0-620)	0.5±1.2	0 (0-6)
Vinblastine	12	22±40	0 (0-132)	2.5±4	0 (0-11)

Doses are expressed in mg/m² or units (bleomycin) as appropriate. [1]Number of patients exposed to the drug. Only a minority of patients were exposed to mitoxantrone (n = 3), 6-thioguanine (n = 2), L-asparaginase (n = 1), or interferon (n = 2).

Design and Methods

Patients and prior chemotherapy

Forty consecutive patients diagnosed with lymphoma who received BEAM conditioning and autologous BM transplantation at our institution between May 1990 and October 1993 were included in the study. The patients' characteristics and the chemotherapy they received before transplant are summarized in Table 1. Patients received a median of two lines (range 1 to 4) of chemotherapy according to standard protocols (Table 1). The doses and number of cycles of the different drugs to which patients had been exposed before transplant are summarized in Table 2. Written informed consent to inclusion in this study was obtained from all patients using institutionally approved forms.

Bone marrow harvest

BM cells were harvested from the posterior iliac crests under general anesthesia according to standard procedures, with a minimum target harvest of 1×10^8 nucleated cells per kilogram body weight. Following collection, cells were resuspended in 10% dimethylsulfoxide (DMSO) with autologous plasma and frozen in a controlled rate freezer at -1°C / min and then stored in liquid nitrogen at -196°C until the day of transplantation. All grafts were frozen without further manipulation.

Progenitor cell assays

The counts of total nucleated cells and mononuclear cells (MNC) were determined using an automated cell counter with an incorporated high resolution flow cytometer. The BM aspirate differentials were performed simultaneously with the counter and on a light microscope; only lymphocytes and monocytes were included in the MNC. Assays of colony-forming unit (CFU-GM) were performed using the method described by Iscove *et al.* (data available for 28 patients).[23] Marrow samples were collected in sterile and preservative-free heparin tubes and separated by Ficoll-Hypaque (d=1070) density gradient centrifugation. Mononuclear cells were plated on methylcellulose and incubated for 14 days. Colonies were considered when more than 40 cells were counted in the aggregates.

Transplant procedure

All the patients received BEAM[24] as the conditioning regimen: BCNU 300 mg/m^2, day 6; etoposide 200 mg/m^2, days 5 to 2 (800 mg/m ^{2}; three patients received 1600 mg/m^2); cytarabine 400 mg/m^2, days 5 to 2 (1600 mg/m ^{2}); and melphalan 140 mg/m^2, day 1. The autologous marrow was reinfused on day 0. A median number of 1.68×10^8/Kg (range: 0.6 to 3.83) MNC, 1.46×10^4/Kg (0.16 to 30) CFU-GM, and 1.54×10^6/Kg (0.46-4.28) CD34$^+$ cells were infused. The number of CFU-GM and CD34$^+$ cells infused was available in only 28 and 8 cases, respectively. G-CSF (5 μg/Kg/day) was administered to 21 patients after transplant, from day +6 to hematologic recovery (neutrophil count $>1\times10^9$/L for 3 consecutive days).

Toxicity and supportive care

Neutrophil recovery was defined as the first of three consecutive days with an absolute neutrophil count (ANC) greater than 0.5×10^9/L. Platelet recovery was defined as the first of three consecutive days with an unsupported platelet count greater than 20×10^9/L. Engraftment was considered stable when patients reached normal counts in peripheral blood (hemoglobin >12 g/dL + leukocytes $>4\times10^9$/L + granulocytes $>1.5\times10^9$/L + platelets $>100\times10^9$/L). For evaluation of long-term hematologic recovery, patients who died due to transplant toxicity not related to graft failure or who developed progressive disease during the first year after transplantation were excluded. Red blood cell (RBC) units were transfused when hemoglobin values decreased to less than 8.0 g/dL or above this limit when symptomatic anemia developed. Platelets were transfused in cases of platelet counts lower than 10×10^9/L, or in cases of fever or hemorrhage with platelet counts lower than 20×10^9/L. Non-hematologic toxicities were evaluated according to the World Health Organization (WHO) grading. Only grade 3 or more toxicities were considered. Transplant-related mortality (TRM) was considered at any time if it was due to a recognized complication of the procedure. Early TRM was defined as death from any cause other than lymphoma occurring within 100 days after high-dose therapy.

