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Original article

Bone marrow reconstitution after high-dose chemotherapy and autologous peripheral blood progenitor cell transplantation: Effect of graft size

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Summary

Background: Peripheral blood progenitor cell transplantation is rapidly replacing autologous bone marrow transplantation as hematological support after high-dose chemotherapy for lymphoma or solid tumors. Controversy exists concerning the number of progenitor cells required for rapid and sustained bone marrow recovery, and as to which of the widely available methods for estimating this number should be employed.

Methods: Forty consecutive patients with solid tumors or lymphomas received high-dose chemotherapy followed by autologous peripheral stem cell reinfusion. All stem cell harvests had been performed after mobilization with standard-dose chemotherapy followed by 300 μg G-CSF daily. Hematopoietic reconstitution was studied in relation to pertinent patient characteristics, to the size of the graft (in terms of the total number of mononuclear cells (MNC), the number of granulocyte/macrophage colony-forming units (CFU-GM) and the number of $CD34^+$ cells, and to the use of G-CSF after stem cell reinfusion.

Results: Both the numbers of CFU-GM and $CD34^+$ cells reinfused, but not those of the MNC, correlated with granulocyte and platelet recovery. Patients who received at least 5×10^6 $CD34^+$ cells/kg body weight achieved platelet transfusion independence on day 12 after reinfusion (range: day 7–37), significantly earlier than patients who had received less ($p = 0.001$). Thirty patients who received G-CSF (300 μg s.c. daily) after reinfusion achieved granulocyte recovery ($\geq 500 \times 10^6$/l) on day 9 (range: day 8–12), while this took a median of 15 days (range: day 10–28) in 10 consecutive patients not receiving G-CSF ($p = 0.0003$). In one patient who had received 1.4×10^6 $CD34^+$ cells/kg, secondary bone marrow failure developed 3 months after transplantation. Reinfusion of cryopreserved autologous bone marrow was followed by prompt recovery.

Conclusion: Peripheral stem cells, mobilized by moderate-dose chemotherapy and G-CSF, lead to rapid and durable engraftment after high-dose chemotherapy when at least $3–5 \times 10^6$ $CD34^+$ cells/kg are reinfused. Lower numbers may also be satisfactory, but are associated with slower granulocyte and platelet recoveries. A moderate dose of G-CSF after reinfusion significantly hastens granulocyte recovery without interfering with platelet recovery.

Key words: peripheral blood progenitor cell transplantation, $CD34^+$ cells, G-CSF

Introduction

High-dose chemotherapy with autologous bone marrow support is increasingly being accepted as a potentially curative treatment modality in the salvage treatment of malignant lymphomas and germ cell tumors. The introduction and wide availability of hematopoietic growth factors has facilitated the development of autologous peripheral blood progenitor transplantations and it is now clear that this technique leads to clinically important enhancement of granulocyte and platelet recoveries [1, 2], with associated reduced morbidity and duration of hospital stay [3, 4]. This has been particularly important for the acceptance of dose-intensification strategies in the adjuvant treatment setting of breast cancer, where the morbidity and mortality associated with conventional bone marrow transplantations could be considered excessive.

Because the experience with peripheral blood progenitor cell transplantation without simultaneous bone marrow transplantation has only recently extended to a multitude of centers, a number of practical questions still need to be settled. Controversy continues regarding the optimal stem cell mobilization regimens and the minimum size of the harvest that is required for rapid and durable engraftment. It is also unclear as to the methods which should be used routinely to determine the number of hematopoietic progenitor cells available for reinfusion and whether or not G-CSF or GM-CSF should be administered after reinfusion. It may well be that the answers to these questions depend in part on the type of stem cell mobilizing chemotherapy regimen

and on the hematologic growth factor(s) used for that purpose [5].

Here we describe our experience with a practical approach in which peripheral stem cells were mobilized using standard-dosage chemotherapy (as appropriate for the tumor type to be treated) and a moderate dose of G-CSF. The number of progenitor cells available for reinfusion was determined using the three most widely available methods: the mononuclear cell count (MNC), the number of granulocyte/macrophage colony-forming units (CFU-GM) and the number of CD34$^+$ cells.

Patients, materials and methods

Patients

At the start of the high-dose chemotherapy, all patients were in chemotherapy-induced partial or complete remissions of either a malignant lymphoma (4 × Hodgkin's disease, 4 × non-Hodgkin's Lymphoma) or a solid cancer type (18 × breast cancer, 10 × germ cell cancer, 2 × ovarian cancer, 1 × neuroblastoma, 1 × medulloblastoma). Sixteen of the 18 breast cancer patients were undergoing intensive adjuvant chemotherapy for high risk N_+M_0 disease [6]. The other patients were in second partial or complete remissions of advanced disease. The patients with germ cell cancer underwent a tandem transplantation procedure with a 5-week interval, the details of which will be described elsewhere [S.R. et al., submitted]. For this report, only the data of the first transplantation procedure have been included.

