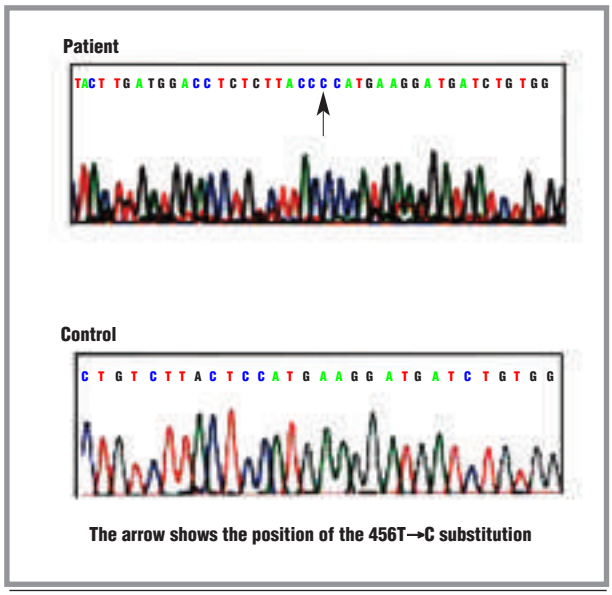

Figure 2. Electropherogram of exon 3 of the GPIIIa gene from the patient and from a control.

although it has been reported that Ser121Ala[10] affected neither αIIβ3 expression nor the ability of the heterodimer to interact with the complex specific monoclonal antibody. Although we could not do expression studies of the mutated integrin on CHO cells, the presence of this mutation in the parents of the patient and the plausibility of it interfering with platelet function makes it a genuine candidate for this important abnormality. In conclusion the patient appeared to carry a homozygous mutation in the GPIIIa gene, which may be responsible for her variant GT-like phenotype. The mutation described here will help further understanding of the GPIIb-IIIa structure and functions and GPIIb-IIIa.

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Stem Cell Transplantation

Basal CD34+ cell count predicts peripheral blood progenitor cell mobilization and collection in healthy donors after administration of granulocyte colony-stimulating factor

We analyzed factors predicting CD34+ cell mobilization and collection after granulocyte colony-stimulating factor (G-CSF) administration in 47 healthy donors. Basal CD34+ cell count and sex were the two variables that significantly predicted a better CD34+ cell mobilization, and greater age was the only variable associated with lower CD34+ cell yields.

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Despite the extensive use of granulocyte colony-stimulating factor (G-CSF) in healthy donors for peripheral blood progenitor cell (PBPC) mobilization, there is still a lack of consistent pre-mobilization variables that accurately predict a donor's response to this cytokine.[1-4] Among such variables, the number of CD34+ cells circulating in steady state, i.e., before G-CSF administration, has only rarely been reported.[5] The purpose of this study was to try to identify clinically significant factors that could influence the effectiveness of CD34+ cell mobilization and collection with special focus on the value of the basal CD34+ cell count in 47 healthy first-time donors from 12 centers undergoing PBPC mobilization and collection. Donors received G-CSF subcutaneously at a median (range) dose of 12 (10-22) μg/kg per day in two separated doses. PBPC collections were started on day five, i.e., after four days of filgrastim in every donor. The median blood volume processed was three times the donor's total blood volume and 20 donors (43%) underwent large volume leukapheresis (LVL) (Table 1). Three different determinations of CD34+ cells were done in each donor: first, baseline CD34+ cell count (before G-CSF administration); second, enumeration of CD34+ cells in peripheral blood on the morning of collection (after G-CSF); finally the number of CD34+ cells in the apheresis bag (CD34+ cells collected). Enumeration of CD34+ hematopoietic cells was performed by a single platform method based on the ISHAGE protocol.[6] The total number of CD34+ cells/mL in peripheral blood on the first day of apheresis (after G-CSF administration) was used to evaluate the effectiveness of mobilization. This variable was examined separately by linear regression analysis against independent variables (sex, age, weight, dose of G-CSF, baseline white cell count, and baseline CD34+ cell count). A p level <0.05 was considered sta-

Table 1. Donors' characteristics.

	No.	*Median (range)*
Age (years)		44 (4-74)
Sex		
Male/female	22/25	
Baseline CBC		
WBC (×10⁹/L)		6.3 (4.3-17.2)
Hb (g/dL)		14.1 (10.2-17.6)
Platelets (×10⁹/L)		248 (134-359)
Baseline CD34+ (cells/μL)		2.25 (0.34-7.12)
G-CSF		
Dose (μg/kg)		12 (10-22)
Days		4 (4-6)
≤12/>12 (μg/kg/day)	26/21	
Body weight (kg)		66.5 (22-110)
Apheresis		1 (1-2)
Blood volume processed (L)		12.3 (4.39-31.68)
Required CVA	4	

CBC: complete blood count; WBC: white blood cell count; CVA: central venous access

Table 2. Factors affecting PBPC mobilization.

