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Identification of factors associated with poor peripheral blood progenitor cell mobilization in Hodgkin's disease

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Background and Objectives. Although the use of drugs which damage stem cells is common in patients with Hodgkin's disease (HD), factors affecting peripheral blood progenitor cell (PBPC) mobilization have not been clearly established in this group of patients. The aim of this study was to identify factors associated with poor PBPC mobilization in patients with HD.

Design and Methods. In order to address this issue we have evaluated in 54 patients with HD mobilized with G-CSF alone the following factors: sex, age, histologic subtype, B symptoms at diagnosis, status of remission, previous chemotherapy and radiotherapy, interval from diagnosis and last chemotherapy cycle to harvest, and dose of G-CSF. Univariate analysis was performed using Student's t-test, Pearson's correlation and Spearman's correlation. A stepwise regression model was used to determine which of the variables was the most predictive of PBPC mobilization.

Results. In univariate analysis poorer PBPC mobilization was observed in patients who had previously received at least two courses of mini-BEAM (p=0.006), a high number of different chemotherapy regimens (p=0.002), a chemotherapy score >30 (p=0.02) and more than 9 months of alkylating agents (p=0.07). We did not find radiotherapy to be a significant factor affecting progenitor cell yield (p=0.59). In the stepwise regression model, only the previous administration of two or more mini-BEAM cycles predicted a poor PBPC yield (p=0.006).

Interpretation and Conclusions. Previous chemotherapy, principally exposure to a mini-BEAM regimen, seems to be the principal factor affecting collection of PBPC in patients with HD mobilized with G-CSF alone. Since mini-BEAM is an effective salvage regimen in relapsed or refractory HD, collection of PBPC should be planned when there has been no or only minimal exposure to a mini-BEAM regimen.
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Key words: chemotherapy, Hodgkin's disease, mini-BEAM, mobilization, peripheral blood progenitor cells

Autologous peripheral blood progenitor cell (PBPC) transplantation has become the standard option for those patients diagnosed as having Hodgkin's disease (HD) who fail to achieve complete remission or those relapsing within the first year after remission.[1,2] Moreover autologous PBPC transplantation can be an appropriate option for some patients with late relapse or those in complete remission presenting with adverse prognostic features.[3] Remission status at transplant is an important predictor of outcome and different salvage regimens have been employed in order to reach a state of minimal residual disease prior to transplant in patients with relapsed or refractory HD.[4,5] Accordingly, most patients who are eligible for transplant are subjected to both prior induction and salvage chemotherapy regimens containing agents such as nitrogen mustard, procarbazine, nitrosoureas or melphalan that potentially affect hematopoietic progenitor cell collection and consequently the rate of hematopoietic reconstitution. From this point of view it seems reasonable to identify factors that influence or predict the yield of progenitor cells in a homogeneous population of patients with HD, a disease that traditionally has been associated with low collection efficiency.[6,7] Previous reports focusing on PBPC mobilization have included heterogeneous populations of patients with different lymphoid malignancies such as non-Hodgkin's lymphoma (NHL), HD and even multiple myeloma.[6-11] In the present study we report those factors associated with poor PBPC mobilization in a series of patients with HD who were uniformly mobilized with granulocyte colony-stimulating factor (G-CSF) alone.

Design and Methods

Fifty-four consecutive patients with HD who had undergone priming with recombinant human (rh)G-CSF (filgrastim) for 4 days through leukapheresis in

Table 1. Patient's characteristics.

	Number (%)
No. of patients	54
Sex	
Male	38 (70%)
Female	16 (30%)
Histologic subtypes	
Nodular sclerosis	38 (70%)
Mixed cellularity	9 (17%)
Others	7 (13%)
Stage of disease	
III, IV	38 (70%)
B symptoms	30 (56%)
First-line regimens	
MOPP/ABVD	11 (21%)
COPP/ABVD	20 (37%)
ABVD	18 (33%)
Others	5 (9%)
No. of previous regimens of chemotherapy	
1 regimen	22 (41%)
2 regimens	19 (35%)
≥ 3 regimens	13 (24%)
Disease status at harvest	
First complete remission	18 (33%)
Second or later complete remission	15 (28%)
Partial remission	14 (26%)
Others	7 (13%)

