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Factors affecting hemopoietic recovery after high-dose therapy and autologous peripheral blood progenitor cell transplantation: a single center experience

ATTILIO OLIVIERI, MASSIMO OFFIDANI, MAURO MONTANARI, LUCA CINIERO, ISABELLA CANTORI, LAURA OMBROSI, CRISTIANA M. MASIA,* RICCARDO CENTURIONI,* STEFANIA MANCINI, MARINO BRUNORI, PIETRO LEONI

*Department of Hematology and *Department of Medicine, University of Ancona, Italy*

ABSTRACT

Background and Objective. **While the minimum number of CD34⁺ cells required for complete and long-lasting engraftment is quite well established, there is not general agreement about the optimal number of CD34⁺ per kg needed in order to obtain engraftment as rapidly as possible. In the present study we assess factors affecting hemopoietic recovery and the optimal peripheral blood progenitor cell (PBPC) number for rapid engraftment in patients treated with high-dose therapy.**

Design and Methods. **We enrolled 80 consecutive patients affected by hematologic and non-hematologic malignancies treated with a median of 10 chemotherapy courses (range 3-38). PBPC collection was performed after mobilization with high-dose chemotherapy and G-CSF 5 μg/kg/day. The circulating and harvested CD34⁺ cells were recognized in the cytofluorimetric CD45⁺/CD14⁻ lymphocyte gate. After myeloablative therapy, PBPC infusion was followed by G-CSF 5 μg/kg/day from day +5 until WBC ≥ 5.0×10^9/L. Univariate and multivariate Cox analyses were performed to investigate factors affecting hemopoietic recovery. The Kaplan-Meier probabilities of hemopoietic reconstitution were compared by log-rank test to assess the optimal CD34⁺ cell number for rapid engraftment.**

Results. **We performed a median of two apheresis (range 1-4) per patient and we infused a median of 6.1×10^6 CD34⁺ cells/kg (range 0.5-30.5). Absolute neutrophil count (ANC) > 0.5×10^9/L was reached after 11 days (range 8-15). The only factor affecting granulocyte recovery proved to be the CD34⁺ cell number; 5.0 to 7.8×10^6 CD34⁺ cells/kg allowed a significantly faster granulocyte recovery than < 2.5×10^6 CD34⁺ cells/kg (p= 0.0312). Platelet transfusion independence (> 20×10^9/L) and 50×10^9/L platelets were reached after 12 (range 8-24) and 15 days (range 9-40), respectively. The CD34⁺ cell number was also the only factor affecting platelet recovery; the number of 5.0 to 7.8 CD34⁺ cells/kg allowed a significantly faster platelet recovery than the lower dose, whereas a higher number did not. No late graft failures were observed. Patients receiving 5.0 to 7.8×10^9 CD34⁺ cells/kg had a significantly shorter duration of neutropenia, fewer platelet transfusions and less time spent in hospital than those receiving lower number did, whereas patients transplanted with a higher number had no advantage.**

Interpretation and Conclusions. **When G-CSF is employed both for PBPC mobilization and after PBPC transplantation, the CD34⁺ cell number is the only factor that affects hemopoietic recovery. Moreover, > 5.0×10^6 CD34⁺ cells/kg is the optimal number for obtaining rapid platelet recovery and reducing the costs of HDT but there is no advantage exceeding the threshold of 7.8×10^6 CD34⁺ cells/kg.**

Key words: PBPC autotransplantation, high-dose therapy, hemopoietic recovery, G-CSF

Correspondence: Attilio Olivieri, MD, Clinica di Ematologia, Università di Ancona, Ospedale Regionale Torrette, 60020 Ancona, Italy.
Phone: international +39-71-5964736 • Fax: international +39-71-889990.

High-dose therapy (HDT) followed by autologous progenitor cell transplantation has been widely used both as salvage strategy[1-3] and, more recently, as upfront treatment for many malignancies.[4,5]

Peripheral blood progenitor cell (PBPC) rescue is being increasingly used instead of bone marrow transplantation mainly after the introduction of cytokines such as GM-CSF[6] or G-CSF,[7] which mobilize very large amounts of committed PBPC. This significantly accelerates the early phase of the hemopoietic reconstitution and consequently reduces the morbidity and mortality of HDT.[8]

The CD34⁺ cell number contained in the PBPC collection seems to be the most powerful predictor of hemopoietic recovery after myeloablative therapy, while mononuclear cells (MNCs) are unreliable and the clonogenic assay of committed granulocyte-macrophage progenitors (CFU-GM) is affected by a wide range of variability.[9,10]

