Autologous Progenitor Cell Transplantation: Prior Exposure to Stem Cell-Toxic Drugs Determines Yield and Engraftment of Peripheral Blood Progenitor Cell But Not of Bone Marrow Grafts

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Agents with stem cell-toxic potential are frequently used for salvage therapy of Hodgkin's disease (HD) and high-grade non-Hodgkin's lymphoma (NHL). Because many patients with relapsed or refractory lymphoma are candidates for autologous progenitor cell transplantation, possible toxic effects of salvage chemotherapy on progenitor cells must be taken into account. In a retrospective study, we have analyzed the influence of a salvage regimen containing the stem cell-toxic drugs BCNU and melphalan (Dexa-BEAM) on subsequently harvested bone marrow (BM)- and peripheral blood-derived progenitor cell grafts (PBPC) and compared it with other factors. Progenitor cells were collected from 96 patients with HD or high-grade NHL. Seventy-nine grafts were reinfused (35 PBPC and 44 BM) after high-dose chemotherapy. Compared with patients autografted with BM, hematopoietic recovery was significantly accelerated in recipients of PBPC. For PBPC, the number of Dexa-BEAM cycles (≤ 1 *v* >1) was the predominate prognostic factor affecting colony-forming unit–granulocyte-macrophage (CFU-GM) yield (66 *v* 6.8 $\times$ 10^4/kg, *P* = .0001), $CD34^+$ cell yield (6.6 *v* 1.6 $\times$ 10^6/kg, *P* = .0001), neutrophil recovery to $>0.5 \times 10^9$/L (9 *v.* 11 days, *P* = .0086), platelet recovery to $>20 \times 10^9$/L (10 *v* 15.5 days, *P* = .0002), and platelet count on day +100 after transplantation (190 *v* 107 $\times$ 10^9/L, *P* = .031) using univariate analysis. Previous radiotherapy was associated with significantly lower CFU-GM and $CD34^+$ cell yields but had no influence on engraftment. Patient age, patient sex, disease activity, or chemotherapy other than Dexa-BEAM did not have any prognostic impact. Multivariate analysis confirmed that Dexa-BEAM chemotherapy was the overriding factor adversely influencing CFU-GM yield ($P < .0001$), $CD34^+$ cell yield ($P < .0001$), and platelet engraftment ($P < .0001$). BM grafts were not significantly affected by previous Dexa-BEAM chemotherapy or any other variable tested. However, prognostic factors favoring the use of BM instead of PBPC were not identified using joint regression models involving interaction terms between the graft type (PBPC or BM) and the explanatory variables investigated. We conclude that, in contrast to previous radiotherapy or other chemotherapy, exposure to salvage regimens containing stem cell-toxic drugs, such as BCNU and melphalan, is a critical factor adversely affecting yields and performance of PBPC grafts. Marrow progenitor cells appear to be less sensitive to stem cell-toxic chemotherapy. PBPC should be harvested before repeated courses of salvage chemotherapy involving stem cell-toxic drugs to preserve the favorable repopulation kinetics of PBPC in comparison with BM.
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AUTOLOGOUS stem cell transplantation after myeloablative chemotherapy is increasingly being used for treatment of Hodgkin's disease (HD) and high-grade non-Hodgkin's lymphoma (NHL). In the majority of cases, autologous bone marrow (BM) has been used as a source of stem cells,[1-5] but transplantation of peripheral blood progenitor cells (PBPC) has become an important alternative.[6-9] PBPC (similar to BM) grafts harvested from lymphoma patients are characterized by a wide variation in terms of progenitor cell content and speed of engraftment,[3,8-11] emphasizing the need to identify factors that influence or predict the yield of progenitor cells and hematopoietic recovery. Although addressed previously, this problem remains controversial because most studies were hampered by small patient numbers and heterogeneity of the study population with regard to underlying disease, mobilization protocol, and conditioning regimens. Moreover, two important aspects have not been covered by previous studies. First, an analysis of the differential effects of various chemotherapeutic drugs on PBPC mobilization and engraftment is lacking. In general, chemotherapy is believed to adversely affect subsequent harvesting of BM or PBPC grafts.[3,11-13] However, animal studies have shown that a variety of cytotoxic agents, including busulfan, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), melphalan, and platinum derivatives, reduce the repopulating capacity of BM, whereas others do not.[14-17] Thus, salvage chemotherapy regimens that contain agents that potentially affect stem cells would be expected to compromise the yield of PBPC collection, whereas regimens not involving stem cell-toxic drugs would not. Testing this hypothesis is of particular importance, because many patients with lymphoma who are eligible for high-dose therapy are subjected to prior salvage chemotherapy including stem cell-toxic components.[4,18,19] Second, a direct comparison of growth factor-mobilized PBPC and BM with respect to the variables influencing progenitor cell yield may identify scenarios in which PBPC are particularly preferable to BM grafts or, vice versa, BM promises to be superior to PBPC.

To address these issues, we have analyzed retrospectively a large population of patients with HD or high-grade NHL treated at a single center. The impact of a salvage regimen containing the stem cell-toxic drugs BCNU and melphalan (Dexa-BEAM) on subsequently harvested PBPC or BM was investigated and compared with other variables that might be relevant in this context. In addition, the differential effects of prognostic factors on PBPC and BM grafts, respectively, were studied using univariate and multivariate analyses.