Statistical analysis

The primary objectives of this study were to determine the factors influencing progenitor-cell yield (number of MNC and CFU-GM collected) and hematologic recovery after transplant (days to reach an ANC $>0.5\times10^9$/L, platelet count $>20\times10^9$/L, and normal counts in peripheral blood). The secondary objectives for analysis were to determine the factors influencing transfusion requirements, incidence of grade 3-4 toxicities, and overall and early TRM rates. Differences in hematologic recovery were analyzed by Kaplan-Maier and log-rank tests. A forward stepwise Cox proportional hazards regression model was used for multivariate analysis, and performed in two phases. Firstly, we only included the 28 patients whose number of CFU-GM had been recorded. If this variable was not significant in the Cox regression model, we repeated the multivariate analysis in a second phase excluding the CFU-GM variable (40 cases). Quantitative parameters were analyzed by a non-parametric Mann-Whitney test and multiple linear regression. Qualitative parameters were analyzed using a χ^2 test and multiple logistic regression. All p values reported are two-sided and statistical significance is defined as a p value less than 0.05. The statistical analyses were computed with SPSS statistical software (SPSS, Inc, Chicago, IL, USA).

The following characteristics were evaluated in the analysis: diagnosis (non-Hodgkin's lymphoma *vs* Hodgkin's disease), sex, age at transplantation (≤40 *vs* >40 years), extranodal involvement at transplantation, disease status at transplant, previous chemotherapy, previous radiotherapy, blood cell counts before harvest, use of G-CSF after transplantation, and number of MNC and CFU-GM infused. For assessment of previous chemotherapy, the cumulative dose of each individual agent was estimated. The number of

chemotherapy lines (1 *vs* 2 *vs* ≥3) and the type of salvage treatment, MiniBEAM (yes *vs* no) and ESHAP (yes *vs* no), were also analyzed. Quantitative variables (dose of each chemotherapeutic drug, and number of MNC and CFU-GM infused) were transformed into binary variables using the quartiles as cut-off values. The relationship between these continuous variables and the time of hematologic recovery was assessed using Pearson's correlation test.

Results

Hematologic recovery

One patient died before engraftment, due to transplant toxicity unrelated to graft failure. Among the remaining 39 evaluable patients, all obtained engraftment. The median time to reach an ANC > 0.5×10^9/L and a platelet count >20×10^9/L was 20 days (10 to 52) and 23 days (10 to 251), respectively. For assessment of long-term hematologic recovery, two patients who died due to transplant toxicity unrelated to graft failure, and eight patients who developed progressive disease during the first year after transplant were excluded. Among the resulting 30 evaluable patients, all achieved normal blood counts, after a median of 155 days (range 49 to 1817). After a median follow-up of 8.9 years, no cases of secondary myelodysplastic syndrome/acute myeloid leukemia were diagnosed.

Factors influencing hematologic recovery

The results of the univariate and multivariate analyses of variables affecting early and long-term engraftment are shown in Table 3. Regarding the impact of previous chemotherapy on early engraftment, only cumulative doses of cytarabine and cisplatin had a negative influence on neutrophil engraftment, but this was not maintained in multivariate analysis. Univariate analysis failed to identify any chemotherapeutic drug or regimen that negatively affected platelet engraftment. Surprisingly, administration of epidoxorubicin (VECOP regimen) had a favorable impact on both neutrophil and platelet engraftment, although the correlation found was weak (r=0.38 and 0.20, r espectively). With multivariate analysis, previous epidoxorubicin was the only factor significantly affecting neutrophil engraftment, whereas previous epidoxorubicin and previous radiotherapy had an independent influence on platelet engraftment (Table 3). By contrast, administration of several drugs, including doxorubicin, procarbazine, nitrogen mustard, cytarabine and cisplatin, significantly delayed complete trilineage reconstitution. In multivariate analysis, only previous administration of doxorubicin retained statistical significance (Table 3).

Factors influencing progenitor-cell content

Univariate analysis failed to identify any variable that significantly affected progenitor-cell content (CFU-GM and MNC).