A suitable CD34⁺ cell number can be easily collected by only one apheresis in most patients when mobilization is performed by chemotherapy followed by growth factors, and patients have not been heav-

ily pretreated.[11-14] However, the optimal mobilization regimen has yet to be settled. Moreover, while the minimum number of $CD34^+$ cells required for complete and long-lasting engraftment is quite well established (i. e. 2×10^6/kg),[15] there is not general agreement about the optimal number of $CD34^+$ per kg needed in order to obtain engraftment as rapidly as possible both for neutrophils and for platelets. This is a critical issue because the main advantage of PBPC transplantation over bone marrow transplantation, i.e. faster hemopoietic recovery, will probably be lost if a suitable number of $CD34^+$ cells is not infused.

The optimal number of $CD34^+$ cells required for rapid engraftment should be carefully evaluated in patients eligible for transplantation; therefore the choice of mobilization regimen, the number of apheresis to perform and, perhaps, the potential load of neoplastic cells reinfused should be taken into account when planning the high-dose therapy.

We studied 80 patients, all of them mobilized by chemotherapy plus G-CSF, who underwent HDT followed by PBPC rescue and G-CSF, in order to assess the factors affecting hemopoietic recovery and the optimal progenitor dose required for rapid engraftment.

Patients and Methods

Patient characteristics

Between June 1991 and January 1996 we enrolled 80 patients eligible for high-dose therapy (Table 1). Thirty-six were males and 44 females, with age ranging from 16 to 67 years (median 44.5). Forty-five patients were affected by non-Hodgkin's lymphoma (NHL: 18 relapsed, 13 partial responders and 11 high-risk complete responders), 12 by Hodgkin's disease (HD: 7 relapsed, 3 partial responders and 2 high-risk complete responders), 16 by multiple myeloma (MM: 4 relapsed, 12 partial remission), 6 stage I breast cancer, 3 metastatic breast cancer and one by relapsed testicular cancer. Patients had been previously treated with a number of chemotherapy courses ranging from 3 to 38 (median 10). Patients affected by NHL received, as induction chemotherapy, VACOP-B (19 pts), CHOP (12 pts), PROMACE-MOPP (7 pts), CVP (2 pts) and chlorambucil plus prednisone (2 pts). Patients affected by HD received MOPP-ABVD (7 pts) and ABVD (5 pts). DHAP regimen was employed as salvage chemotherapy both for NHL and HD. Patients affected by MM received VAD regimen (10 pts) and melphalan plus prednisone (6 pts). Patients affected by breast cancer were treated with FEC (6 pts) and CMF (3 pts). The only one testicular cancer was treated with PEB. Eleven patients (14%) received previous radiotherapy; 28 patients (35%) received only one regimen whereas 52 patients (65%) received 2 or more regimen (Table 2).

Oral informed consent was obtained from all patients according to the institutional guidelines.

Table 1. Patient characteristics.

Characteristic	*Median (range)*	*No (%)*
All patients		80 (100)
Age	44.5 (16-67)	
Sex		
male		36 (45)
female		44 (55)
Performance status (WHO)		
0-1		72 (90)
≥ 2		8 (10)
Disease		
NHL		42 (52.5)
MM		16 (20)
HD		12 (15)
solid tumor		10 (12.5)
Prior chemotherapy courses	10 (3-38)	
Prior chemotherapy regimens		
1		28 (35)
≥ 2		52 (65)
Prior radiotherapy		
yes		11 (13.7)
no		69 (86.3)

PBPC mobilization, collection and cryopreservation

Priming chemotherapy included high-dose drugs such as cyclophosphamide 7 g/sm in 44 patients, etoposide 2 g/sm in 13 patients and DHAP regimen in 23 patients.[16] Starting 24 hours after the discontinuation of chemotherapy, G-CSF (Granulokine, Roche, Basel, Switzerland) 5 μg/kg/day subcutaneously was administered until leukapheresis.

From the 5th day of G-CSF administration, patients were monitored daily for the following parameters: WBC, platelet counts and circulating MNC; from day +9 after priming, circulating CFU-GM, BFU-E and $CD34^+$ cells were monitored daily. The following parameters were used as criteria for the beginning of leukapheresis: $CD34^+$ cells ≥ 20/μL and/or MNC ≥ 3000/μL, leukocyte count ≥ 5,000/μL, platelet count ≥ 50,000/μL.