All patients had ECOG/ZUBROD WHO performance status of 0 or 1, adequate renal and hepatic functions (creatinine clearance ≥50 ml/min, bilirubin ≤25 µmol/L) and normal bone marrow functions (white blood cell count, WBC ≥3.5 × 10^9/L, platelets ≥100 × 10^9/L). All patients were treated in clinical studies approved by the Institutional Ethical Committee with peer-reviewed treatment protocols. Written informed consent was obtained from all patients according to institutional guidelines.

Mobilization procedure

Autologous peripheral stem cells were mobilized by administering chemotherapy, followed by daily subcutaneous administration of 300 µg granulocyte colony-stimulating factor (G-CSF; Neupogen, Amgen Inc. Thousand Oaks, CA) until completion of the leukocytaphereses. In the patients with breast cancer, the mobilizing regimen consisted of fluorouracil (500 mg/m^2), epirubicin (120 mg/m^2) and cyclophosphamide (500 mg/m^2) given on day 1, with G-CSF starting on day 2 [6]. In the remaining patients, PBPC were mobilized using ifosfamide (4 g/m^2) on day 1 and etoposide (100 mg/m^2) on days 1 through 3, followed by G-CSF from day 4 on.

Peripheral stem cell harvest

From the 7th day of G-CSF administration, the percentage of CD34$^+$ cells in the peripheral blood was determined daily. As soon as the WBC count exceeded 3.0 × 10^9/L and an unequivocal rise in CD34$^+$ cell percentage was observed, leukocytapheresis procedures were started and continued for 2 to 4 consecutive days, depending on the number of CD34$^+$ cells harvested, which was determined at the end of each pheresis day. Based on the experience of others, we initially attempted to harvest a minimum total number of CD34$^+$ cells of 7.8 × 10^6/kg [7]. If necessary, a second mobilization procedure was performed to fulfill this requirement.

Prior to the mobilization procedure, a Hickman double lumen catheter (13.5 French) was inserted in the subclavian vein. The leukocytaphereses were performed as an outpatient procedure using a continuous-flow blood cell separator (Fenwal CS3000, Baxter Deutschland GmbH, Germany). At the start of leukocytapheresis, the number of platelets had to be ≥50 × 10^9/L. The total blood volume processed in each session was between 7 and 10 L. This resulted in harvested cell suspensions of 200 ml per run. The cell yields (routinely consisting of over 95% mononuclear cells) were counted, the numbers of CD34$^+$ cells were determined by flow cytometry and the number of CFU-GM was measured. The cells were cryopreserved in physiological saline solution, enriched with 0.1% glucose, 0.38% trisodium citrate, 10% human serum albumin and 10% dimethylsulfoxide (DMSO) at a cell concentration of approximately 50 × 10^6 MNC/ml. For cryopreservation, the cell suspensions were frozen at a controlled rate using a Kryo10 (Cryotech, Schagen, The Netherlands). The frozen cells were stored in the vapor phase of liquid nitrogen until reinfusion.

Bone marrow harvest

In the first 20 patients, bone marrow was harvested by multiple aspirations from the iliac crests under general anesthesia. A minimum number of 2 × 10^8 nucleated cells/kg body weight was required. Mononuclear cells were separated by centrifugation over ficoll-hypaque, washed and cryopreserved. The bone marrow was kept in storage in case the peripheral blood progenitor cell transplantation alone did not lead to full hematopoietic recovery.

CD34$^+$ cell counts

Ten ml of EDTA blood was centrifuged to remove the platelet-rich plasma, followed by lysis of the pellet in 25 ml of isotonic NH_4Cl for 10 minutes at 0 °C. For leukocytapheresis material, only 1–2 ml NH_4Cl was added to a 300 µl leukocytapheresis cell suspension containing about 4–8 × 10^6 cells. Phosphate buffered saline (PBS) containing bovine serum albumin (BSA) 0.2% (w/v) was added to a final volume of 50 ml, followed by centrifugation and resuspension of the pellet in PBS/BSA 0.2% to a cell concentration of 20 × 10^6/ml. 0.5–1.0 × 10^6 cells were incubated for 30 minutes at 4 °C with the fluorescein isothiocyanate (FITC) conjugated monoclonal antibody 8 G 12 (CD 34, kindly donated by Dr. P. M. Lansdorp, Terry Fox Laboratory, Vancouver, Canada). When peripheral blood was analyzed, incubation with a biotinylated CD66 antibody was performed as well to exclude CD66$^+$-granulocytes, thus enhancing the sensitivity of the assay. The cells were washed with PBS/BSA and thereafter incubated with streptavidine-phycoerythrin (streptavidine-PE) for 20 minutes at 4 °C. Flow cytometry was performed using a FACScan (Becton and Dickinson, San Jose, CA). For the determination of the percentage of CD34$^+$ cells in the leukocyte fraction, at least 20,000 cells were acquired in list mode; in the mononuclear cell fraction, the percentage CD34$^+$ cells were determined setting a live gate using single histogram on PE-negative cells and storing the data of at least 10,000 CD66$^-$ cells.

