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

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 >0.5×10⁹/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 >20×10⁹/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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on BM seem to be much weaker than those on PBPC, 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, 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.

### 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 $1 \times 10^9$ 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). Marrow samples were collected in sterile test tubes as the conditioning cells infused was ≤ values reported in patients. Toxities and supportive care

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 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 anaemia 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.

Transplant procedure
All the patients received BEAM as the conditioning regimen: BCNU 300 mg/m², day 6; etoposide 200 mg/m², days 5 to 2 (800 mg/m²); three patients received 1600 mg/m²; cytarabine 400 mg/m², days 5 to 2 (1600 mg/m²); and melphalan 140 mg/m², day 1. The autologous marrow was reinfused on day 0. A median number of 1.68×10^9/Kg (range: 0.6 to 3.83) MNC, 1.46×10^9/Kg (0.16 to 30) CFU-GM, and 1.54×10^9/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 >1×10^9/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 >4×10^9/L + granulocytes >1.5×10^9/L + platelets >100×10^9/L). For evaluation of 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 χ² 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).

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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 >0.5×10^9/L, platelet count >20×10^9/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 hematoologic recovery were analyzed by Kaplan-Meier 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 χ² 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).
Factors influencing prophylactic-cell content

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 cyclophosphamide 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, respectively). With multivariate analysis, previous epidoxorubicin was the only factor significantly affecting neutrophil engraftment, whereas previous epirubicin 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).

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. 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. 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. 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...
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
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.\textsuperscript{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.\textsuperscript{20} Rabinow 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.\textsuperscript{21} Chopra et al. found that platelet recovery in 25 patients with Hodgkin’s disease harvested after treatment with Mini-BEAM was significantly later than in comparison to historical controls.\textsuperscript{22} 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.\textsuperscript{23} 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.\textsuperscript{13}

Our results on short-term hematologic recovery are in line with those reported by Dreger et al.,\textsuperscript{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.\textsuperscript{9} 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\textsuperscript{+} hematopoietic progenitor-cell subsets in BM and the leukapheresis product could be relevant. It is well established that the CD34\textsuperscript{+} 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.\textsuperscript{26-33} After stimulation with either G-CSF or GM-CSF, with or without mobilizing chemotherapy, the mobilized blood CD34\textsuperscript{+} cells are predominantly myeloid progenitors, with very limited quantities of progeni-
itors of other lineages and of the earliest stem cells. 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. 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. 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 engraftment is the damage to the stem-cell compartment induced by chemotherapy, 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. This stromal damage can persist for a long period, even six years after transplantation. In our series the rate of early TRM was 5%, which is within the range commonly observed after autologous stem-cell transplantation for lymphomas. 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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