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CD34+ cell dose and CD33- subsets: collection and engraftment kinetics in autologous peripheral blood stem cells transplantation

ANTONIA SAMPOL MAYOL, JOAN BESALDUCH VITAL, ANTONI GALMÉS LLODRÁ, JOAN BARGAY LLEONART, NURIA MATAMOROS FLORI,* MIQUEL MOREY SUREDA, ANDRÉS NOVO GARCÍA, MARTÍ MASCARÓ RIERA, ELENA GONZÁLEZ BACH, PEDRO MARTÍNEZ GARCÍA*

*Servicio de Hematología y Hemoterapia y *Servicio de Inmunologia, Hospital Son Dureta, Palma de Mallorca, Spain*

Abstract

Background and Objective. **We analyzed the factors that affected the number and quality of peripheral blood stem cells (PBSC) collected for transplant in order to establish a minimum threshold for rapid hematopoietic recovery.**

Design and Methods. **From January 1995 to November 1996, a consecutive series of 67 patients, with hematologic and solid tumors underwent autologous PBSC transplantation. Collection of PBSC was performed after mobilization with granulocyte-colony stimulating factor (G-CSF) or with chemotherapy (CT) plus G-CSF. We calculated the factors that influenced PBSC collection, the kinetics of granulocyte and platelet recovery and the threshold value of CD34+ cells for a rapid recovery. The data were analyzed by means of multivariate Cox regression model and the receiver operating characteristic (ROC) methodology.**

Results. **Our results showed that mobilization with chemotherapy plus G-CSF was associated with a higher yield of PBSC in comparison with mobilization with G-CSF alone. Disease status, fewer cycles of conventional prior chemotherapy and absence of prior radiation therapy also influenced the yield of PBSC. The number of CD34+ cells, CD34+CD33- cell subsets, the mobilization schedule, and the conditioning regimen correlated significantly with time to hematopoietic recovery. In the multivariate analysis only the CD34+CD33- cell content and the total number of CD34+ were related with rapid neutrophil and platelet recovery, respectively. Use of G-CSF after**

Autologous peripheral blood stem cells (PBSC) are increasingly being used as a source of hematopoietic stem cells to rescue the hematopoietic tissue after high-dose therapy. The easier collection and faster engraftment were some of the advantages observed in comparison with the use of the bone marrow. Mobilized peripheral blood is a rich source of both primitive and committed stem cells.[1]

The capacity of reconstitution of the hematopoietic system has been found to reside within the CD34+ cell population.[2] However, the hematopoietic cells expressing the CD34 antigen constitute a heterogeneous group of cells some of them committed to a particular lineage. Further characterization of the CD34+ cell populations by flow cytometry has identified early multipotent stem cells by the expression of CD45RO and by the lack of expression of CD38, HLA-DR, CD33 and CD13.[3] The phenotype CD34+CD33- has been found to give rise to precursors of colony-forming cells and blast colony-forming cells.[4] The relative proportions of specific subsets of CD34+ cells may provide an explanation for the rapid engraftment observed with mobilized PBSC. Patient characteristics, as well as mobilization techniques, have a significant influence on the ability to collect adequate quantities of CD34+ cells.[5] Measurement of CD34+ cells is one of the best indicators for the reconstitutive capacity of the graft.[6]

Table 1. Patient characteristics.

Characteristic	*N. of patients (n=67)*
Diagnosis	
Breast cancer	26
Non Hodgkin's lymphoma	18
Hodgkin's disease	5
Multiple myeloma	11
Acute myeloblastic leukemia	2
Acute lymphoblastic leukemia	1
*Others**	4
Age (yrs)	
Median (range)	43 (4-62)
Sex	
(Male/female)	24/43
Prior chemotherapy regimens	
Median (range)	2 (0-4)
Status at transplantation	
Complete remission	44
Partial remission	15
Relapse	6
Primary refractory	2

**Others: Ewing' sarcoma (1), seminoma (1), neuroblastoma (2).*

Table 2. Treatment characteristics.