When inadequate antecubital venous access was available, the aphereses were performed using a double lumen catheter (Mahurkar, Quinton, USA) for hemodialysis. Aphereses were carried out using an automatic blood cell separator Fenwall CS 3000 Plus (Baxter, Santa Ana, CA, USA), with 50 mL/min blood volume flow rate, according to the modified program 1 for lymphocyto-apheresis as previously described; the total volume processed ranged from 7 to 11 liters (median 10) and a final volume of 50 ml was collected using the Baxter small volume chamber. The final product was cryopreserved in 10% DMSO with a Planer R201 controlled-rate freezing device and stored at 196°C in liquid nitrogen.

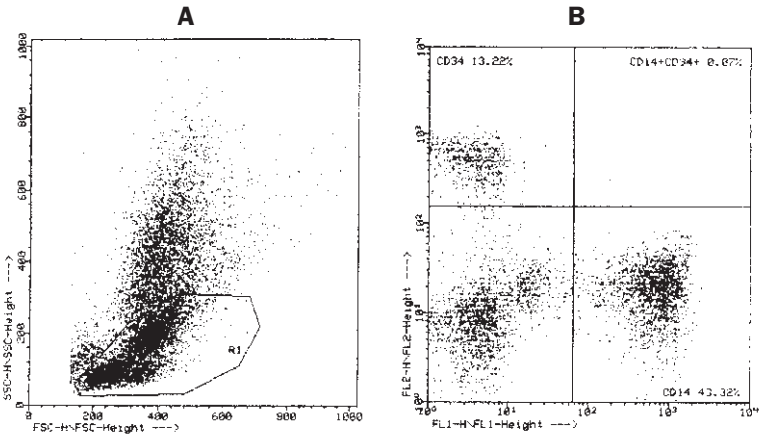

Figure 1. Cytogram scatter showing in R1: lymphomonocytic cells gated from peripheral blood (A) and the whole CD34⁺ cell population (B) with a small fraction of CD34⁺/14⁺ cells which is sometimes detectable, but has been excluded in the CD34⁺ enumeration.

Cell and PBPC measurement in the harvest

The harvest size was calculated after each leukapheresis by total nucleated cell count, MNC count, clonogenic assay of committed progenitors and cytofluorimetric count of CD34⁺ cells. MNC were identified by basophil channel H*1-Technicon coulter as elsewhere described.[17]

The CD34⁺ cells were counted using a Becton-Dickinson FACScan after erythrocyte lysis (EDTA-ammonium-chloride solution) and direct incubation for 30 minutes with phycoerythrin (PhE) conjugated monoclonal anti-CD34⁺ (HPCA2, Becton-Dickinson, San Jose, Ca, USA). CD34⁺ cell counts were performed both in open gate, considering the whole cell population, and by choosing a proper lymphomonocyte population as follows: the entire population was gated using double staining with anti-CD45/14 PhE-FITC conjugated antibodies; in this way only those cells with lymphomonocyte scatter characteristics were acquired, and the double staining with anti-CD34/14 PhE-FITC conjugated antibodies ruled out non-specific binding by the monocytes (Figure 1). A negative control for non specific fluorescence (Simultest; Becton Dickinson, Erembodegem-Aalsi, Belgium) was used with unstained cells; the minimal number of events acquired for each determination was 20,000 and the entire procedure was performed at 4 °C. In all cases only the CD34⁺ cell count performed in the lymphomonocyte gate has been considered for data analysis.

Colony growth was evaluated in 0.9% methyl-cellulose plating 5×10^4 cells in duplicate; the medium consisted of 10% bovine serum albumin, 20% fetal calf serum, 3 U/mL erythropoietin (Eprex; Janssen, Latina, Italy) ,10 ng/mL GM-CSF (Schering-Plough, Milan, Italy) and IL-3 (Genzyme, MA, USA) and 20% Iscove modified Dulbecco's medium.

Cultures were scored using an inverted Zeiss microscope after 14 days of incubation at 37°C in 5% CO_2 humidified atmosphere; aggregates with more than 40 cells were considered as colonies.

High-dose therapy and patient care

Conditioning regimens included fractioned total body irradiation (TBI 1200 cGy) in 8 patients, high-dose melphalan (200 mg/sm) in 9, melphalan (140 mg/sm) and thiotepa (15 mg/kg) in 12, melphalan (120 mg/sm) and busulfan (12 mg/kg) in 7, BEAM (BCNU 300 mg/sm; VP-16 800 mg/sm; aracytin 1600 mg/sm; melphalan 140 mg/sm) in 28, CVB (cyclophosphamide 6 g/sm; VP-16 1600 mg/sm; BCNU 600 mg/sm) in 9 and ICE (ifosfamide 12 g/sm; VP-16 1200 mg/sm; carboplatin 1500 mg/sm) in 7 cases.