CFU-GM assay

The number of CFU-GM was measured in standard colony-assay systems using human placental-conditioned medium as a source of growth factors [8]. 1–3 × 10^5 peripheral blood mononuclear cells were plated per 35-mm Petri dish in 1 ml of culture medium consisting of Isocoves Modified Dulbecco's Medium supplemented with 20% (v/v) fetal calf serum, 2 mercaptoethanol (0.1 Mm), penicillin (100 IU/ml), streptomycin (100 µg/ml), bovine serum albumin 0.83% (w/v), transferrin (0.77 mg/ml), gluthation (0.08 mg/ml) and methylcellulose 0.9% (v/v). Human placental-conditioned medium, final concentration of 10% (v/v), was added as the source of colony-stimulating factors. All cultures were plated in duplicate. After 12 days of culture at 37 °C and 5% CO_2, colonies (>40 cells) were counted using an inverted microscope.

Intensification regimen and PBPC-transplantation

Patients with solid tumors (N = 32) received a high-dose chemotherapy regimen, described previously [9], consisting of carboplatin 1600 mg/m^2 i.v., thiotepa 480 mg/m^2 i.v. and cyclophosphamide 6000 mg/m^2 i.v., divided over four days ('CTC'). Forty-eight hours after the completion of chemotherapy, patients received autologous peripheral blood progenitor cell transplantations.

Patients with Hodgkin's disease or non-Hodgkin's lymphoma (N = 8) received the BEAM regimen (BCNU 300 mg/m^2, etoposide 800 mg/m^2, Ara-C 800 mg/m^2, melphalan 140 mg/m^2) [10], followed by PBPC reinfusion one day after the completion of chemotherapy.

The PBPC-containing bags were thawed in a 37 °C water bath at bed-side, an equal volume of normal saline was added and the cell suspension was infused rapidly. All infusions were administered through the double-lumen Hickman catheter. No simultaneous bone marrow transplantation procedure was performed. In 30 of 40 patients, G-CSF (Neupogen, received as a gift from Amgen-Roche, Breda, The Netherlands) was administered from the day of PBPC-T until the WBC count in the peripheral blood exceeded 5×10^9/L.

PBPC and hematopoietic recovery

The relationships between the number of mononuclear cells, the number of CD34$^+$ cells and the number of CFU-GM reinfused were analyzed in relation to time to platelet transfusion independence (defined as the number of the days after reinfusion during which the platelet count remained $\geq 20 \times 10^9$/l without platelet transfusions), days to granulocyte recovery ($\geq 500 \times 10^6$/l, transfusion requirements and duration of hospitalization.

Primary graft failure was defined as continued requirement of platelet and/or red blood cell transfusions 3 months after PBPC-T, or continued WBC counts below 3.0×10^9/l at that time. Secondary graft failure was defined as a secondary significant fall in blood count for at least one month, occurring at any time after initial full engraftment (hemoglobin level ≥ 7.0 mmol/l, WBC count $\geq 3.0 \times 10^9$/l and platelets count $\geq 100 \times 10^9$/l, for at least one month).

Statistical analysis

The logrank test was used to study the association of a range of patient characteristics (see Table 1) with the time to granulocyte recovery (to a level of 500×10^6/l) and with the time to platelet transfusion independence. To simultaneously determine the relative influence of these factors (multivariate analysis), a Cox's proportional hazards model was used. The models were built using a stepwise procedure.

Results

Between January 1990 and August 1993, 40 patients underwent transplantation with autologous peripheral blood progenitor cells (PBPC-T.) Pertinent patient characteristics are listed in Table 1.

A median of 3.5 (range 1–8) leukocytapheresis procedures were performed to harvest the PBPC. The number of mononucleated cells and of CD34$^+$ cells were determined in the grafts of all patients, and the CFU-GM assays in the grafts of all but 4 patients. The median number of CD34$^+$ cells available for reinfusion was 7.1×10^6/kg (range: 0.8–39.4). The median reinfusion numbers of CFU-GM and MNC were 97×10^4/kg (range: 4.39–419) and 4.0×10^8/kg (range: 1.9–13.5), respectively.