Characteristic	*N. of patients (n=67)*
Mobilization regimens	
Chemotherapy + G-CSF	8
Cy + G-CSF	35
G-CSF alone	24
Transplant preprataive regimens	
HDMF	11
BEAM	18
CBV	9
BUCy-4	3
CTCb	26
G-CSF after transplant	

sensitive partial remission and 2 (3%) patients being in primary refractory disease before harvest and PBSC transplantation. Marrow disease was assessed by histologic examination of marrow biopsies. Twenty (29%) of patients had bone marrow positive for tumor before harvest and PBSC transplantation.

Mobilization procedure and PBSC harvesting

The mobilization techniques are listed in Table 2. In 24 patients, PBSC's were collected after the administration of G-CSF alone at doses of 5 µg/kg/day. On day 5, PBSC collections started on G-CSF treatment which was maintained until the apheresis were completed. In 43 patients, the mobilization consisted in chemotherapy (cyclophosphamide 4 g/m^2 and specific chemotherapy regimens given to 8 patients with non Hodgkin's lymphoma and 3 patients with acute leukemia) plus G-CSF at doses of 5 µg/kg/day started at the fifth day of the chemotherapy cycle. PBSC's collections were initiated when the WBC count reached to 1×10^9/L.

In all cases, the PBSC were collected during an outpatient leukapheresis procedure using a continuous-flow blood cell separator (CS-3000 plus, Fenwal). The median number of apheresis was 4 (1-9). The initial target value for adequacy of PBSC collection was 3×10^8/kg MNC. So, we stopped the process of apheresis when this goal was achieved. In addition, we analyzed a posteriori the graft content of CD34$^+$ cells and CFU-GM. The target values were 3×10^6/kg and 3×10^4/kg for CD34$^+$ cells and CFU-GM, respectively. We only proceeded to transplantation if 2 out of 3 of these values were obtained. In nine patients for whom it was not possible to collect the target number of MNC, CD34$^+$ cells, or CFU-GM, alternative mobilization strategies were performed. PBSC harvests from 67 patients were analyzed and cryopreserved using a simplified method developed in our institution, in an –80°C mechanical freezer without rate-controlled freezing.[9,10]

Immunophenotyping

of 50 or more cells were enumerated under an inverted microscope on day 14 of culture.

Conditioning regimens and reinfusion

Patients received one of several myeloablative regimens that were disease and protocol specific (Table 2).

PBSC were thawed and infused over 1 to 2 days, depending on the volume, 36 to 48 hours after the last dose of chemotherapy. The day of the first PBSC infusion was designated day 0. Twenty patients received G-CSF 5 μ/kg/day beginning on day 5 after infusion of PBSC until the absolute neutrophil count was greater than 0.5×10^9/L, as a part of an ongoing randomized trial, contemporary with this study.

Engraftment was defined as the first of two consecutive days on which the patient's neutrophil count was greater than 0.5×10^9/L following the nadir, and the first of seven consecutive days on which the patient's unsupported platelet transfusion showed a platelet count greater than 20×10^9/L.

Statistical analysis

We used the Student t-test to evaluate pre-mobilization patient variables and their influence on PBSC collections. The variables studied were: mobilization method, age, sex, diagnosis, remission or relapse status at transplant, presence of marrow disease, previous radiation therapy and prior number of chemotherapy cycles.

Probabilities of achieving neutrophil and platelet counts of 0.5×10^9/L and 20×10^9/L respectively, were calculated and compared using the Kaplan-Meier method.[11] The effect of variables that could potentially influence the *tempo* of engraftment was examined by multivariate analysis using Cox regression models.[12] The variables included patient diagnosis, remission or relapse status, marrow disease, prior radiation and chemotherapy cycles, mobilization method, conditioning regimens (CTCb used in breast cancer vs the other regimens used in hematologic malignancies), number of $CD34^+$ cells infused, $CD34^+CD33^-$ subsets, CFU-GM, MNC, and hematovariables with a univariate p value less than 0.05 were added Backward-Stepwise at the model. To ascertain which cell dose of $CD34^+$ cells per kilogram predicts for rapid or slow recovery, we calculated threshold values of $CD34^+$ and $CD34^+CD33^-$ cells/kg by using the *receiver operating characteristic* (ROC) methodology for clinical decision making.[13] We defined a rapid recovery when the patients recovered neutrophils $>0.5\times10^9$/L and platelets $>20\times10^9$/L before or on 11th day after infusion. On the contrary, we defined slow recovery when the patients recovered the same values after the 11th day.