order to mobilize and collect hematopoietic progenitor cells were included in the study. Written informed consent using institutionally approved forms was obtained from each patient scheduled for progenitor cell harvesting. The patient's characteristics are summarized in Table 1. Their median age was 33 years (range 18 to 63). The intervals between diagnosis and harvest and last chemotherapy and harvest were 395 days (range 135 to 4362) and 50 days (range 12 to 182), respectively. At harvest, absence of bone marrow involvement was evidenced in all cases and 33 patients were in complete remission. Eighteen out of these thirty-three were in first complete remission. Twenty-two patients received radiation therapy to various extents before harvest. Nineteen patients received either mediastinal or mantle-field irradiation and 3 patients received total nodal irradiation. Twenty out of the 54 patients received at least two cycles of mini-BEAM to reduce tumor burden before collection and seven other patients received one course of mini-BEAM. Mobilization of progenitor cells was performed by administering rhG-CSF to patients in steady-state phase, after their recovery from the last cycle of chemotherapy, at a dose of 5-10 µg per kg per day. PBPC collection was initiated in every patient 5 days after the start of rhG-CSF administration. Leukapheresis were performed on consecutive days using a continuous-flow cell separator (Fenwal CS 3000 Plus, Baxter or Cobe Spectra, Cobe Laboratories). After collection, the cells were resuspended in 10% dimethylsulfoxide with autologous plasma, frozen in a controlled-rate freezer at –1°C per minute (n=17) or following an uncontrolled-rate method (n=37) as previously described,[12,13] and cryopreserved in liquid nitrogen until the day of transplantation. The number of $CD34^+$ cells was measured in red cell-lysed leukapheresis samples. Cells were stained with the phycoerythrin-conjugated CD34 monoclonal antibody HPCA-2 and analyzed by flow cytometry using the Becton Dickinson FACScan as previously described.[12-14]

In order to evaluate the influence on mobilization and collection of progenitor cells, the following characteristics were evaluated: sex, age, histologic subtype (nodular sclerosis vs. others), presence or absence of B symptoms at diagnosis, status of remission at harvest, previous chemotherapy and radiation therapy, interval from diagnosis to harvest and last chemotherapy cycle to harvest, and dose of G-CSF for mobilization. Prior treatment, excluding the mini-BEAM regimen and radiotherapy, was analyzed according to the scoring system proposed by Drake *et al.*,[15] categorized into binary categories (≤30 vs. >30) according to the median. We also analyzed the number of different chemotherapy regimens prior to mobilization and exposure to more than 9 months of alkylating agents following previously established criteria.[9]

Statistical analysis was performed using the SPSS software. Univariate analysis was performed using Student's t test, Pearson's correlation and Spearman's correlation. A stepwise regression model was used to determine which of the variables was the most predictive of PBPC mobilization. The total number of $CD34^+$ cells per kg divided by number of aphereses was used as the dependent variable.

Results

A median of 1.95×10^6/kg $CD34^+$ cells (range 0.16 to 11.37) were collected with a median of 2 aphereses (range 1 to 7). A poor mobilization of progenitor cells was observed in patients who had been intensively treated with chemotherapy. Thus, in those patients who had received a greater number of different chemotherapy regimens prior to harvest, the number of $CD34^+$ cells collected was lower. Besides, lower progenitor cell yields were obtained in patients who had received two courses or more of the mini-BEAM regimen, more than 9 months of alkylating agents, and a Drake's score >30, than in patients without these features (Table 2). By contrast, we did not find prior radiotherapy to be a significant factor affecting PBPC yield. In previously irradiated patients the mean number of $CD34^+$ cells per apheresis was 1.55×10^6/kg while in those who did not

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Table 2. Factors that influence mobilization of peripheral blood progenitor cells.

	CD34×10⁶/kg per collection (mean±SE)	*p*
Sex		
Male	1.60±0.29	
Female	0.93±0.21	0.15
Histology		
Nodular sclerosis	1.39±0.25	
Others	1.10±0.39	0.57
B symptoms		
Presence	1.13±0.14	
Absence	1.70±0.50	0.28
Alkylating agents		
≥ 9 months	0.90±0.20	
< 9 months	1.70±0.31	0.07
Drake's score		
> 30	0.99±0.15	
≤ 30	2.00±0.46	0.02
No. of mini-BEAM courses		
≥ 2 courses	0.65±0.12	
None or 1 course	1.85±0.31	0.006
No. of previous chemotherapy regimens		
1 regimen	1.92±0.38	
2 regimens	1.29±0.37	
3 regimens	0.71±0.24	
4 regimens	0.80±0.38	
5 regimens (1 patient)	0.24	0.002
Radiotherapy		
Presence	1.55±0.44	
Absence	1.31±0.20	0.59
Dose of G-CSF		
5 µg/kg/day	1.16±0.26	
10 µg/kg/day	1.35±0.27	0.70
Disease status at harvest		
Complete remission (1st, 2nd or later)	1.34±0.25	
Absence of complete remission	1.50±0.40	0.72
Harvest in first complete remission		
Yes	1.77±0.42	
No	1.22±0.25	0.23

receive radiation therapy the mean number was 1.31×10^6/kg (Table 2). Moreover, age (p=0.5), interval from diagnosis to harvest (p=0.3) and time from last chemotherapy cycle to harvest (p=0.5) did not affect the yield of filgrastim-mobilized hematopoietic progenitor cells. The results of univariate analysis are summarized in Table 2. The regression analysis (Table 3) revealed that only the previous administration of two or more mini-BEAM cycles predicted a poor PBPC yield (p=0.006).