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PATIENTS AND METHODS

Patients. Patients with a diagnosis of HD or high-grade NHL referred to our center for autologous transplantation were included in this retrospective study if colony-forming unit–granulocyte-macrophage (CFU-GM) and/or $CD34^+$ cell data were available, and (in cases of PBPC donation) mobilization was performed with one of the two mobilization protocols described below. Between June 1990 and July 1994, 96 consecutive patients fulfilling these criteria donated 102 progenitor cell grafts. PBPC were harvested from 52 patients, 6 of whom underwent an additional BM harvest. Forty-four patients donated BM only. Seventy-three patients had HD and 23 patients had high-grade NHL according to the updated Kiel classification.[20] Fifty-five patients had received radiotherapy to various extents before harvest. All patients had been pretreated with chemotherapy for primary or salvage treatment of lymphoma. A median of 8 cycles (range, 2 to 115 cycles) of 2 different regimens (range, 1 to 5) had been administered. The most common regimens were COPP (cyclophosphamide, vincristine, procarbazine, prednisolone; 17 patients), ABVD (doxorubicin, bleomycin, vinblastine, dimethyltriazeno-imidazole-carboxamide [DTIC]; 12 patients), COPP/ABVD (24 patients), COPP/ABV/IMEP (ifosfamide, methotrexate, etoposide, prednisolone [IMEP]; 19 patients), COPBLAM (cyclophosphamide, vincristine, prednisolone, bleomycin, doxorubicin; 16 patients), CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone; 8 patients), and CHOEP (cyclophosphamide, doxorubicin, vincristine, etoposide, prednisolone; 5 patients). Altogether, 96 patients had received cyclophosphamide, 96 had received doxorubicin, 82 had received bleomycin, 82 had received procarbazine, 42 had received DTIC, 38 had received etoposide, 33 had received methotrexate, and 30 had received ifosfamide. Vinca alkaloids had been administered to all patients. Only a minority had been exposed to regimens containing cytarabine (n = 7), nitrosoureas (n = 7), nitrogen mustard (n = 4), chlorambucil (n = 1), or mitoxantrone (n = 1). No patient had been treated with platinum derivatives or melphalan before harvest. In addition, 23 patients had received 1 cycle and 30 patients had received 2 or more cycles of Dexa-BEAM[18] before being referred to our hospital for progenitor cell collection; an additional 31 of the remaining 43 patients received Dexa-BEAM thereafter. With 2 exceptions, all patients receiving more than 1 cycle of Dexa-BEAM before harvest had HD.

Twenty-one progenitor cell grafts were obtained from patients presenting with active disease (refractory disease or untreated relapse), whereas all other grafts were harvested from patients in complete or partial remission. The cohorts of patients donating PBPC or BM, respectively, were comparable with respect to age, diagnosis, previous radiotherapy, previous chemotherapy, and disease activity at time of harvest, but not for sex (Table 1).

Written informed consent using institutionally approved forms was obtained from each patient scheduled for progenitor cell harvesting. Twenty-three patients analyzed in this study participated in a randomized clinical trial comparing autologous transplantation of granulocyte colony-stimulating factor (G-CSF)–mobilized PBPC with autologous BM transplantation.[21] This trial was also approved by the institutional review board.

Progenitor cell collection. Mobilization of PBPC was performed using two different protocols. Fifteen grafts were obtained after mobilization with chemotherapy in conjunction with G-CSF. Patients received filgrastim (5l μg/kg subcutaneously [SC] daily; AMGEN, Munich, Germany) from day 8 (d8) after the start of Dexa-BEAM treatment until the last day of PBPC collection. Leukapheresis was started as soon as the white blood count exceeded 10×10^9/L.[10] The Dexa-BEAM regimen included dexamethasone 3× at 8 mg on d1 through 10, BCNU at 60 mg/m^2 on d2, etoposide at 150 to 400 mg/m^2 on d4 through 7, cytarabine at 100 mg/m^2 every 12 hours on d4 through 7, and melphalan at 20 mg/m^2 on d3. In the other 37 cases, filgrastim alone (10 μg/kg SC daily) was used for mobilization. PBPC collection was initiated after four doses of G-CSF (eg, on d5 after the start of G-CSF administration). At least 4×10^8/kg mononuclear cells (MNC) and 2×10^6/kg $CD34^+$ cells, respectively, were collected. A median of 3 leukapheresis procedures (range, 2 to 6 leukapheresis procedures) was performed on consecutive days using a Fenwal CS3000 blood component separator (Baxter, Munich, Germany). Usually, 10 L of blood was processed daily at a flow rate of 30 to 60 mL/min. Each leukapheresis product was cryopreserved at a cell concentration of 5×10^7/mL to 2×10^8/mL in liquid nitrogen until the day of transplantation.

Marrow grafts were collected from the posterior iliac crests according to standard procedures. A buffy coat was produced from the unfractionated harvest using a Haemonetics V50 cell separator (Haemonetics, Braintree, MA) and subjected to cryopreservation similar to the PBPC grafts. All grafts were frozen without further manipulation or purging.

High-dose chemotherapy and progenitor cell reinfusion. Eighty grafts were reinfused after high-dose chemotherapy to 79 patients. One patient (a 33-year-old man with HD harvested after 2 cycles of Dexa-BEAM) received both a PBPC and a BM graft. Although BM autografting was originally intended in this case, an additional PBPC graft containing 0.6×10^6/kg $CD34^+$ cells was infused because of poor MNC content of the marrow harvest. For the purpose of this