Results

Factors that influence PBSC collection

The PBSC collection data of the initial mobilization are listed in Table 3. There was no difference in number of apheresis between the 2 types of mobilization. The yield of collected $CD34^+$ cells $CD34^+CD33^-$ subsets and CFU-GM in patients mobilized with G-CSF alone was inferior to patients who received CT plus G-CSF for mobilization. However, the MNC collected per day were higher in patients with G-CSF mobilization. Nine patients needed a subsequent mobilization procedure to obtain the target value of MNC, $CD34^+$ cells or CFU-GM.

The influence of patient characteristics on PBSC collection is showed in Table 4. CT plus G-CSF mobilization and fewer cycles of chemotherapy before mobilization were favorable features for the collection of higher numbers of $CD34^+$ cells. Absence of prior radiation therapy, remission disease status and mobilization with G-CSF alone were favorable features for harvesting a higher number of MNC per day. Mobilization with CT plus G-CSF was also associated to a higher number of $CD34^+CD33^-$ cells and CFU-GM collected than G-CSF alone.

Factors that influence engraftment kinetics

With regard to engraftment kinetics, patients who received chemotherapy plus G-CSF had a quicker neutrophil and platelet recovery than patients who

Table 4. Influence of mobilization and patient characteristics on PBSC collection.

	$CD34^+$ cells$\times10^6$/kg			$CD34^+CD33^-$ cells$\times10^6$/kg			CFU-GM$\times10^4$/kg			CMN$\times10^8$/kg		
	n	*mean±SE*	*p*	*n*	*mean±SE*	*p*	*n*	*mean±SE*	*p*	*n*	*mean±SE*	*p*
Mobilization												
CT+G-CSF	43	3.5±0.61	< 0.001	30	1.5±0.48	< 0.004	40	21.3±2.7	< 0.01	43	0.9±0.1	< 0.02
G-CSF	21	1.4±0.16		20	0.6±0.93		21	15.3±1.6		21	1.8±0.2	
Prior CT												
< 2	44	3.6±0.57	< 0.01	34	1.4±0.38	NS	22	14.7±2.8	NS	33	0.9±0.1	NS
> 2	33	1.4±0.37		19	0.7±0.38		42	23.1±2.7		44	1.4±0.2	
Prior radiation												
Yes	17	2.2±0.52	NS	14	1.3±0.52	NS	17	22.7±3.6	NS	17	0.6±0.1	< 0.003
No	37	2.9±0.61		28	0.9±0.23		35	17.7±2.9		37	1.5±0.2	
Disease status*												
Remission	49	2.9±0.46	NS	38	0.9±0.16	NS	47	20.7±2.4	NS	49	1.4±0.1	< 0.002
Relapse	15	2.2±1.4		12	1.9±1.1		14	14.6±3.2		15	0.7±0.1	

Disease status at transplant. Abbreviations: NS=not significant; CT=chemotherapy; SE= standard error.

Table 5. Engraftment kinetics.

	All patients		*G-CSF mobilized*		*Chemotherapy + G-CSF mobilized*		
Variable	*Mean*	*Range*	*Mean*	*Range*	*Mean*	*Range*	*p*
N. of patients	67		24		43		
Days to ANC >0.5$\times10^9$/L	11	7-39	14	10-39	11	7-22	< 0.005
Day of platelet independence	12	3-28	15	9-24	11	3-38	< 0.02

Abbreviations: ANC=absolute neutrophil count.

The analysis of the factors that influenced engraftment kinetics by means of univariate regression showed that absolute number of $CD34^+$ cells and $CD34^+CD33^-$ cells reinfused, mobilization schedule used in PBSC collection, and chemotherapy conditioning regimen, were significantly related with a rapid neutrophil and platelet recovery. Large number of infused CFU-GM produced a significant early neurecovery 9 days (8-28) versus 12 days (8-24), p<0.001 (Figure 2). Eighty-eight per cent of patients who received more than 0.86$\times10^6$/kg $CD34^+CD33^-$ cells showed shorter time to neutrophil and platelet engraftment, whereas only 45% who received less than these number of cells had recovered within 11 days.