To compare granulocyte and platelet recoveries in patients who had received low numbers of CD34$^+$ cells versus patients who had received high numbers, several cutoff points were studied, including 3.0×10^6/kg [11], 5.0×10^6/kg [12] and 7.8×10^6/kg [7] CD34$^+$ cells. The numbers have been used by others for a similar purpose. In all three groups we observed a significant difference in recovery rate of granulocytes, WBC and platelets. For the purpose of this report, we have selected 5.0×10^6 CD34$^+$/kg as cutoff value to divide the 40 patients into two groups of similar size. A reinfusion number of 100×10^4 CFU-GM/kg was selected as cutoff value for the statistical analysis, because this number divided the patients into two different groups with respect to granulocyte and platelet recoveries. There

Table 1. Univariate analysis of patients and graft characteristics as predictors of granulocyte recovery (500×10^6/l) and platelet transfusion independence.

		Number of patients	p-values	
			granulocyte recovery	Platelet transfusion independence
Age (years)	<25 26–35 36–45 >45	6 13 13 	NS	NS
Sex	male female	13 27	NS	NS
Number of prior regimens	1 ≥2	21 19	NS	0.0197
Mobilization regimen	FEC IME	22 18	NS	0.0480
High-dose regimen	CTC other	7 33	NS	NS
G-CSF after reinfusion	no yes	10 30	0.0012	NS
CD34$^+$ cells $\times 10^6$/kg	<7.8 ≥7.8	24 16	NS	0.0388
CD34$^+$ cells $\times 10^6$/kg	<5.0 ≥5.0	16 24	NS	0.0089
CD34$^+$ cells $\times 10^6$/kg	<3.0 ≥3.0	8 32	NS	0.0219
CFU-GM $\times 10^4$/kg	<50 ≥50 unknown	7 29 4	NS	0.0594
CFU-GM $\times 10^4$/kg	<80 ≥80 unknown	14 22 4	NS	0.0779
CFU-GM $\times 10^4$/kg	<100 ≥100 unknown	22 14 4	NS	0.0001
Mononuclear cells $\times 10^8$/kg	<20 ≥20	2 38	NS	NS
Mononuclear cells $\times 10^8$/kg	<42 ≥42	22 18	NS	NS

was no statistically significant difference between the groups when cutoff values of either 50×10^4/kg or 80×10^4/kg were used (Table 1), in contrast to what has been reported in the literature [3, 7].

Granulocyte recovery

In the 30 patients who received G-CSF after PBPC-T, the granulocyte counts had recovered to at least 500×10^6/l by day 10 after reinfusion (median; range: 8–13 days). In the ten patients who did not receive G-CSF following PBPC-T, the granulocyte recovery occurred later, with a median time of 15 days before this level was reached (range: 10–28 days ($p = 0.0003$). This highly significant difference was observed despite the fact that the median number of $CD34^+$ cells reinfused in the non-G-CSF group was more than twice the number of that in the G-CSF-positive group: 10×10^6/kg (range: 1.4–15.8) versus 4.9×10^6/kg (range: 0.8–39.4).

In the patients who received G-CSF after PBPC-T ($N = 30$), reinfusion of $\geqslant 5.0 \times 10^6$ $CD34^+$ cells/kg resulted in a significantly faster granulocyte recovery to 500×10^6 cells/l (median: 9 days, range: 8–12) compared with the time to recovery after reinfusion of $< 5.0 \times 10^6$ $CD34^+$ cells (median: 11 days, range 10–13) ($p = 0.0001$, Fig. 1A). The discriminating power of the CFU-GM numbers was similar. When the number of CFU-GM reinfused was $\geqslant 100 \times 10^4$/kg, granulocyte recovery to 500×10^6 cells/l took a median of 9 days (range: 8–12), while after reinfusion of $< 100 \times 10^4$/kg recovery to this level took a median of 11 days (range: 9–13 days, $p = 0.0009$, Fig. 1B).

In the group of patients who did not receive G-CSF after reinfusion ($N = 10$), only a single patient received less than 5.0×10^6 $CD34^+$ cells/kg. This patient, who was transplanted with only 1.4×10^6 $CD34^+$ cells/kg, later experienced secondary graft failure (see below). In the patients who did not receive G-CSF after transplantation, a relationship between the number of $CD34^+$ cells reinfused and the rate of granulocyte recovery could not be established (data not shown). However, it should be noted that the number of patients in this group ($N = 10$) was too small for a statistically meaningful analysis.

Platelet recovery

A platelet count of $\geqslant 20 \times 10^9$/l was achieved within 40 days after PBPC-T in all 40 patients, with a median of 14.5 days (range 7–40). The administration of G-CSF after reinfusion did not influence the rate of platelet recovery (data not shown).