Table 1. Patient Characteristics and Treatment Before Harvest

	PBPC Median (range)	BM Median (range)	*P*
Patients harvested	52	50	
M/F	39/13	23/27	<.01*
Age (yr)	29 (15-55)	30.5 (13-58)	NS†
HD/NHL	40/12	39/11	NS*
Previous radiotherapy (no/yes)	23/29	17/32	NS*
Previous Dexa-BEAM (≤1/>1 cycle)	36/16	31/19	NS*
Previous chemotherapy other than Dexa-BEAM (≤6/>6 cycles)	26/26	21/29	NS*
No. of cycles			
Total	6 (2-21)	8 (2-115)	NS†
With vinca alkaloids	6 (2-16)	8 (2-20)	NS†
With cyclophasphamide	4 (0-10)	4 (0-12)	NS†
With doxorubicin	4 (0-12)	4 (0-9)	NS†
With bleomycin	3 (0-12)	4 (0-8)	NS†
With procarbazine	4 (0-10)	4 (0-12)	NS†
With DTIC	0 (0-12)	0 (0-7)	NS†
With etoposide	0 (0-7)	0 (0-111)	NS†
With methotrexate	0 (0-7)	0 (0-111)	NS†
With ifosfamide	0 (0-7)	0 (0-111)	NS†
Active disease at time of harvest (no/yes)	43/9	38/12	NS*
Mobilisation regimen (G-CSF 10 μg/Dexa-BEAM + G-CSF 5 μg)	37/15	—	
Patients proceeding to transplant	35	44	
Preparative regimen (BEAM/CBV)	23/11‡	19/25	<.05*

Abbreviation: NS, not significant.

* χ^2 test.

† Mann-Whitney Test.

‡ One additional patient received busulfan 4 × 4 mg/kg plus cyclophosphamide 2 × 60 mg/kg.

analysis, the patient was included in the PBPC group. Another patient (an 18-year-old man with HD harvested after 2 cycles of Dexa-BEAM) received 0.4×10^6/kg CD34$^+$ cells mobilized with granulocyte-macrophage colony-stimulating factor (GM-CSF; Sandoz, Nuremberg, Germany; 250 μg/m^2 for 7 days) in addition to 2.3×10^6/kg CD34$^+$ cells mobilized with G-CSF. Five grafts (1 PBPC and 4 BM) were stored as a back-up for patients receiving transplants of BM and PBPC, respectively; five other grafts were obtained for possible use later in the course of the disease. Eleven patients became ineligible for high-dose therapy because of progressive disease after harvest; 1 patient underwent allogeneic transplantation. High-dose chemotherapy consisted of 6 g/m^2 cyclophosphamide, 1,000 mg/m^2 etoposide, and 300 mg/m^2 BCNU (CVB; n = 36) or 300 mg/m^2 BCNU, 800 mg/m^2 etoposide, 1,600 mg/m^2 cytarabine, and 140 mg/m^2 melphalan (BEAM; n = 42).[5] A single patient (a 15-year-old boy with T-immunoblastic NHL) received busulfan (16 mg/kg) plus cyclophosphamide (120 mg/kg). Supportive care was performed as described previously.[5] Daily filgrastim (5 to 30 μg/kg) was administered to 64 patients after transplantation to accelerate neutrophil recovery. Neutrophil recovery was defined as the first of 3 consecutive days with a neutrophil count greater than 0.5×10^9/L; platelet recovery was defined as the first of 3 consecutive days with an unsupported platelet count greater than 20×10^9/L. Patients were discharged from hospital after the white blood count had recovered (3 days $>1 \times 10^9$/L) in the absence of fever, parenteral nutrition, or intravenous antibiotics.

Progenitor cell assays. CFU-GM were grown by plating 1×10^5 MNC in 0.3% agar culture medium consisting of 20% fetal calf serum (FCS) and 5% human placenta-conditioned medium in supplemented Iscove's modified Dulbecco's medium (IMDM). All cultures were performed in triplicate. After 7 and 14 days (BM) or 14 days (PBPC) of incubation in a humidified atmosphere of 5% CO_2 at 37°C, colonies (>40 cells) were counted. For burst-forming unit-erythroid (BFU-E), MNC were plated in methylcellulose containing 30% FCS, purified human erythropoietin (Terry Fox Laboratories, Vancouver, British Columbia, Canada), and phytohemagglutinin (PHA)-leukocyte–conditioned medium as supplements. The remaining procedure was similar to that described for CFU-GM; BFU-E were counted after 14 days.

Immunophenotyping. Preparation of MNC for flow cytometry has been described elsewhere.[22] Cells were suspended with phycoerythrin (PE)-and fluorescein isothiocyanate (FITC)-conjugated specific monoclonal antibodies (MoAbs) or PE/FITC-conjugated irrelevant isotype-specific antibodies (DAKO, Hamburg, Germany) in phosphate-buffered saline containing 0.2% sodium azide. After 30 minutes of incubation and fixation with 1% formaldehyde, flow cytometry was performed with a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany). The antibodies used were 8G12-PE, 8G12-FITC (HPCA-2/CD34), Leu-M3-PE (CD33), and HLe-1-FITC (CD45; all from Becton Dickinson). Nucleated cells were defined as CD45$^+$ and gated using a forward scatter versus CD45-FITC fluorescence dot plot or, in cases in which CD45 labeling was not available, using a gate established from the analysis of forward scatter/right-angle light scatter characteristics. Absolute numbers of CD34$^+$ cells were calculated from the total percentage of 8G12-PE brightly stained cells using a right-angle light scatter/red fluorescence quadrant that excludes virtually all background fluorescence (0.02% or less positive cells in the unspecific control) and all events that were right-angle light scatter high.

For evaluation of CD33 expression on CD34$^+$ subpopulations, MNC were subjected to double labeling using CD34-FITC versus CD33-PE. After appropriate compensation, a life gate was set on CD34$^+$ cells, as defined by right-angle light scatter (low) and green fluorescence (bright) characteristics. Five hundred to ten thousand events were acquired and analyzed for red fluorescence versus green fluorescence intensity. To discriminate between CD33 bright (CD33$^+$) and CD33 dim (CD33$^-$) CD34$^+$ cells, a quadrant was set using a red versus green fluorescence dot plot of ungated cells. Events exhibiting a red fluorescence that was lower than that of neutrophils were regarded as CD33$^-$. (Neutrophils display an intermediate fluorescence and monocytes display a bright fluorescence when stained with Leu M3-PE.) Using this quadrant, the percentage of CD34$^+$ cells that were CD33$^-$ was measured. Absolute numbers of CD34$^+$CD33$^-$ cells were calculated by multiplying this percentage with the absolute number of CD34$^+$ cells, as described above.