During the aplastic phase all patients were kept in a positive pressure HEPA filtered room and received antimicrobial prophylaxis with ciprofloxacin 1000 mg/day and fluconazole 100 mg/day orally and acycloguanosine 15 mg/kg/day intravenously.

All patients received G-CSF 5 µg/kg/day until WBC count reached 5×10^9/L starting five days after BPC reinfusion; irradiated blood products were given in order to maintain the hemoglobin and platelet levels over 8 g/dL and 20×10^9/L, respectively.

Statistical methods

Neutrophil engraftment was defined as the first day on which the absolute neutrophil count (ANC) exceeded 0.5×10^9/L for two consecutive days. Platelet transfusion independence was defined as the first day on which platelet count excedeed 20×10^9/L unsupported by transfusion.

Probabilities of achieving ANC > 0.5×10^9/L, platelets > 20×10^9/L and platelets > 50×10^9/L were calculated using the Kaplan-Meier method[18] and the curves were compared by the log-rank test.

Factors affecting hemopoietic recovery were investigated using the Cox proportional hazard regression model[19] including the following variables: age, sex, diagnosis, prior chemotherapy course, prior chemotherapy regimens, mobilization regimen, type of high-dose therapy, antimicrobial therapy, CFU-GM prethawing (arbitrarily dichotomized at the dose of 30

$\times 10^4$/kg) and CD34$^+$ cells $\times 10^6$/kg. CFU-GM post-thawing was not included in the analysis because it strongly correlated with the CFU-GM pre-thawing (data not shown). Since the relation between CD34$^+$ cells/kg and the tempo of ANC and platelet recovery proved to be not linear but logarithmic, log CD34$^+$ cells/kg was used to obtain the best fitting. Moreover, CD34$^+$ cells/kg was considered as a continuous variable in the first step analysis; the CD34$^+$ cells/kg cut-off values were chosen on the basis of previous published data[9,12,20,21] and included in the second step Cox analysis. Probability curves of hemopoietic recovery were compared by log-rank test to assess the optimal CD34$^+$ cell cut-off value for rapid engraftment.

P < 0.05 was considered as significant. Data were analyzed using SPSS statistical package.

Results

PBPC infused

A median of 2 aphereses (range 1-4) was performed to collect BPC. The median number of MNC, CD34$^+$ cells and CFU-GM infused per patient was 2.1×10^8/kg (range 0.3-7.2), 6.1×10^6/kg (range 0.5-30.5) and 41.5×10^4/kg (range 0.6-410), respectively. Cut-off values of CD34$^+$ cell infused are listed in Table 3.

Granulocyte recovery

All patients achieved an ANC > 0.5×10^9/L with a median of 11 days (range 8-15). Univariate and step-wise Cox analysis found CD34$^+$ cell dose as the only factor affecting granulocyte recovery; considering the previously established cut-offs, values of CD34$^+$ cells < 2.5×10^6/kg were predictive of significantly delayed engraftment compared to CD34$^+$ values ranging from 5.0 to 7.8 and to CD34$^+$ values > 7.8×10^6/kg (p= 0.0312 and p= 0.0026, respectively). When the CD34$^+$ cells infused were 5.0 to 7.8×10^6/kg the 95% probability of achieving granulocyte recovery was 12 days (Table 3).

Probability curves of neutrophils engraftment as a function of CD34$^+$ cell dose are shown in Figure 2.

Platelet recovery

One patient (1.25%), given only 0.5×10^9 CD34$^+$ cells/kg, did not achieve platelet transfusion independence. Seventy-nine patients achieved platelet transfusion independence within a median of 12 days (range 8-24) (Table 3). Univariate Cox analysis showed that previous chemotherapy courses, number of CFU-GM infused (< 30 vs ≥ 30×10^4/kg) and number of CD34$^+$/kg infused significantly affected the tempo of platelet transfusion independence; however, step-wise Cox analysis selected only CD34$^+$ cells/kg (Table 4): values < 2.5×10^6/kg and from 2.5 to 4.9 were predictive of significantly delayed engraftment compared to values > 4.9 (p< 0.0001), whereas the difference between 5 to 7.8 and > 7.8×10^6/kg was not significant (Table 3).

When the CD34$^+$ cell dose ranged from 2.5 to 4.9×10^6/kg, the 95% probability of achieving the platelet transfusion-independence was 20 days, versus 16 days in patients reinfused with a CD34$^+$ cell number ranging from 5.0 to 7.8 (Table 3). Probabili-

Table 2. Treatment characteristics.