Reinfusion of at least 5×10^6 $CD34^+$ cells/kg was associated with a significantly shorter time to platelet transfusion independence. The median day after reinfusion on which this was achieved was day 12 (range: day 7–37), while it took a median of 21 days (range 15–40) in patients who had received $< 5.0 \times 10^6$ $CD34^+$ cells/kg ($p = 0.0012$, Fig. 2A).

The number of CFU-GM that was reinfused could be used in a similar way. Patients who had received at least 100×10^4/kg CFU-GM achieved platelet transfusion independence by day 11 after reinfusion (range: day 7–15), while this took a median of 19 days (range: 9–40 days) in patients who had received less than that ($p = 0.0002$, Fig. 2B).

MNC and hematologic reconstitution

No relationship could be detected between the number of MNC reinfused and one of the parameters of the times to blood cell recovery (data not shown).

$CD34^+$ cells, CFU-GM and MNC

As might be expected, a significant correlation existed between number of $CD34^+$ cells and the numbers of

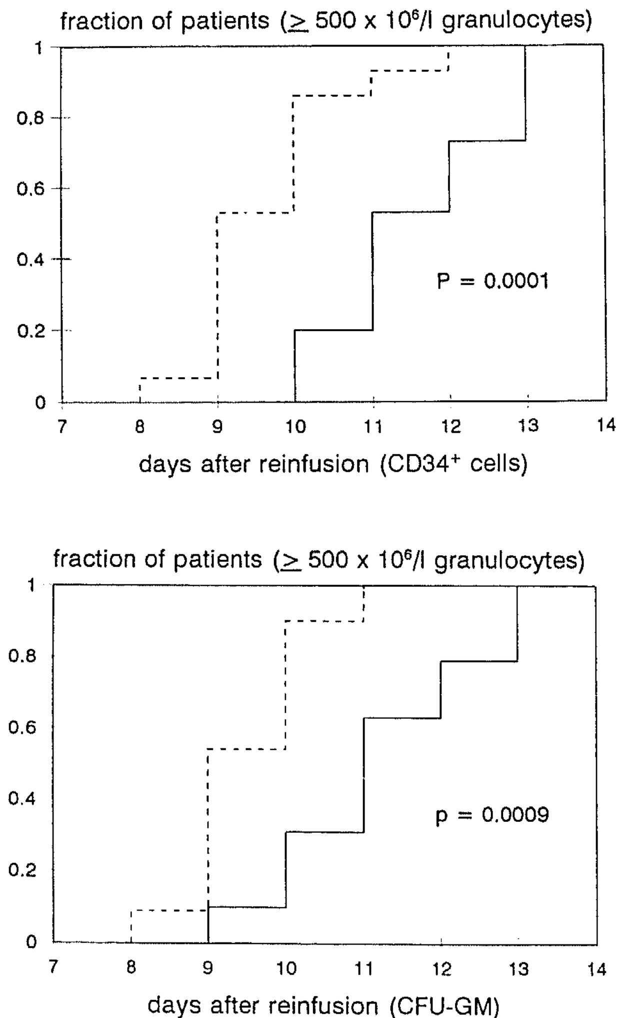
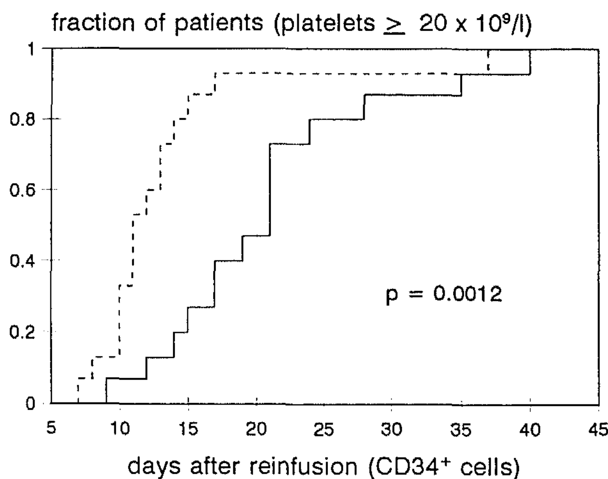


Fig. 1. Granulocyte count recovery to 500×10^6/l after peripheral blood progenitor cell transplantation in patients who received G-CSF after reinfusion. (A) Dotted line: $\geqslant 5.0 \times 10^6$ $CD34^+$ cells/kg grafted ($N = 15$), uninterrupted line: $< 5.0 \times 10^6$ $CD34^+$ cells/kg grafted ($n = 15$). (B) Dotted line: $\geqslant 100 \times 10^4$ CFU-GM/kg grafted ($N = 11$), uninterrupted line: $< 100 \times 10^4$ CFU-GM/kg grafted ($N = 19$).