Statistical analysis. χ^2 test statistics were used to compare qualitative and nonparametric Mann-Whitney tests to compare quantitative parameters between the two types of stem cell grafts (PBPC and BM). If appropriate, quantitative variables were transformed into binary variables using the median as a cut-off value. For assessment of chemotherapeutic pretreatment, we decided to count the number of cycles per each individual agent administered rather than the number of chemotherapy regimens to better take into account the qualitative and quantitative variations between different regimens. Because BCNU, cytarabine, and melphalan had been administered as components of the Dexa-BEAM regimen in the vast majority of patients, they were not analyzed separately. Instead, complete Dexa-BEAM cycles were studied.

To separate the independent contributions of factors associated with progenitor cell yield, multivariate analysis was performed using multiple linear regression. Log transformation of the values for CFU-GM and CD34$^+$ cells was neccessary to achieve an approximately normal distribution of these variables. The coding of the explanatory variables was dichotomous, using the same cut-off values as in the univariate analysis. The implementation of a joint regression model involving the type of graft (PBPC = 0; BM = 1) as a explanatory variable in addition to diagnosis (HD = 0; NHL = 1), previous radiotherapy (no = 0; yes = 1), previous Dexa-BEAM therapy ($\leq$1 cycle = 0; >1 cycle = 1), and previous chemotherapy other than Dexa-BEAM ($\leq$6 cycles = 0; >6 cycles = 1) was possible because the residual variances of the individual regression models of both subgroups (PBPC and BM) were homogeneous. Besides linear terms, the interaction terms graft type * diagnosis, graft type * radiotherapy, graft type * Dexa-BEAM, and graft type * previous chemotherapy were also included in the model to identify qualitative or quantitative differences of the explanatory variables between the different graft types (PBPC and BM, respectively). Significant variables and interaction terms were identified using backward elimination. Proportional hazards models (Cox regression) were set up to investigate the factors prognostic for engraftment (days to reach a platelet count $>20 \times 10^9$/L). The explanatory variables analyzed were type of graft, previous radiotherapy, previous Dexa-BEAM chemotherapy, previous chemotherapy other than Dexa-BEAM, and interaction terms between type of graft and the other variables. The coding was dichotomous, as described above. In addition, the number of CFU-GM actually transplanted ($\leq 12.6 \times 10^4$/kg = 0; $>12.6 \times 10^4$/kg = 1) was included in the models. Backward and forward elimination resulted in similar models. All analyses were performed using SPSS for Windows (release 6.0; SPSS Inc, Chicago, IL). Significance levels were set at 0.05.

RESULTS

Yields of harvest and recovery after transplantation. Fifty-two patients underwent collection of mobilized PBPC. In the majority of cases (37 patients), three 10-L leukapheresis procedures were performed. Harvesting of PBPC was terminated after 2 aphereses in 5 individuals, whereas 10 patients needed more than 3 apheresis procedures to collect an adequate number of PBPC. The yields in terms of CFU-GM, BFU-E,

Table 2. Harvest Yields and Hematopoietic Recovery

	PBPC Median (range)	BM Median (range)	P*	n (PBPC/BM)†
A. Yield‡				
CFU-GM (×10⁴/kg)	33 (0-238)	8 (1-51)	.0001	47/48
BFU-E (×10⁴/kg)	29 (0-148)	8 (0.5-53)	.0001	44/50
$CD34^+$ cells (×10⁶/kg)	4.1 (0.1-58.5)	1.8 (0.5-8.9)	.0018	45/18
$CD34^+CD33^+$ cells (×10⁶/kg)	2.6 (0-17.2)	0.9 (0.2-7.4)	.011	35/13
$CD34^+CD33^-$ cells (×10⁶/kg)	1.4 (0.1-42.8)	0.6 (0.1-1.5)	.024	35/13
B. Outcome				
ANC >0.5 × 10⁹/L (all patients; d)	10 (8-17)	14.5 (9-43)	.0001	35/44
ANC >0.5 × 10⁹/L (G-CSF posttransplant; d)	10 (8-14)	14 (9-27)	.0001	32/33
Platelets >20 × 10⁹/L (d)	11 (7-69)	22.5 (13-143)	.0001	35/44
Hospitalization (d)	16 (11-31)	21.5 (14-100)	.0002	35/44
Platelet count on day +100 (×10⁹/L)	167 (23-479)	107 (17-394)	.065 (NS)	30/36

Abbreviation: NS, not significant.
* Mann-Whitney Test.
† Number of patients with data available.
‡ For PBPC calculated per 30 L leukapheresis volume.

and $CD34^+$ cells per kilogram of body weight are shown in Table 2. To allow an unbiased comparison of mobilization efficacy, values are given per 30 L leukapheresis volume. In comparison to 50 BM grafts, PBPC grafts contained significantly higher amounts of CFU-GM, BFU-E, and $CD34^+$ cells. $CD34^+CD33^+$ and $CD34^+CD33^-$ cell numbers were available for 35 PBPC and 13 BM grafts; again, a significant difference in favor of PBPC was observed (Table 2).

Seventy-nine patients proceeded to high-dose chemotherapy followed by progenitor cell reinfusion. Patients reconstituted with PBPC (n = 35) experienced a faster recovery of neutrophils (even after adjustment for G-CSF after transplantation) and platelets and a shorter duration of hospitalization when compared with patients grafted with BM (n = 44; Table 2). The platelet count on day +100 after transplantation was considered a surrogate marker for the stability of engraftment. This value was also higher in the cohort reinfused with PBPC, although statistical significance was not reached ($P = .065$): Seventeen of 30 patients with data available (57%) had normal platelet levels (>150 × 10⁹/L) on day +100 after transplantation. In contrast, a normal platelet count at day +100 was observed in only 12 of 36 BM recipients (33%).