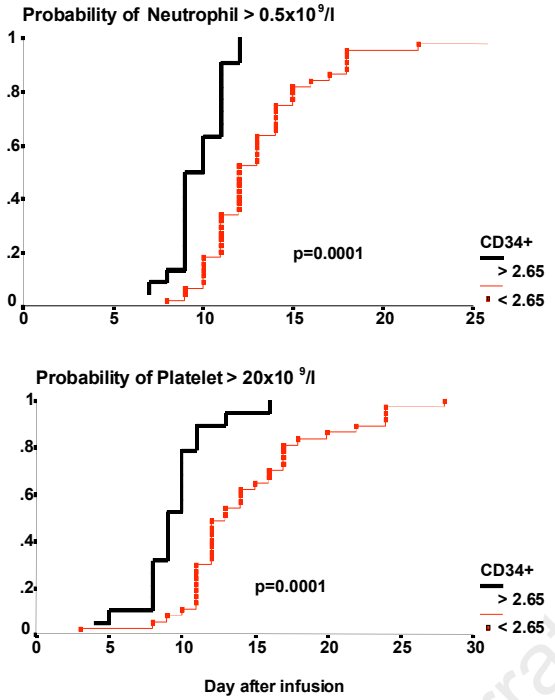
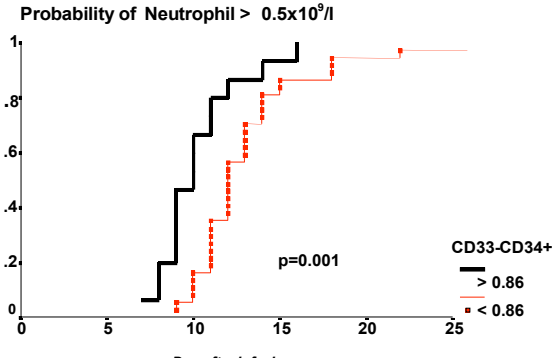


Figure 1. Kaplan-Meier probability of achieving neutrophil and platelet independence separated by CD34⁺ (×10⁶/kg) cell doses infused.



In the Cox multivariate analysis, the predictive parameters for rapid neutrophil recovery were the number of CD34⁺ that lacked expression of the CD33 antigen and the conditioning regimen. With regard to platelet engraftment, only the total number of CD34⁺ cells was predictive for rapid recovery (Table 6). The use of post-transplant G-CSF did not show any significant influence on engraftment kinetics of neutrophils and platelets. But, if we consider only the patients who were transplanted with suboptimal dose of CD34⁺ and CD34⁺CD33⁻ cells we observe a significant difference in terms of neutrophil recovery between the group who received post transplant G-CSF and those who did not receive it (Figures 3 and 4). We did not observe any effect of G-CSF on platelets recovery with regard the dose of cells infused.

Discussion

The utilization of autologous PBSC for hematopoietic transplantation has increased during the past years. Advantages over the bone marrow are well know and include shorter time to recovery, easy collection, and supposedly, less chance of tumoral contamination.[14] However, the timing to harvest the PBSC remains a matter of controversy and repeated leukapheresis are often required to obtain the sufficient amount of cells to proceed to transplantation. On the other hand, the minimal cell dose for a rapid and sustained recovery remains to be established. Several approaches have been used to calculate the dose of cells necessary for a successful grafting. Some authors predicted the harvest of CD34⁺ cells according to the concentration of these cells in the peripheral blood before leukapheresis.[17,18] Timing based on CFU-GM assays are not practical due the length of time required for results. Similarly, the most employed targets for adequacy of graft were the number of MNC,[7] the number of CFU-GM,[6] and more recently, the content of CD34⁺ cells.[8] Some studies indicated that the minimum number of progenitor cells necessary for a successful transplantation ranges between 3 and 5×10⁶ CD34⁺ cells/kg.[8,15,16]