CFU-GM ($r = 0.70$, $p < 0.0001$, Fig. 4). There was no correlation between the number of MNC and the numbers of either CFU-GM or $CD34^+$ cells (data not shown).

Multivariate analysis

The factors studied and the results of the univariate analysis are presented in Table 1. The only factor predictive of a rapid granulocyte recovery in the univariate recovery was the use of G-CSF after reinfusion ($p = 0.0012$). When the factors were studied in the Cox's model, however, and the patients were stratified for the use of G-CSF after reinfusion, the number of $CD34^+$ cells/kg was also significantly associated with granulocyte recovery ($p = 0.0001$). None of the other factors in Table 1 improved the model in a statistically significant manner.



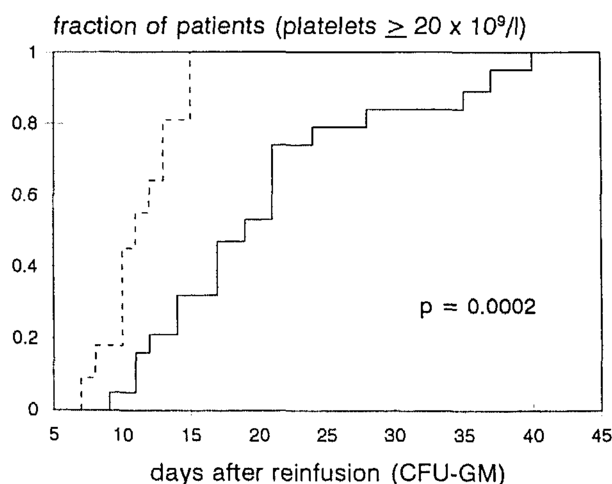


Fig. 2. Time to platelet transfusion independence after peripheral blood progenitor cell transplantation. (A) Dotted line: $\geq 5.0 \times 10^6$ $CD34^+$ cells/kg grafted (N = 24), uninterrupted line: $< 5.0 \times 10^6$ $CD34^+$ cells/kg grafted (n = 16). (B) Dotted line: $\geq 100 \times 10^4$ CFU-GM/kg grafted (N = 11), uninterrupted line: $< 100 \times 10^4$ CFU-GM/kg grafted (N = 19).

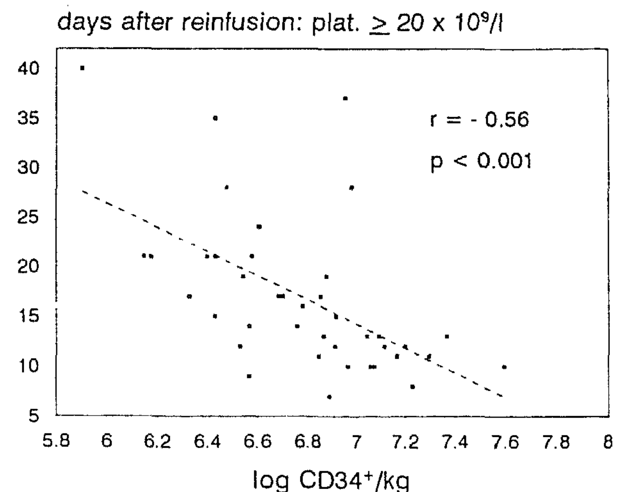


Fig. 3. Time to platelet transfusion independence after peripheral blood progenitor cell transplantation, as a function of the logarithm of the number of $CD34^+$ cells/kg grafted.

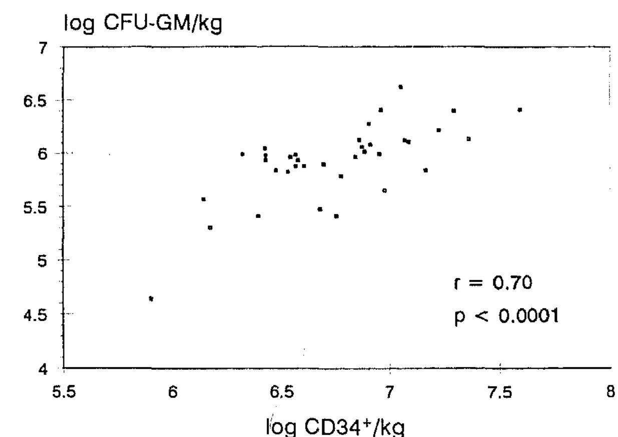


Fig. 4. Correlation between the number of $CD34^+$ cells and CFU-GMs grafted.