Univariate analysis of variables influencing progenitor cell content and engraftment. The results of an univariate analysis of variables affecting CFU-GM yield, $CD34^+$ cell yield, neutrophil (ANC) recovery, platelet recovery, and the platelet count on day +100 after transplantation are shown in Table 3. For PBPC, a diagnosis of HD and previous radiotherapy adversely influenced the yields of both CFU-GM and $CD34^+$ cells. Intensive pretreatment with bleomycin was associated with reduced CFU-GM numbers. Whereas neither any other single agent nor the number of cycles of regimens other than Dexa-BEAM significantly affected progenitor cell yield and/or engraftment, Dexa-BEAM chemotherapy had a strong impact on the quality of subsequently

Table 3. Factors Adversely Influencing Progenitor Cell Yield and Engraftment (Univariate Analysis)

	Explanatory Variables*			
Endpoint	PBPC	P	BM	P
CFU-GM (×10⁴/kg)	Dexa-BEAM cycles >1	.0001	DTIC cycles ≥1	.02
	Radiotherapy yes	.0016		
	Diagnosis HD	.0028		
	Bleomycin cycles >3	.02		
$CD34^+$ cells (×10⁶/kg)	Dexa-BEAM cycles >1	.0001	None	
	Diagnosis HD	.0018		
	Radiotherapy yes	.0074		
ANC recovery (days to >0.5 × 10⁹/L)	Dexa-BEAM cycles >1	.0086	None	
Platelet recovery (days to >20 × 10⁹/L)	Dexa-BEAM cycles >1	.0002	None	
Day 100 platelet count (×10⁹/L)	Dexa-BEAM cycles >1	.031	None	

The Mann-Whitney Test was used.
* Factors included in the analysis: sex; age (<30/≥30); diagnosis; previous radiotherapy; number of previous Dexa-BEAM cycles (≤1/>1); number of total previous chemotherapy cycles other than Dexa-BEAM (≤6/>6); number of previous cycles containing vinca alkaloids (≤6/>6), cyclophosphamide (≤4/>4), procarbazine (≤4/>4), doxorubicin (≤4/>4), bleomycin (≤3/>3, DTIC (0/≥1), etoposide (0/≥1), ifosfamide (0/≥1), methotrexate (0/≥1); disease activity at time of mobilization/harvest; preparative regimen (BEAM/CBV).