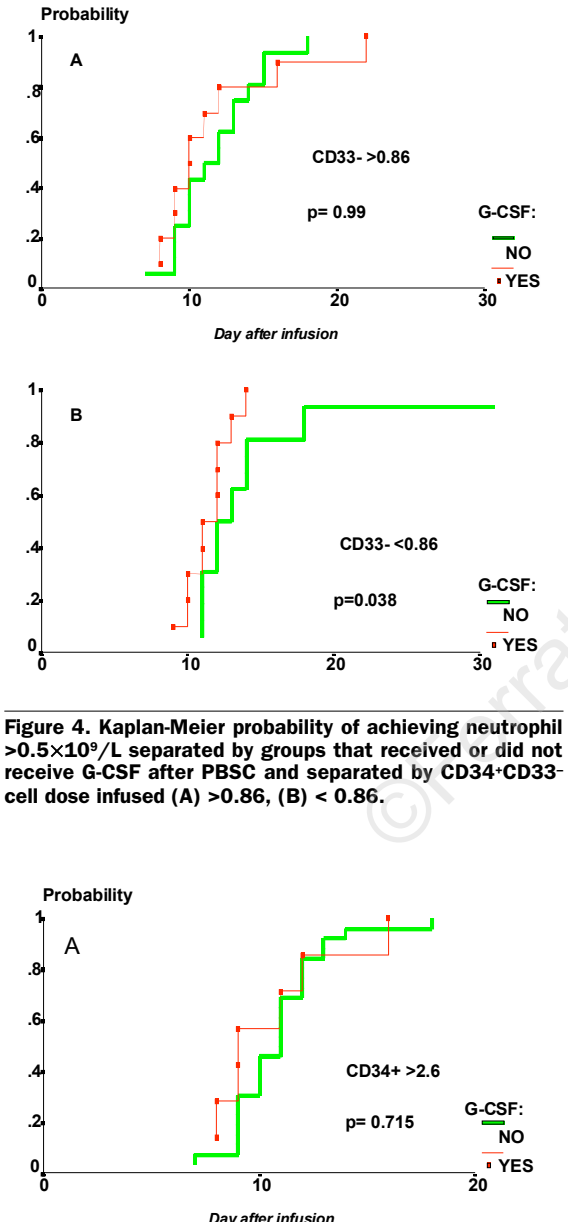


Figure 4. Kaplan-Meier probability of achieving neutrophil >0.5×10⁹/L separated by groups that received or did not receive G-CSF after PBSC and separated by $CD34^+CD33^-$ cell dose infused (A) >0.86, (B) < 0.86.

related with rapid platelet recovery. One can speculate in the hierarchy of hematopoietic cells, and their relationship on the recovery of hematopoiesis. The $CD34^+$ population probably includes different lineages with a mixture between immature stem cells and more differentiated ones. We can suppose that the $CD34^+CD33^-$ cells represent a more immature population. In a recent study, the number of $CD34^+CD33^-$ cells correlated better with neutrophil recovery than the total number of $CD34^+$ cells.[20] Although our data also support this finding, we cannot draw any explanation for this. The importance of the $CD34^+$ cell subsets in defining the hematopoietic recovery merits further investigation.

An important remark in the present study is the ROC methodology employed to calculate the threshold value for the $CD34^+$ and $CD34^+33^-$ cell content of graft. The minimum threshold value obtained in our study was 2.65×10^6/kg for $CD34^+$ cells and 0.86×10^6/kg for $CD34^+33^-$ cells. These data will help us to determine the minimal dose for a rapid engraftment. These issues assume greater importance in the context of heavily treated patients in whom we can expect an exhaust marrow. For this reason it is appropriate to provide specific recommendations on minimum numbers of $CD34^+$ cells. However, due to the variability of cell counts between different laboratories, each team must calculate its minimal dose for a successful transplant.

Although the utilization of post-transplant G-CSF, as suggested in this work, would be useful only in those patients infused with low numbers of hematopoietic precursors, we must await the completion of the underway randomized study to extract definitive conclusions. Patients infused with large number of PBSC probably did not need the help of growth factors for a quick recovery.

Contributions and Acknowledgments

AS formulated the design of this study and carried out the statistical analysis. JBa, AN and MMo were involved in the clinical assessment of patients. NM and PM developed and

Disclosures

Conflict of interest:

Redundant publications: no substantial overlapping with previous papers.

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