Characteristic	*No (%)*
Mobilization regimen	
Cyclophosphamide 7 g/sm	44 (55)
DHAP regimen	23 (29)
Etoposide 2 g/sm	13 (16)
CD34$^+$ cells (x10^6/kg)	
< 2.5	9 (11.3)
2.5-4.9	22 (27.5)
5-7.8	19 (23.7)
> 7.8	30 (37.5)
Preparative regimen*	
BEAM	28 (35.0)
Melphalan + thiotepa	12 (15.0)
Melphalan 200 mg/sm	9 (11.25)
CVB	9 (11.25)
Total body irradiation	8 (10.0)
Melphalan + busulphan	7 (8.75)
ICE	7 (8.75)

**see text for details.*

Table 3. Time to ANC and platelet recovery.

CD34+ cells (x10^6/kg)	*No (patients)*	*Median (days)*	*95% CI (days)*
ANC > 0.5×10^9/L			
< 2.5	9	11*	15
2.5-4.9	22	11	14
5-7.8	19	11	12
> 7.8	30	10*	12
platelets > 20×10^9/L			
< 2.5	8	17*	24
2.5-4.9	22	13*	20
5-7.8	19	12*	16
> 7.8	30	11	15
platelets > 50×10^9/L			
< 2.5	7	30*	34
2.5-4.9	20	16*	31
5-7.8	19	14*	17
> 7.8	30	14	20

**p< 0.05.*

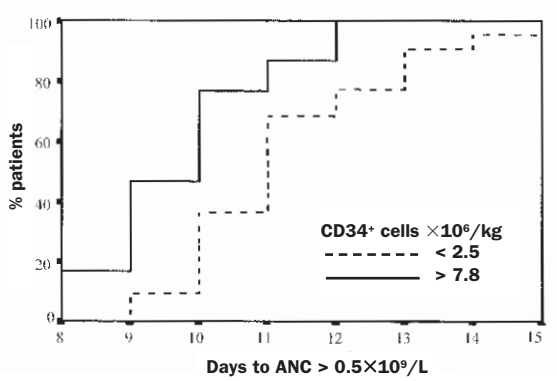

Figure 2. Kaplan-Meier probability of achieving an ANC >0.5×10⁹/L according to the CD34⁺ cell dose: there is a significant advantage in the group of patients reinfused with a dose of > 7.8×10⁶ CD34⁺ cells/kg compared with a group of patients reinfused with < 2.5×10⁶ CD34⁺ cells/kg (p = 0.0008).

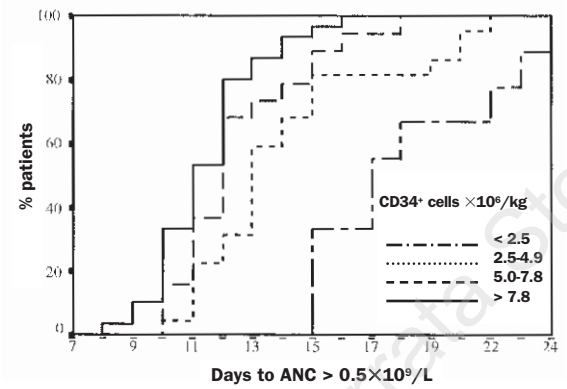

Figure 3. Kaplan-Meier probability of achieving platelets >20×10⁹/L according to the CD34⁺ cell dose: there is a significant advantage in the group of patients reinfused with a dose of > 5.0 to 7.8×10⁶ CD34⁺ cells/kg compared with a group of patients reinfused with 2.5 to 4.9×10⁶ CD34⁺ cells/kg (p = 0.0312); there os a significant difference in favor to the latter group compared to the group reinfused with 2.5×10⁶ CD34⁺ cells/kg (p = 0.0021).

ty curves of achieving 20×10⁹/L platelets for the above mentioned CD34⁺ cell dose are shown in Figure 3.

Four patients (5%) never achieved 50×10⁹/L platelets: two patients because the very low number of CD34⁺ cells infused (0.5 and 1.5×10⁶/kg), one patient had early disease progression and one had autoimmune thrombocytopenia (documented by circulating antiplatelet autoantibody and hypermegakariocytic bone marrow) successfully treated with prednisone.