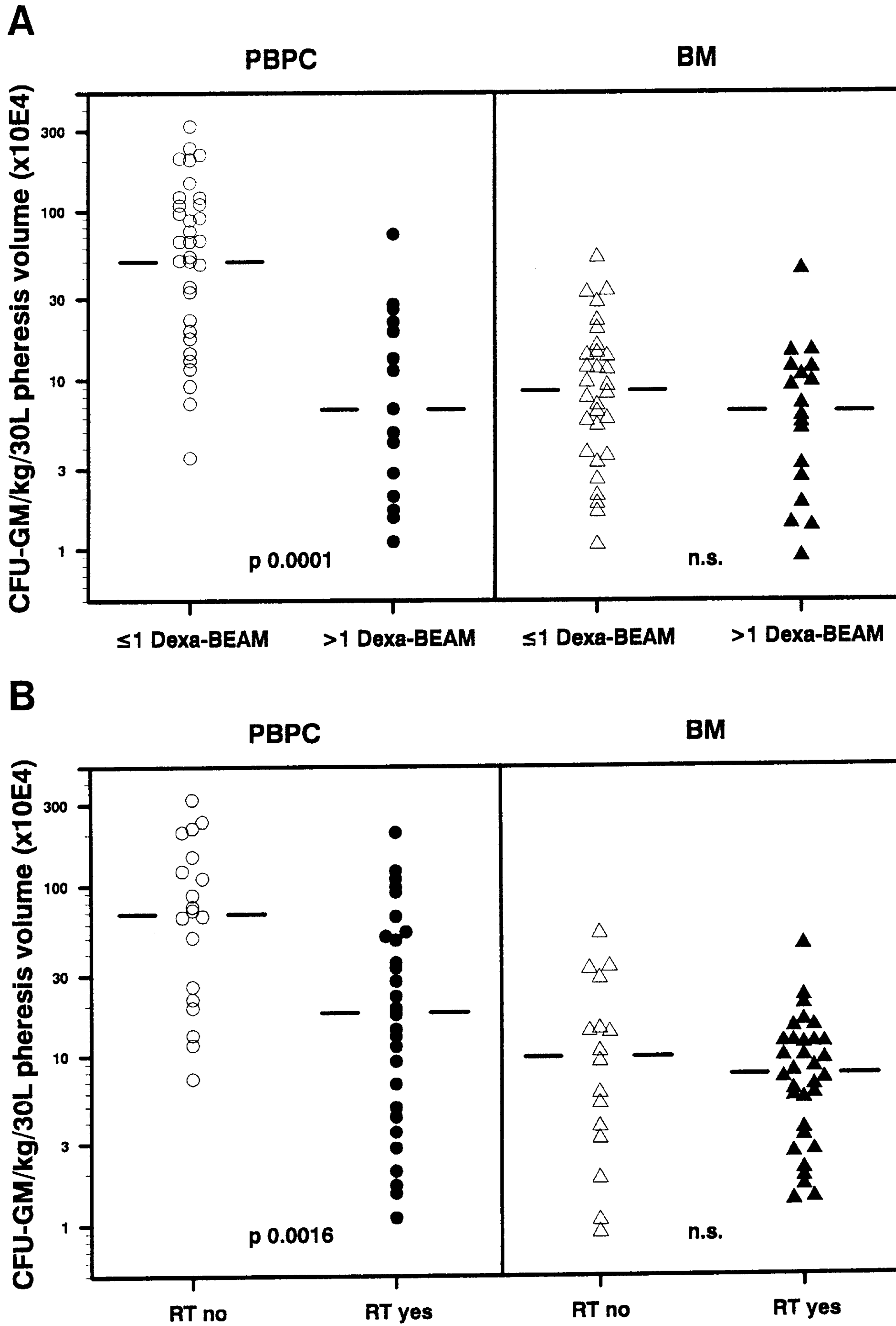

Fig 1. Influence of previous Dexa-BEAM therapy (A) and previous radiotherapy (B) on CFU-GM contents of PBPC and BM grafts, respectively (univariate analysis).

harvested PBPC grafts (Fig 1). Highly significant differences in favor of less pretreatment emerged when patients having received less than 2 cycles of Dexa-BEAM were compared with those having received 2 or more cycles (CFU-GM yield [66 *v* 6.8 $\times$ 10^4/kg, *P* = .0001]; $CD34^+$ cell yield [6.6 *v* 1.6 $\times$ 10^6/kg, *P* = .0001]; ANC recovery to >0.5 $\times$ 10^9/L [9 *v* 11 days, *P* = .0086]; platelet recovery to >20 $\times$ 10^9/L [10 *v* 15.5 days, *P* = .0002], and platelet count on day +100 after transplantation [190 *v* 107 $\times$ 10^9/L, *P* = .031]).

Univariate analysis failed to identify any variable that significantly affected progenitor cell yield and engraftment of BM grafts (Table 3), although there was a trend towards delayed platelet recovery after repeated courses of Dexa-BEAM (21 *v* 26 days, *P* = .08; Fig 2).

Multivariate analysis of variables influencing progenitor cell yield. To evaluate the independent contributions of each of the explanatory variables, a multiple linear regression analysis was performed. Stepwise backward elimination of explanatory variables/interaction terms from the regression model resulted in selection of variables/interaction terms sig-

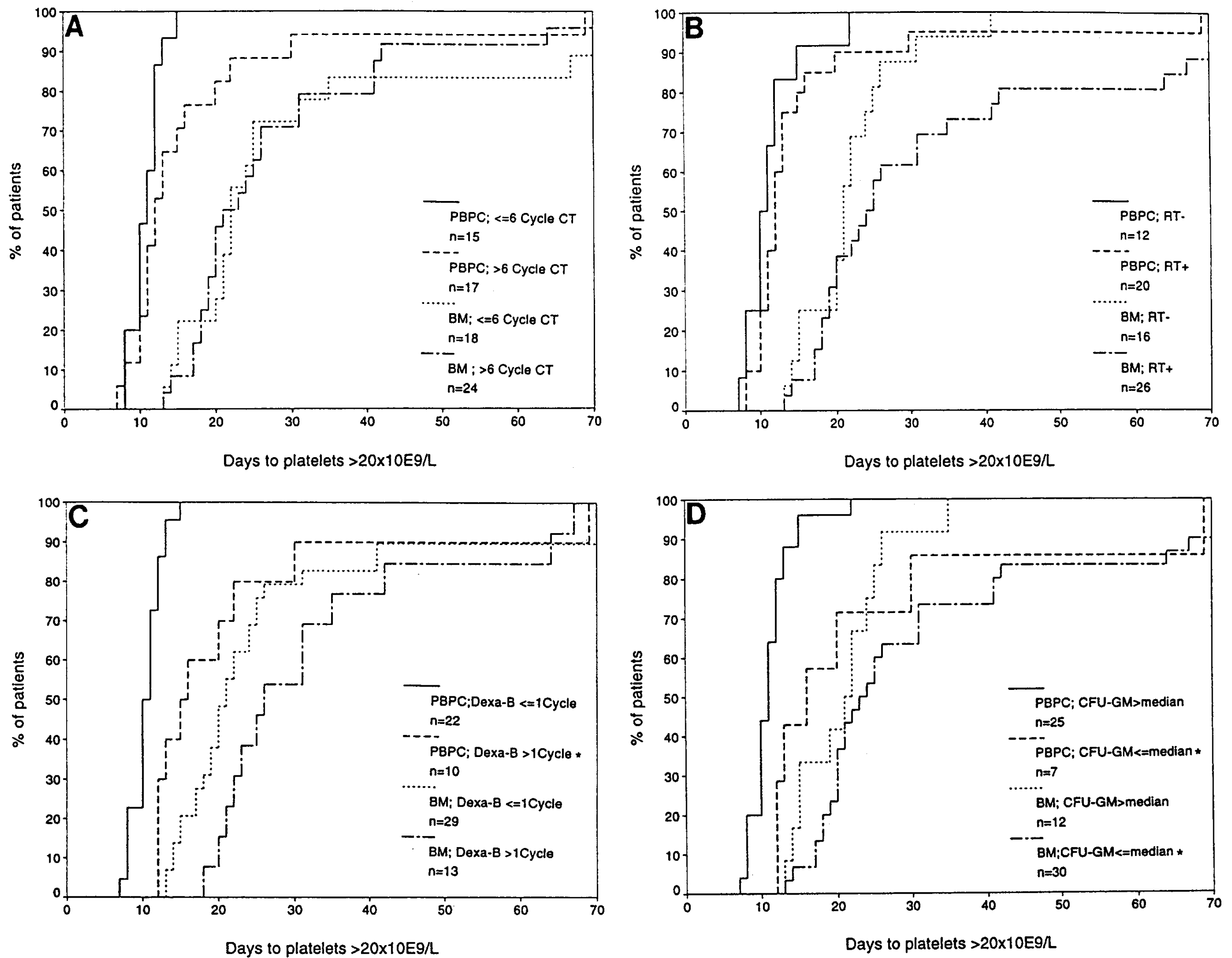

Fig 2. Influence of previous chemotherapy other than Dexa-BEAM (A), previous radiotherapy (B), previous Dexa-BEAM (C), and CFU-GM infused (D) on platelet engraftment (univariate analysis). Significant differences are indicated by an asterisk.