Table 3. Results of multivariate analysis of factors that influence mobilization of peripheral blood progenitor cells.

	p value
No. of previous chemotherapy regimens	0.38
Alkylating agents ≥ 9 months	0.35
Drake's score > 30	0.07
No. of mini-BEAM courses ≥ 2 courses	0.006

Discussion

Different reports have previously addressed factors influencing PBPC mobilization in patients with lymphoma.[6-11] However these factors, as some authors point out, remain controversial because most studies were hampered by the heterogeneity of the patients' underlying diseases (NHL, HD and myeloma) and mobilization strategies. In previous reports, except for one, patients were principally mobilized either with cytotoxic chemotherapy or chemotherapy plus G-CSF or GM-CSF whereas only a minority of patients received G-CSF alone.[6-10] In the single report on a series of patients all mobilized with G-CSF, only 11 patients with HD were included and the doses of G-CSF ranged from 12.5 to 50 µg/kg.[11] Mobilized cells collected from lymphoma patients are characterized by a wide variation in terms of progenitor cell content and speed of engraftment.[16] Indeed, there is a significantly lower collection efficacy in patients with HD than in those with NHL.[6,7] Thus, it seems of particular interest to identify factors that influence the yield of progenitor cells in homogeneous groups of patients. In the present study we analyzed these factors in a homogenous population of patients with HD mobilized with G-CSF alone.

In our series the number of progenitor cells was lower in those patients who had been heavily pretreated. Studies evaluating the impact of prior chemotherapy on PBPC yields have yielded conflicting results. The main reason for these contradictory results may be that it is difficult to achieve a useful quantification of chemotherapy because of the large number of different drugs administered, the different doses and the various combinations. In addition, chemotherapeutic agents differ widely in their toxicity to hematopoietic progenitor cells.[7,10,17] In order to avoid these problems, in our study we chose the score proposed by Drake *et al.* to classify patients according to their previous therapy.[15] Our patients had been pretreated with different first-line and salvage chemotherapy regimens, but 27 out of 36 refractory or relapsed patients (17 of them as early salvage regimen and 10 after two or more regimens) had received 1 to 4 cycles of mini-BEAM before harvest.

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Therefore it was of particular interest to evaluate the influence of this regimen, which contains several stem cell-toxic agents (i.e. BCNU and melphalan), on PBPC mobilization. As previously reported by Dreger *et al.* in patients receiving Dexa-BEAM,[7] our data clearly reveal that more than 1 cycle of mini-BEAM reduces the number of CD34+ cells in the harvest confirming that BCNU and melphalan may reduce the amount of hematopoietic progenitor cells that can be mobilized by growth factors. Indeed, there is evidence suggesting that immature and committed progenitor cells are damaged by these regimens and both adversely affect PBPC mobilization in patients with lymphoma.[7,18]

Haas *et al.* and Dreger *et al.* pointed out that previous radiotherapy is the main adverse factor influencing the collection of CD34+ cells.[6,7] However, Moskowitz *et al.* reported that the number of CD34+ cells collected is similar in patients who had received radiation therapy to that obtained in patients who had not had prior radiotherapy.[10] In our series prior radiation therapy did not affect the yield of progenitor cells. In order to explain these conflicting results, it must be considered that previous studies included patients with NHL, and doses and fields of radiation therapy may not be comparable among studies. This situation once again highlights the need for studies conducted in homogeneous groups of patients.

Others tested variables did not affect the yield of progenitor cells. However, the impact of G-CSF dose on PBPC mobilization has been addressed in patients with hematologic malignancies as well as in healthy donor.[19] In our series there was no difference between patients receiving 5 or 10 µg/kg of G-CSF but only 12 patients received the lower dose.

In summary, our data provide evidence that prior therapy with stem cell-toxic drugs is the overriding factor negatively affecting the yield of PBPC collection in patients with HD mobilized with G-CSF alone. Since mini-BEAM is an effective salvage chemotherapy regimen widely used prior to autologous PBPC transplantation,[20-22] but one that negatively affects PBPC mobilization, it could be suggested that it would be opportune to collect progenitor cells just before or after only one course of mini-BEAM and then to continue with further treatment in order to achieve the best possible response prior to transplant. In this setting some authors have reported that PBPC collection after a mini-BEAM regimen is feasible.[23]

Contributions and Acknowledgments

MAC and RA were responsible for the conception of the study and interpretation of its results. MAC wrote the manuscript. MCF-J, AM, MDC, EQ, and JG-B collected clinical data. JD performed the statistical analyses. JFSM and FH-N 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 and FH-N, as heads of department, are cited last.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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Potential implications for clinical practice

Identification of factors implicated in mobilization and collection of progenitor cells is important to optimize the harvest. These factors may be different in each disease, thus highlighting the need for studies conducted in homogeneous groups of patients. Our results indicate that collection of PBPC from patients with Hodgkin's disease should be planned when there has been no or only minimal exposure to stem cell-toxic drugs.

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