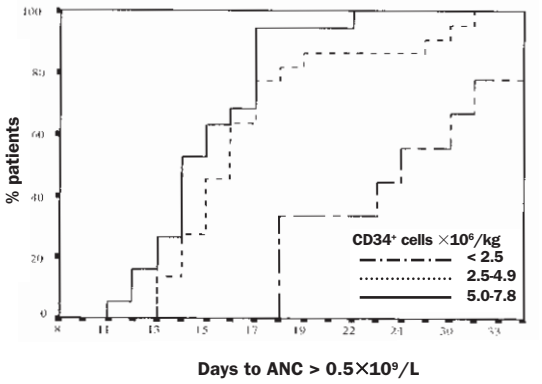

Figure 4. Kaplan-Meier probability of achieving platelets >50×10⁹/L according to the CD34⁺ cell dose: there is a significant advantage in the group of patients reinfused with a dose of > 5.0 to 7.8×10⁶ CD34⁺ cells/kg compared with a group of patients reinfused with 2.5 to 4.9×10⁶ CD34⁺ cells/kg (p = 0.0209).

Seventy-six patients achieved 50×10⁹/L platelets within a median of 15 days (range 9-40). Univariate Cox analysis showed a statistically significant difference according to diagnosis (HD vs solid tumor), previous chemotherapy course, CFU-GM/kg and CD34⁺ cells/kg (Table 4); once again, step-wise Cox analysis selected CD34⁺ cells /kg as the only factor affecting platelet recovery. When the CD34⁺ cell number was between 2.5 and 4.9×10⁶/kg, the 95% probability of achieving 50×10⁹/L platelet was 31 days, whereas it was 17 days in patients reinfused with a CD34⁺ cell number ranging from 5.0 to 7.8×10⁶/kg (Table 3).

The Kaplan-Meier probability curves according to the different groups of patients reinfused with the above CD34⁺ cell ranges are shown in Figure 4.

Clinical course

One patient (1.25%) died from CMV infection at 40th day before achieving 50×10⁹/L platelets. No grade III-IV organ toxicity occurred, but 5 episodes of cardiac supraventricular arrhythmia were observed. Nineteen patients (23.9%) needed total parenteral nutrition for grade IV mucositis and weight loss.

During the neutropenia period (ANC < 0.5×10⁹/L) ranging from 3 to 20 days, sixty-five patients (81.2%) had fever > 38°C for a median of 4 days (range 2-16); these patients underwent empirical broad spectrum antibiotic therapy for a median of 8 days (range 7-18). A median of 2 single donor platelet units (range 0-13) and a median of 2 red cell units were transfused. A median of 14 days (range 10-37) was needed for patient discharge.

No late graft failures were observed with a median follow-up of 24 months (range 6-60); however, one

Table 4. Univariate and stepwise Cox regression analysis of factors affecting hemopoietic recovery.

	Days to ANC > 0.5×10⁹/L		*Days to platelets > 20×10⁹/L*		*Days to platelets > 50×10⁹/L*	
	Univariate	*Multivariate*	*Univariate*	*Multivariate*	*Univariate*	*Multivariate*
Age	ns	ns	ns	ns	ns	ns
Sex	ns	ns	ns	ns	ns	ns
Disease *NHL* *MM* *HD* *solid tumor*	ns	ns	ns	ns	0.0282	ns
Prior chemotherapy courses	ns	ns	0.0452	ns	0.0321	ns
Prior chemotherapy regimens *0-1* *≥ 21*	ns	ns	ns	ns	ns	ns
Mobilization regimen *cyclophosphamide 7 g/sm* *DHAP* *etoposide 2 g/sm*	ns	ns	ns	ns	ns	ns
Preparative regimen	ns	ns	ns	ns	ns	ns
Antibiotic therapy *yes* *no*	ns	ns	ns	ns	ns	ns
CFU-GM x 10^4/kg *< 30* *≥ 30*	ns	ns	0.0121	ns	0.0029	ns
$CD34^+$ cells × 10^6/kg	0.0001	0.0002	< 0.0001	< 0.0001	0.0072	0.0023
$CD34^+$ cells × 10^6/kg *< 2.5* *2.5-4.9* *5.0-7.8* *> 7.8*	0.0175	0.0183	0.0001	< 0.0001	0.0026	0.0003

patient still requires red cell transfusion periodically.

As shown in Table 5, patients given 5.0 to 7.8 $CD34^+$ cells $\times 10^6$/kg had a significantly shorter duration of neutropenia, fewer platelet transfusions and less time spent in hospital than patients receiving 2.5 to 4.9 $CD34^+$ cells $\times 10^6$/kg; no difference was found between the above mentioned group of patients receiving these two different $CD34^+$ cell doses, as regards days of fever, days on antibiotics and the number of red cell units transfused.

Discussion

There are many retrospective studies aimed to define the most effective parameters for predicting hemopoietic reconstitution after PBPC transplantation; the most common parameters used have been derived from the experience in bone marrow transplantation where total nucleated cells or MNC harvested are currently considered the most reliable. Although these two parameters were adopted for predicting engraftment in the preliminary experiences

Table 5. Clinical course of the 80 patients transplantated with autologous PBPC.