nificantly influencing CFU-GM yield. Harvesting of BM instead of PBPC, previous radiotherapy, and repeated administration of Dexa-BEAM were significant in predicting a low CFU-GM yield (Table 4). The interaction terms graft type * radiotherapy and graft type * Dexa-BEAM also remained in the regression model, implying that quantitative differences exist between PBPC and BM grafts with regard to the variables Dexa-BEAM and radiotherapy, eg, the adverse influence of these factors on BM grafts is much smaller or absent. Although in patients pretreated with multiple cycles of Dexa-BEAM or in irradiated patients the CFU-GM content of PBPC harvests was not higher than that of BM harvests, a constellation of prognostic factors in which BM promises to be superior to PBPC could not be identified.

Using log $CD34^+$ cell yield as the dependent variable, multiple regression analysis again identified BM grafts ($P < .0001$), previous Dexa-BEAM chemotherapy ($P < .0001$), and previous radiotherapy as independent predictors for a low yield and graft type * radiotherapy as a significant interaction term ($P = .025$). The interaction between graft type and Dexa-BEAM was only borderline ($P = .063$); the low number of BM donors with CD34 data available may have accounted for this.

Multivariate analysis of variables influencing engraftment. To investigate the independent impact of prognostic factors with respect to engraftment, a multivariate proportional hazard model was established. Cox regression analysis involving graft type, log CFU-GM infused, previous radiotherapy, previous Dexa-BEAM therapy, previous chemotherapy other than Dexa-BEAM, and the respective interaction terms as independent variables and platelet engraftment as dependent variable was used. Graft type, number of CFU-GM infused, and repeated administration of Dexa-BEAM were the only prognostic factors significantly influencing platelet engraftment (Table 5). Because the interaction term graft type * Dexa-BEAM also remained in the model, it can be concluded that the adverse effect of Dexa-BEAM on engraftment is significantly stronger in PBPC recipients compared with patients grafted with BM (Fig 2). Again, a constellation of prognostic factors favoring the use of BM instead of PBPC was not identified.

DISCUSSION

Studies evaluating the impact of prior chemotherapy on PBPC yields have shown conflicting results. Although some investigators observed a reduction of CFU-GM or CD34 counts (but not of the speed of engraftment) after intensive chemotherapy,[12,13,23,24] others have been unable to detect such effects.[25-27] The main reason for these contradictory results may be that a useful quantification of chemotherapy is difficult to achieve, given the large number of different drugs administered at different doses and in various combinations. In addition (and this aspect has not been addressed so far), chemotherapeutic agents widely differ in their toxicity to hematopoietic progenitor cells.[14-17] Some drugs even when used repeatedly should not affect PBPC harvesting, whereas others may effectively reduce the pool of hematopoietic progenitor cells that can be mobilized by growth factors. Furthermore, confounding factors such as underlying disease, age, irrradiation fields and doses, and the preparative regimens used may mask the effects of chemotherapy.

In the present study, we were able to analyze a large cohort of homogeneously treated patients with HD or high-grade NHL receiving a transplant at a single institution. Although our patients also had been pretreated with a variety of primary and salvage chemotherapy regimens, more than 50% had received 1 to 4 cycles of the Dexa-BEAM protocol before harvest, giving us the unique opportunity to evaluate the influence of this regimen, which contains the stem cell-toxic components BCNU and melphalan, on progenitor cell yields and engraftment of PBPC. Our data show that more than 1 cycle of Dexa-BEAM effectively reduces CFU-GM and $CD34^+$ cell yields of PBPC collections and leads to significantly delayed platelet recoveries. Because the detrimental effect of Dexa-BEAM on late engraftment (day +100 platelet count) of PBPC and on BM engraftment was less pronounced, one could speculate that committed rather than immature progenitor cells are preferentially affected by Dexa-BEAM. However, there is evidence suggesting that immature progenitors are damaged by this regimen as well. The toxicity of melphalan and particularly BCNU for primitive hematopoietic cells has been shown in various animal models.[14-17] Moreover, the number of $CD34^+CD33^-$ cells present in PBPC harvests was strongly reduced after repeated courses of Dexa-BEAM when compared with grafts obtained from patients with less Dexa-BEAM pretreatment (median, 0.4 *v* 1.9×10^6/kg; $P < .0001$). The fact that both the number of CFU-GM infused and the number of Dexa-BEAM cycles remained as significant variables in the Cox model suggests that Dexa-BEAM affects not only the quantity but also the quality of progenitor cells.