Factors influencing transfusion requirements

The results of the univariate and multivariate analyses of variables affecting transfusion requirements are shown in Table 4. Factors significantly increasing RBC and platelet transfusion requirements were previous radiotherapy, previous chemotherapy, and active disease as status at transplant. In multivariate analysis, cumulative doses of cytarabine and cumulative doses of cisplatin were the only factors significantly related to RBC and platelet transfusion requirements, respectively (Table 4).

Non-hematologic toxicity

Two patients (5%) died early of transplant-related causes, one of a bacterial infection and the other of a fungal infection. Another patient (2.5% of the series) died more 100 days after transplantation from a transplant-related cause (secondary malignancy: T-lymphoproliferative disease). The overall TRM was 5% (95% CI: 0% to 11.8%) at 5 years by Kaplan-Meier analysis. Univariate analysis did not identify any factor related to an increase in the incidence of TRM. Two patients (5%) developed an infection above grade 3 during their hospital stay and died. Hepatic, pulmonary, cardiac, renal or central nervous system toxicities (≥grade 3) each occurred in less than 5% of patients. Female sex was the single factor associated with a higher incidence of severe toxicities (67% vs. 21%, p=0.006). Univariate analysis did not identify any chemotherapeutic drug or regimen that increased the incidence of severe toxicities.

Discussion

One major clinical problem in PBPC transplantation is that a significant proportion of patients either do not mobilize or only poorly mobilize progenitor cells into the peripheral blood.[12] Although not all the reasons for poor mobilization are known, there is compelling evidence that pretreatment with cytotoxic agents is the overriding factor adversely affecting yields and performance of PBPC grafts.[13-18,25] By contrast, Lemoli *et al.* recently showed that BM transplantation after stimulation with G-CSF was feasible in heavily pretreated patients who had failed to mobilize PBPC.[11] The results of this study suggest that marrow progenitor cells are not as sensitive to chemotherapy as are PBPC. However, information regarding the impact of previous chemotherapy on

Table 3. Factors that influence hematologic recovery.

	Neutrophils >0.5×10⁹/L	*p*	*Platelets >20×10⁹/L*	*p*	*Normal blood counts*	*p*
Total	20 (10-52)		23 (10-251)		155 (49-1817)	
Previous treatment						
Radiotherapy						
Yes (n = 7)			41 (23-251)		418 (147-1817)	
No (n = 32)			22 (10-68)	0.01	131 (49-1128)	0.05
Doxorubicin						
Yes (n = 31)					190 (58-1817)	
No (n = 8)					97 (49-177)	.008
Epidoxorubicin						
Yes (n = 8)	14.5 (10-20)		16 (11-32)		70 (49-364)	
No (n = 31)	22 (10-52)	.0004	24 (10-251)	0.003	177 (65-1817)	0.008
Cumulative doses	r = –0.38	.009			r = –0.28	0.06
Procarbazine						
Yes (n = 18)					206 (70-1817)	
No (n = 21)					124 (49-412)	0.011
Nitrogen mustard						
Yes (n = 17)					190 (70-1817)	
No (n = 22)					131 (49-435)	0.049
Cytarabine						
Cumulative doses	r = 0.28	0.04			r = 0.47	0.004
Cisplatin						
Cumulative doses	r = 0.32	0.02			r = 0.61	< 0.001
Characteristics at transplant						
CFU-GM infused						
≤ 5×10⁴/Kg (n = 21)			24 (10-63)			
> 5×10⁴/Kg (n = 7)			16 (11-43)	0.017		
Cox regression analysis*						
Radiotherapy (yes)			HR = 2.90 95% CI: 1.06 to 7.93	0.037		
Doxorubicin (yes)					HR = 3.49 95% CI: 1.29 to 9.47	0.014
Epidoxorubicin (no)	HR = 4.30 95% CI: 1.74 to 10.60	0.002	HR = 2.71 95% CI: 1.14 to 6.41	0.023		