	< 2.5 Median (range)	2.5-4.9 Median (range)	5-7.8 Median (range)	> 7.8 Median (range)	p*
ANC < 0.1x10⁹/L	6 (3-10)	5.5 (3-12)*	5 (1-6)*	5 (2-9)	0.0224
ANC < 0.5x10⁹/L	9 (7-13)	8 (5-20)*	7 (3-9)*	7 (3-9)	0.0267
Days fever ≥ 38°C	1 (0-9)	2 (0-16)	2 (0-4)	2 (0-6)	ns
Days on antibiotics	6 (0-17)	7.5 (0-18)	6 (0-9)	6 (0-13)	ns
Units of red cell transfusion	2 (0-16)	2 (0-10)	2 (0-6)	2 (0-10)	ns
Units of platelet transfusion	5 (3-13)	3 (1-12)*	2 (1-5)*	2 (0-6)	0.0108
Days of hospitalization	16 (15-24)	16 (11-37)*	13 (10-17)*	13 (10-16)	0.0149

of PBPC transplantation, there is now clear evidence that both parameters, although simple and cheap, are not at all reliable.[10,14,21,22]

Reiffers *et al*.[23] reported a good correlation between CFU-GM per kg reinfused and hemopoietic recovery, while some authors did not, especially when they considered platelet recovery.[24-27] Indeed, the CFU-GM assay seems more useful for the evaluation of committed myeloid progenitor clonogenic potential; moreover, the result of this assay vary according to different laboratory standards and finally (even more important) this method is not suitable for a real-time graft-size measurement because it requires 10 to 14 days to evaluate results. On the contrary the $CD34^+$ cells obtained from apheresis can be measured in real time and this count is easily reproducible in many laboratories.

Even though some authors keep a different opinion,[28,29] several experiences in PBPC transplantation suggest that $CD34^+$ cell number is the most reliable measure of graft-size for predicting the kinetics of hematopoietic reconstitution.[9,10,11,19,22,24,27,28] According to these reports there should be a strong positive relationship, not only between the $CD34^+$ cell number and the tempo for neutrophil engraftment, but also for the platelet recovery.

There is still controversy about the minimum threshold for obtaining rapid and full engraftment and the upper cut-off above which there should not be any benefits in terms of acceleration of hemopoietic reconstitution. Some authors propose different thresholds ranging from 2 to 7.8×10^6/kg $CD34^+$ cells[20,30] and to date it is not completely settled whether the minimum $CD34^+$ cell dose should be 2.5 or 5×10^6/kg or a different value.[9-11,19] This safety threshold can be probably better defined in a patient population which is at least homogeneous as regards growth factor used both for mobilization and after the PBPC reinfusion.

We enrolled 80 patients, all of them mobilized with high-dose chemotherapy followed by filgrastim, administered at the constant dose of 5 μg/kg/day subcutaneously; moreover, non-glycosylated G-CSF was always administered at the same dosage after the myeloablative therapy, with an identical schedule (starting from day +5). In this population we found that the only significant factor affecting both neutrophil and platelet engraftment proved to be the $CD34^+$ cell dose reinfused; as above mentioned 5 and 2.5×10^6/kg have been largely reported as minimum safe threshold to obtain a full and stable engraftment after PBPC transplantation[9,11,20,21] and 7.8×10^6/kg has been considered the optimal threshold by Siena *et al*. to obtain a quick, complete and stable engraftment.[21] Based on these findings we planned to validate the reliability of these well established three cut-offs, using them to separate four groups of patients transplanted with very low (>2.5×10^6/kg), low (2.5-4.9×10^6/kg), high (5-7.8×10^6/kg) and very high (>7.8×10^6/kg) $CD34^+$ cell dose. It should be noted that the relationship between $CD34^+$ cell dose and tempo to ANC > 0.5×10^9/L was hardly detectable because patients also receiving a very low $CD34^+$ cell dose (< 1.0×10^6/kg) reached ANC < 0.5×10^9/L in a short time; however, when almost all patients (95%) had been considered, the dose-tempo relationship proved to be statistically significant. Indeed patients receiving less than 2.5×10^6 $CD34^+$/kg cells showed a significant delay in neutrophil engraftment (48 hours) compared with those receiving a dose ranging from 5 to 7.8×10^6/kg; we did not observe any significant advantages in terms of acceleration of engraftment in patients receiving more than 7.8×10^6/kg $CD34^+$ cells. This observation confirms the data by Bensinger *et al*.[12] who found ≥ 5×10^6 $CD34^+$ cells/kg an optimal threshold to ensure rapid hematological recovery.