Several factors were associated with platelet recovery, as also indicated in Table 1. When these factors were studied together in a Cox's model, the most statistically significant one was the number of CFU-GM/kg in the graft ($p = 0.0002$). Four patients could not be included in this part of the analysis because this number was unknown for their grafts. No other factor improved the model significantly. Since the number of CFU-GM and the number of $CD34^+$ cells were closely correlated (Fig. 5) and because it is easier to obtain the number of $CD34^+$ cells/kg than that of the CFU-GM/kg, a similar model was studied in which the number of CFU-GM was ignored. This model was based on the data from all 40 patients. In this case, the number of $CD34^+$ cells/kg was found to be the only statistically significant factor for platelet recovery ($p = 0.0008$).

Transfusion requirements and duration of hospitalization

Patients who received over 5.0×10^6 $CD34^+$ cells/kg required significantly fewer red blood cell and platelet

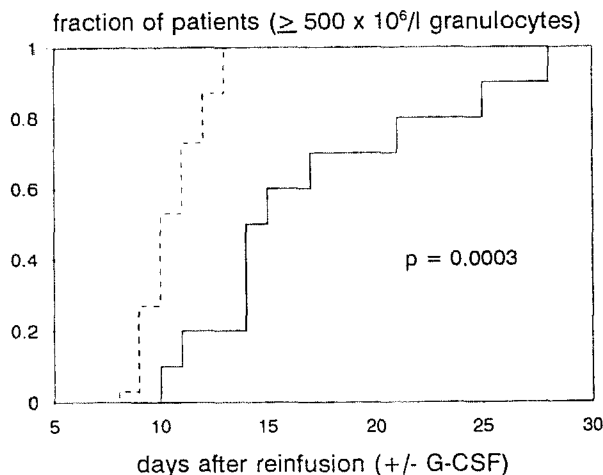


Fig. 5. Time to granulocyte recovery (500×10^6/l) after peripheral blood progenitor cell transplantation. Dotted line: patients who received G-CSF after reinfusion (N = 30); uninterrupted line: patients who did not receive G-CSF (N = 10).

transfusions, and had fewer days with fever over 38°C. On average, they also spent less time in hospital, but this difference was not statistically significant (Table 2).

Graft failures

Primary graft failures (see materials and methods for definition) were not observed. In one patient with breast cancer, however, secondary graft failure occurred. She had been reinfused with only 1.4×10^6/kg $CD34^+$ cells and 37×10^4 CFU-GM/kg. The viability of the leukocytapheresis-product after cryopreservation had been adequate: 89%. After an unremarkable recovery with platelet numbers up to 98×10^9/l and a maximum WBC count of 5.3×10^9/L, cytopenias recurred. From day 92 after PBPC-T on, the number of platelets fell to a level of 15×10^9/l, and the WBC counts decreased to 2.0×10^9/l. Transfusions of red blood cells were repeatedly required. Bone marrow, which had been stored prior to high-dose chemotherapy, was reinfused on the 197th day after transplantation. This resulted in rapid hematologic reconstitution. Blood transfusions were no longer necessary, the granulocyte count exceeded 3.0×10^9/l four weeks after transplantation and the platelet count rose about 50×10^9/l within 8 weeks after transplantation.

Table 2. Fever, transfusion requirements and day of discharge in 40 patients, depending on number of $CD34^+$ cells/kg reinfused (means).

	$\leq 5.0 \times 10^6$/kg $CD34^+$ cells (N = 16)	$>5.0 \times 10^6$/kg $CD34^+$ cells (N = 24)	p-value[a]
Days fever >38°C	6.7 (range: 1–12)	4.0 (range: 0–12)	p = 0.05
Units of red blood cells transfused	7.1 (range: 4–16)	4.6 (range: 2–9)	p = 0.015
Number of platelet transfusions	6.4 (range: 2–11)	3.9 (range: 2–13)	p = 0.005
Days of hospitalization after stem cell reinfusion	15.6 (range: 11–37)	11.7 (range 11–13)	NS

[a] Mann-Whitney test.

Discussion

As reported by others [13], the reinfusion of peripheral stem cells resulted in rapid granulocyte reconstitution in all patients and in swift achievement of platelet transfusion independence in most. The recovey kinetics compare favorable with historic series of patients who received autologous bone marrow alone after high-dose therapy. The number of mononuclear cells that were reinfused did not correlate with the time to granulocyte and platelet recovery, but both the CFU-GM numbers and the numbers of $CD34^+$ cells reinfused did. There was a satisfactory correlation between the CFU-GM determinations and the $CD34^+$ counts (r = 0.71), while neither of these was clearly superior to the other in predicting recovery. Consequently, there appears to be no need to perform both assays if one merely wishes to establish the adequacy in size of the stem cell harvest. Although the CFU-GM number was slightly better in predicting platelet recovery, the $CD34^+$ cell counts can be rapidly performed by flow cytometry and are less tedious than CFU-GM assays. Because of this, we have discontinued the routine use of CFU-GM assays in the setting of uncomplicated single-procedure stem cell reinfusions. A second reason for preferring $CD34^+$ cell counts over CFU-GM assays is that the latter may be more difficult to standardize than the former. The wide variations in results between laboratories could obviously preclude comparisons of mobilizing regimens and frustate the conduct of urgently needed multi-center studies of high-dose therapy.