*Results are expressed as median of days (range), except cumulative doses (r: Pearson's correlation coefficient). HR: hazard ratio. CI: confidence interval. *Multivariate analysis was performed in two phases. First, we only included the 28 patients for whom the number of CFU-GM was recorded. As this variable was not significant in any of the Cox regression models, we repeated the multivariate analysis in a second phase excluding the CFU-GM variable (40 cases).*

the performance of BM grafts is scanty. In the present study, we analyzed 40 patients with lymphoma consecutively treated with the BEAM regimen and autologous BM transplantation at a single center. To the best of our knowledge, this is the first study which analyzes the effects of the individual cumulative doses of the different chemotherapeutic drugs on progenitor-cell harvest and hematologic recovery. Our results show that higher pre-transplant cumulative doses of cytarabine and cisplatin significantly increased transfusion requirements. However, previous chemotherapy had no influence on progenitor-cell yield (in terms of CFU-GM and MNC), and had little impact on short-term engraftment. Only cumulative doses of cytarabine and cisplatin had a negative influence on neutrophil engraftment, but this significance was not maintained in multivariate analysis. Our analysis failed to identify any chemotherapeutic drug or regimen that negatively affected platelet engraftment. By contrast, previous chemotherapy had a greater impact on long-term engraftment, since administration of several drugs significantly delayed complete trilineage reconstitution, including doxorubicin, procarbazine, nitrogen mustard, cytarabine and cisplatin, although in multivariate analysis only the correlation with previous doxorubicin treatment retained statistical significance. Surprisingly, administration of epidoxorubicin (included only in the

Table 4. Factors that influence transfusion requirements.

	RBC Units	*p*	*Platelet Units*	*p*
Total	4 (0-24)		42 (6-236)	
Characteristics at diagnosis				
Sex				
Male (n=28)	4 (0-20)		42 (6-224)	
Female (n=11)	8 (2-24)	0.013	67 (30-236)	0.05
Previous treatment				
Radiotherapy				
Yes (n=7)	12 (4-20)		67 (35-236)	
No (n=32)	4 (0-24)	0.03	41 (6-224)	0.04
ESHAP				
Yes (n=10)	10 (0-24)		76 (28-236)	
No (n=29)	4 (0-20)	0.026	42 (6-224)	0.047
Epidoxorubicin				
Yes (n=8)	2 (0-6)		21 (6-54)	
No (n=31)	6 (0-24)	0.023	56 (7-236)	0.021
Cumulative doses	r=0.35	0.014	r=0.31	0.026
Cytarabine				
≤1600 mg/m^2 (n=24)	4 (0-16)		41.5 (6.224)	
>1600 mg/m^2 (n=15)	10 (0-24)	0.017	66 (12-236)	0.041
Cumulative doses	r=0.3	0.03	r=0.31	0.025
Cisplatin				
Yes (n=10)	10 (0-24)		76 (28-236)	
No (n=29)	4 (0-20)	0.026	42 (6-224)	0.047
Cumulative doses	r=0.44	0.002	r=0.48	0.001
Characteristics at transplant				
Status at transplant				
First CR (n=11)	2 (0-16)		21 (14-224)	
Second or subsequent CR (n=11)	4 (0-14)		56 (6-126)	
Partial remission (n=11)	6 (0-24)		42 (7-203)	
Refractory disease (n=6)	9 (4-20)	0.014	76 (30-236)	0.027
Multiple regression analysis				
Cytarabine (>1600 mg/m^2)		0.002		
Cisplatin (cumulative doses)				0.002

Results expressed as median (range), except cumulative doses (r: Pearson's correlation coefficient).

VECOP regimen) had a favorable impact on both neutrophil and platelet engraftment, although the correlation found was weak (r=0.38 and 0.20, respectively). This point could reflect the fact that patients receiving epidoxorubicin did not receive other more toxic regimens employed as first-line therapy, such as PROMACE-MOPP or MOPP/ABVD.