In our study, the importance of $CD34^+$ cell dose emerges more significantly when we consider the speed of platelet engraftment; in fact, transfusion independence in 95% of patients was obtained 8 or 4 days later in those receiving less than 2.5 and in those receiving 2.5 to 4.9×10^6/kg $CD34^+$ cells, respectively, compared to patients transplanted with a $CD34^+$ cell dose ranging from 5 to 7.8×10^6/kg $CD34^+$ cells. It should also be noted that in this case that patients given more than 7.8×10^6/kg $CD34^+$ cells did not show any additional platelet take acceleration.

When we considered a threshold of 50×10^9 platelets/L, the discriminant power of the $CD34^+$ cell cut-offs was increased; indeed we observed a delay of 17 and 14 days in those patients transplanted with less than 2.5 and less than 5×10^6 $CD34^+$ cells/kg respectively, and no additional advantage for those patients receiving more than 7.8×10^6/kg $CD34^+$ cells was observed.

Concerning the role of myeloid growth factors after PBPC reinfusion, there is general agreement on the beneficial effect that they have on neutrophil engraftment in the order of 24-48 hours.[31,32] It should be emphasized that our patients who received a suboptimal number of $CD34^+$ cells (< 5×10^6/kg) showed a very short delay of neutrophil recovery compared to those receiving more than 5×10^6/kg; moreover, some authors hypothesized that the administration of G- or GM-CSF can hinder the platelet engraftment in patients receiving a suboptimal number of $CD34^+$ cells (i. e. < 5×10^6/kg).[10,11,19] We cannot confirm these data; however, the appropriate role of growth factor administration after PBPC transplantation in patients receiving a suboptimal number of $CD34^+$ cells can be established only by appropriately planned trials.

As regards to the clinical course, patients receiving the optimal dose of $CD34^+$ cells experienced fewer days of severe and very severe neutropenia (neutrophils < 0.1/L) although this did not significantly reduce febrile episodes, the days of fever and of antibiotic therapy. However, the median number of platelet units and days of hospitalization were significantly

lower, thus leading to a significant cost reduction. No additional benefits were observed in patients receiving more than 7.8×10^6/kg $CD34^+$ cells as regards to the previously considered clinical parameters.

In conclusion, in patients receiving G-CSF both for mobilization and after PBPC infusion, the only significant factor affecting the hematopoietic recovery is the graft size measured by the $CD34^+$ cell number, and the optimal dose for rapid and complete engraftment ranges between 5 and 7.8×10^6/kg $CD34^+$ cells. A graft size ranging from 2.5 to 4.9×10^6/kg is safe for complete, stable and rapid neutrophil engraftment (when followed by G-GSF), but not for a rapid platelet engraftment. When the $CD34^+$ cell dose is $< 2.5\times10^6$/kg, the neutrophil recovery is still optimal although a significant further delay of platelet engraftment is to be expected.

In non-heavily-pretreated patients, a dose of $CD34^+$ cells $> 5\times10^6$/kg can be easily obtained, while it is very difficult to reach this goal in heavily pretreated ones. In this latter subset of patients in which the optimal threshold of 5×10^6/kg cannot be obtained, a longer platelet transfusion dependence period and the increased overall cost of PBPC transplantation should be taken into account, even thought growth factor administration is routinely planned after the graft reinfusion.

Finally, since our results suggest that the reinfusion of a $CD34^+$ cell number exceeding the threshold of 7.8×10^6/kg is not advantageous, it should be kept in mind that in patients with neoplastic bone marrow involvement or with an high tumor burden, tumor cell mobilization is a very frequent event.[33]

In this subset of patients the reinfusion of very large amounts of $CD34^+$ cells (i.e. $> 7.8\times10^6$/kg) could be associated with an increased risk of reinfusing a large number of potentially clonogenic tumor cells without obtaining significant reduction of the transplant-related toxicity.

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AO: conception and design and interpretation of data, patients assessment, drafting and critically revising the article and final approval of the definitive version. MO: design, analysis and interpretation of data, patients assessment, drafting the article and statistical analysis. MM and LC: patients assessment, data collection, $CD34^+$ cell monitoring and blood progenitor cryopreservation. IC, CMM, SM: blood cell manipulation and clonogenic assay (CFU-GM). LO, MB, RC: patients assessment, reinfusion of cryopreserved bag and data collection. PL: critically revising the article and final approval of the definitive version.

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Disclosures

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