With regard to chemotherapy other than Dexa-BEAM, we were unable to detect clear-cut effects on PBPC yield and engraftment. This finding is consistent with animal studies that failed to show toxic effects of agents frequently used in our patients, such as vinca alkaloids, cyclophosphamide, and doxorubicin, on stem cells.[14-16]

Similar to our experience with Dexa-BEAM, Shea et al[28] reported a decrease in circulating progenitor cells after sequential high-dose carboplatin. In contrast, Pettengell et al[27] did not find a reduction of circulating colony-forming cells during repeated cycles of the VAPEC-B regimen (including vincristine, doxorubucin, prednisolone, etoposide, cyclophosphamide, and bleomycin). These findings confirm that chemotherapeutic drugs differ in their capacity to affect the pool of hematopoietic progenitors available for mobilization. Because harvesting was performed immediately after the last cycle in most of the patients having received repeated courses of Dexa-BEAM, time may have played a role for the adverse effects observed. However, when only patients whose cells were harvested within 2 months after the last chemotherapy were taken into consideration, the strong negative influence of multiple cycles of Dexa-BEAM remained

Table 4. Multiple Regression Analysis of CFU-GM Yield

Explanatory Variable	Regression Coefficient	95% Confidence Interval	*P*
Graft type (PBPC = 0; BM = 1)	−1.001	−1.326; −0.676	<.0001
Prognostic factors and interaction terms remaining in the model			
Diagnosis (HD = 0; NHL = 1)	0.23	−0.019; 0.479	.0696
Previous radiotherapy (no = 0; yes = 1)	−0.417	−0.694; −0.139	.0037
Previous Dexa-BEAM (≤1 cycle = 0; >1 cycle = 1)	−0.729	−1.028; −0.43	<.0001
Graft type * radiotherapy	0.418	0.026; 0.81	.0369
Graft type * Dexa-BEAM	0.604	0.204; 1.004	.0035
Constant	1.853	1.614; 2.331	

Prognostic factors and interaction terms not remaining in the model
Previous chemotherapy other than Dexa-BEAM (≥6 *v* >6 cycles); graft type * diagnosis; graft type * previous chemotherapy.

Table 5. Cox Regression Analysis of Platelet Engraftment (Days to >20 × 10^9/L)

Explanatory Variable	Regression Coefficient	95% Confidence Interval	*P*
Graft type (PBPC = 0; BM = 1)	−2.554	−3.446; −1.662	<.0001
Prognostic factors and interaction terms remaining in the model			
CFU-GM (≤12.6 = 0; >12.6 × 10^4/kg = 1)	0.726	0.111; 1.341	.0208
Previous Dexa-BEAM (≤1 cycle = 0; >1 cycle = 1)	−2.052	−3.050; −1.054	.0001
Graft type * Dexa-BEAM	1.798	0.618; 2.978	.0028

Prognostic factors and interaction terms not remaining in the model
Previous radiotherapy (no *v* yes); previous chemotherapy other than Dexa-BEAM (≤6 *v* >6 cycles); graft type * CFU-GM; graft type * radiotherapy; graft type * previous chemotherapy

Relative risk = EXP (regression coefficient).

unchanged (CFU-GM, $P = .02$; CD34$^+$ cells, $P = .0002$; days to platelets $>20 \times 10^9$/L, $P = .0078$; n = 20). Despite this finding , it cannot be excluded that the ability to mobilize stem cells will recover with time, but this possibility is of limited clinical relevance because the nature of the disease will not allow us to delay harvesting in most instances.

Among the other preharvest variables tested, only radiotherapy emerged as an independent factor affecting the yield of PBPC but not the speed of platelet recovery. This finding is in agreement with the results of previous studies showing a detrimental effect of irradiation on CD34$^+$ cell yield but not on engraftment.[12,25,29] A possible explanation for this discrepancy is that the decrease of PBPC yields caused by radiotherapy is not strong enough to reduce the numbers of available PBPC below thresholds critical for engraftment.

None of the factors included in this analysis significantly altered the quality of BM grafts, although we observed a trend to delayed platelet recovery in the patients pretreated with repeated courses of Dexa-BEAM. There are few other reports on the influence of previous chemotherapy on the performance of BM grafts. Visani et al[11] as well as Brandwein et al[3] found a significant delay in engraftment if the marrow was harvested after intensive chemotherapy. In both studies pretreatment frequently included BCNU or other agents that have been shown to damage stem cells. Rabinowe et al[30] reported that engraftment was inversely correlated with previous exposure to stem cell-toxic drugs but not with the total number of drugs given. Finally, Chopra et al[4] saw significant delays in platelet recovery in patients harvested after treatment with mini-BEAM, a regimen very similar to Dexa-BEAM. Altogether, stem cell-toxic chemotherapy appears to affect BM progenitor cells, too, but the detrimental effects on BM are much weaker than those on PBPC. This conclusion is supported by the finding that in some heavily pretreated patients with insufficient PBPC yields successful marrow harvesting is still possible.[31] However, prognostic factors favoring the use of BM instead of PBPC were not identified among the variables investigated here.

In accord with previous reports ,[8,21] engraftment was faster in the patients reinfused with PBPC. In the present study, we looked also at the stability of engraftment (defined by day +100 platelet levels) and found a trend in favor of PBPC recipients compared with patients reconstituted with BM. One of the possible explanations for this finding may be that PBPC grafts contain, apart from committed progenitors, also higher numbers of immature hematopoietic cells than BM grafts. Our observation that more CD34$^+$CD33$^-$ cells are present in mobilized PBPC than in marrow harvests supports this notion and is in line with a hypothesis recently posed by To et al.[32]

Taken together, our data provide evidence that pretreatment with stem cell-toxic drugs is the overriding factor adversely affecting yields and performance of PBPC grafts, whereas other factors including pretreatment with other chemotherapy have limited effects on these endpoints. As a practical consequence, it seems advisable to harvest PBPC before repeated courses of salvage chemotherapy involving stem cell-toxic drugs to preserve the favorable repopulation kinetics of PBPC in comparison with BM. The negative effect of stem cell-toxic drugs on BM grafts was much less pronounced, suggesting that (more immature) marrow progenitor cells are not as sensitive to stem cell-toxic chemotherapy as PBPC. Despite this, in heavily pretreated patients collection of PBPC is still more promising than BM harvesting with regard to progenitor cell yield and engraftment. Further studies are required to elucidate the mechanisms involved in the differential sensitivity of PBPC and BM, respectively, to stem cell-toxic drugs.

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