Our data clearly show that stem cell transplantations with relatively low numbers of $CD34^+$ cells can rapidly restore granulocyte counts, although the time to recovery may be one or two days longer than when high numbers of $CD34^+$ cells are reinfused (Fig. 1A). Such a slight prolongation of the absolute neutropenic period is probably not clinically important and it illustrates the practical value of reinfusing small numbers of peripheral stem cells even if an autologous bone marrow transplantation must be performed because of poor mobilization results.

A major difference between low $CD34^+$ cell and high $CD34^+$ cell stem cell transplantations was observed in the platelet recovery data (Fig. 2A). In fact, the number of the $CD34^+$ cells reinfused is a fairly good predictor of the time to platelet transfusion independence (TPTI) (Fig. 3). Clearly, the length of this time depends on the number of divisions of progenitor cells will have to go through to accumulate the cell mass required for adequate mature cell counts. If the popula-

tion doubling times are thought to be constant, the TPTI should be linearly related to the logarithm of the number of reinfused progenitor cells. Although CD34$^+$ counts are obviously not an ideal measure of megakaryocyte-precursors, and despite the fact that considerable inter-patient variation might be expected for many reasons, this linear relationship between the logarithm of CD34$^+$ reinfused cells and TPTI was clearly detectable in our results. In practice, all patients who received at least 10×10^6 CD34$^+$ cells/kg had achieved platelet transfusion independence before day 15 (Fig. 3). Below that number the rate of recovery was highly variable.

Improved quantification of thrombocyte precursors has been reported in studies employing multi-parameter phenotyping of CD34$^+$ cells [14] and more accurate predictions of platelet recovery may become clinically available in the near future.

The establishment of a practical minimum number of CD34$^+$ cells required for rapid and durable engraftment continues to be hampered by differences in CD34$^+$ assay methodology and by differences in stem cell mobilizing regimens. The latter may lead to different distributions of the various progenitor cell types which may not be accurately reflected by the CD34$^+$ cell counts. In addition, factor such as patient age, previous chemotherapy, bone marrow involvement and type of preparative high-dose regimen could lead to differences in recovery kinetics. The population in this report consisted entirely of patients with lymphomas or solid tumors without bone marrow involvement. All patients received stem cell mobilizing regimens based on standard-dose cytotoxic drugs and moderate doses of G-CSF and all patients underwent high-dose chemotherapy without Total Body Irradiation. In this setting, $3.0–5.0 \times 10^6$ CD34$^+$ cells/kg body weight was adequate and was associated with rapid engraftment. When less than this number of CD34$^+$ cells are available for reinfusion, performance of a simultaneous autologous bone marrow transplantation may be prudent to avoid secondary graft failure. Even in that case, the peripheral stem cells reinfused will ensure rapid granulocyte recovery (usually before day 12 after reinfusion) despite their relatively low numbers.

Another unresolved controversy is the use of hematologic growth factors after the reinfusion of peripheral stem cells. Although we have not investigated this in a randomized study, we have withheld G-CSF after reinfusion in 10 consecutive patients who received high-dose chemotherapy in the adjuvant setting for breast cancer. Since these patients had not received previous chemotherapy except the four courses of fluorouracil, epirubicin and cyclophosphamide as part of their adjuvant chemotherapy, delayed reconstitution was not expected on the basis of one of the patient factors mentioned above. Nevertheless, their time to granulocyte recovery was significantly longer when compared to the patient group as a whole ($p = 0.0003$, Fig. 5) or when compared to the other 8 patients receiving high-dose therapy for breast cancer. This difference, which averaged 6 days for recovery of granulocyte counts of 500×10^6/l, was not associated with important morbidity or even mortality in this small series. Early studies aiming to define the effects of colony-stimulating factors in this setting have yielded conflicting results [11, 15]. Definitive trials studying the effects on mortality would require large studies which would rapidly be outdated as the field of stem cell transplantation continues to expand and new growth factors become available. For the time being, we feel that administration of G-CSF is indicated in view of the lack of side effects and the reduced duration of hospitalization.

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