There are few other reports on the influence of previous chemotherapy on hematologic recovery after BM transplantation. Visani *et al.*, in a series of 31 patients with non-Hodgkin's lymphoma, found that engraftment was significantly later in patients who had received chemotherapy before marrow collection than in patients harvested at diagnosis.[19] Brandwein *et al.*, in a series of 30 lymphomas, also reported a significant delay in engraftment in patients who had received more than six cycles of salvage chemotherapy.[20] Rabinowe *et al.* analyzed neutrophil recovery in a series of 128 patients with lymphoma or acute lymphoblastic leukemia, concluding that engraftment was negatively affected by prior exposure to stem-cell toxic drugs, but not by the total number of drugs given.[22] Chopra *et al.* found that platelet recovery in 23 patients with Hodgkin's disease harvested after treatment with Mini-BEAM was significantly later than in comparison to historical controls.[21] Finally, Dreger *et al.* did not find any significant influence of previous chemotherapy on hematologic recovery in a series of 50 patients diagnosed with lymphoma, although there was a trend to delayed platelet recovery in patients pre-treated with repeated courses of Dexa-BEAM.[13] Altogether, chemotherapy appears to affect BM progenitor cells, but the detrimental effects on BM are apparently much weaker than those previously described for PBPC, as pointed out by Dreger *et al.*[13]

Our results on short-term hematologic recovery are in line with those reported by Dreger *et al.*,[13] showing little influence of previous chemotherapy on early engraftment. We did, however, find an independent influence of cumulative doxorubicin doses on long-term engraftment. To the best of our knowledge, this finding has not been previously described. Interestingly, in a previous study including 103 lymphoma patients treated with the BEAM regimen and autologous PBPC transplantation at our center, we found that prior administration of chemotherapy was a critical factor negatively influencing stem-cell yield and short-term hematologic recovery, but not significantly affecting long-term engraftment.[18] These results suggest a differential sensitivity of PBPC and BM progenitor-cells to chemotherapeutic agents.

Although the exact explanation for these results still needs to be clarified, the distribution of the different CD34$^+$ hematopoietic progenitor-cell subsets in BM and the leukapheresis product could be relevant. It is well established that the CD34$^+$ cells represent a heterogeneous cell population consisting of both committed progenitors, responsible for the first phase of hematologic recovery, as well as primitive uncommitted and pluripotent stem cells, which contribute to the late phases of hematologic recovery.[26-33] After stimulation with either G-CSF or GM-CSF, with or without mobilizing chemotherapy, the mobilized blood CD34$^+$ cells are predominantly myeloid progenitors, with very limited quantities of progeni-

tors of other lineages and of the earliest stem cells.[26,27,30] The relative proportions of specific subsets of CD34+ cells may provide an explanation for the more rapid engraftment observed with mobilized PBPC than with BM.[26,28-30] In the present study, we found little influence of previous chemotherapy on early engraftment, and a greater impact on long-term engraftment, in contrast to the results that we had observed in PBPC transplants.[18] Our results suggest that chemotherapy preferentially affects committed progenitor cells, responsible for the rapid early hematologic recovery in PBPC transplants, whereas more immature BM progenitor cells (responsible for long-term engraftment) are not as sensitive to chemotherapy as are committed progenitor cells. Another factor that could contribute to the delay in complete trilineage recovery, in addition to the damage to the stem-cell compartment induced by chemotherapy, is impairment of the stromal cell compartment (bone marrow microenvironment), identified in both PBPC and BM transplantation settings by long-term marrow culture techniques.[34,35] This stromal damage can persist for a long period, even six years after transplantation.[36] In our series the rate of early TRM was 5%, which is within the range commonly observed after autologous stem-cell transplantation for lymphomas.[6,11,18,37] Univariate analysis did not identify any chemotherapeutic drug or regimen associated with an increased incidence of TRM or severe toxicities, although the low incidence of TRM observed in our series means that larger numbers of patients are needed in order to validate the results.

The results of this study should be interpreted with some caution because the study is based on a retrospective analysis of a heterogeneous group of patients. With this caveat in mind, our results show that pre-transplant chemotherapy has little or no influence on progenitor-cell yield and short-term engraftment after autologous BM transplantation. In contrast, we found that cumulative doxorubicin doses were independently related to long-term engraftment. According to our results, BM transplantation represents an attractive option in heavily pretreated lymphoma patients in whom a poor mobilization is expected.

AM, JAPS and MDC were responsible for the conception of the study and interpretation of the results. AM performed the statistical analyses and wrote the manuscript. All authors critically revised the paper and gave the final approval for its submission. The order in which the names of authors appear is based on their contribution to the study. JFSM, as head of department, is cited last. The authors declare that they have no potential conflicts of interest.

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