Univariate analysis		
Variable	*r*	*p*
Baseline CD34+ cell count	0.30	0.04
Sex	0.23	0.11
Age	-0,27	0.06
Weight	0.08	0.58
Baseline WBC count	0.29	0.05
G-CSF dose	0.09	0.55
G-CSF ≤12 vs. >12 μg/kg/day	0.13	0.38
Multivariate analysis		
Baseline CD34⁺ cell count	0.4	0.012
Sex	0.3	0.03

**Data available in 42 donors.*

tistically significant. In a second step, logistic regression analysis was done to determine the variables influencing the number of CD34⁺ cells collected/kg of donor body weight (<4 vs. ≥4×10⁶ CD34⁺ cells/kg). The independent variables analyzed in this step were the same as those in the previous one with the addition of the volume of blood processed (normal vs. LVL). Again a value <0.05 was considered statistically significant. As concerns mobilization of CD34⁺ cells, the median number of CD34⁺ cells in peripheral blood on the day of the apheresis was 78/μL (range, 5-189). By multivariate analysis, baseline CD34⁺ cell count (p=0.012) and female sex (p=0.03) were the only two variables that correlated with the number of CD34⁺ cells in blood the day of apheresis (R of the model = 0.42) (Table 2). The median number of CD34⁺ cells collected in the whole series was 5.73×10⁶/kg (range, 0.73-17.29) and a CD34⁺ cell count >4×10⁶/kg was obtained in 27 cases. The donor's age was the only variable that significantly correlated with the number of CD34⁺ cells/kg collected (p=0.019), elderly donors being less likely to yield >4×10⁶ CD34⁺ cells/kg (β coefficient for age = -0.056). Our study confirms that some donors are poor responders to G-CSF and that the baseline number of CD34⁺ cells correlated with the number of CD34⁺ cells in blood the day of apheresis. Thus, the number of CD34⁺ cells circulating in peripheral blood in steady state can be used as a useful indicator of CD34⁺ cell mobilization after G-CSF administration, confirming previous results,[5] although a definitive explanation for this finding needs further investigation.

When considering a target dose of 4×10⁶ CD34⁺ cells/kg, the donor's age was found to be the only significant predictive factor for the apheresis yield of CD34⁺ cells in multivariate analysis, showing an age-related decline in CD34⁺ numbers. This is in keeping with data reported recently by our group and with those from studies on the kinetics of CD34⁺ cell collection reported in the literature.[2,4,7-9] The reasons why baseline CD34⁺ cell count predicts mobilization of CD34⁺ cells into peripheral blood but not the final number of progenitors collected are unclear. In normal donors, the correlation between pre-apheresis CD34⁺ cell count and the total harvested CD34⁺ cell count has been reported as weak or moderate, a fact that may explain this finding.[10,11] It seems as if PBPC mobilization and collection could be a two-step process, with variables affecting one of the steps having less influence on the other. Despite this, monitoring the number of steady-state circulating CD34⁺ cells to predict mobilization could be especially useful in older donors.

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The following institutions enrolled donors in the present study: Barcelona, Hospital Clínic (Dr. P. Marín); Barcelona, H. de Sant Pau (Dr. C. Martínez); Barcelona, H. Germans Trias i Pujol (Dr. J.M. Ribera); Barcelona, H. Valle de Hebrón (Dr. J. Zuazu); Madrid, H. La Princesa (Dr. A. Alegre); Madrid, H. Ramón y Cajal (Dr. C. Zamora); Madrid, H. Niño Jesús (Dr. M.A. Díaz); Málaga, H. Carlos Haya (Dr. M.J. Pascual); Palma de Mallorca, H. Universitario Son Dureta (Dr. A.Galmés); Salamanca, H. Clínico (Dr. C. Cañizo); Santander, Banco de Sangre de Cantabria (Dr. A. Insunza); Valencia, Hospital Universitario La Fe (Dr. J. de la Rubia); Valencia, Hospital Clínico (Dr. C. Arbona).

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Key words: CD34⁺ cell count, mobilization, G-CSF, prediction.

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Stem cell transplantation

Successful peripheral blood stem cell harvesting with granulocyte colony-stimulating factor alone after previous mobilization failure

A total of 138 patients whose stem cell mobilization failed following chemotherapy and granulocyte colony--stimulating factor (G-CSF) at a dose of 5 μg/kg/d were given a higher dose of G-CSF (10 μg/kg/d) for 5 days after a 7-day resting period. Stem cell mobilization was successful in 90 patients, who yielded a median of 3.5×10⁶ CD34+ cells/kg, partially successful in 17 patients (1-2.4×10⁶ CD34+ cells/kg) and failed in the remaining 31 patients.

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Autologous peripheral blood stem cells (PBSC) are currently mobilized by administering either chemotherapy (CT) plus granulocyte colony-stimulating factor G-CSF or G-CSF alone.[1,2] The most important problem, occurring in 10%-20% of cases, especially in heavily pretreated patients, is failure to obtain a sufficient number of CD34+ cells after apheresis.[2-4] In 1995, at the 37th annual meeting of the American Society of Hematology, we presented the first successful regimen for PBSC harvesting rescue with G-CSF alone (10 μg/kg/d) a few days after mobilization by CT plus G-CSF had failed.[5] Here we report further results from a large cohort of patients which confirm the relevance and safety of this procedure.

From 1997 to 2002, 1292 patients, referred to St-Louis and Necker hospitals, received CT plus G-CSF (5 μg/kg/d) in order to mobilize PBSC. The peripheral blood (PB) CD34+ cells were quantified when the white blood cell (WBC) count had recovered to 1000/μL after CT. Leukapheresis was initiated when the PB CD34+ cell count reached 10/μL. If PB CD34+ counts remained negative (<10 CD34+ cells/μL) for 6 consecutive days despite continued administration of G-CSF and a high level of WBC (> 20,000/μL), the PBSC harvest was not performed. Successful mobilization was defined by a harvest of at least 2.5×10⁶ CD34+ cells/kg. Partially successful mobilization was defined by a harvest of between 1 and 2.4×10⁶ CD34+ cells/kg. Failure was defined by three consecutive negative PB CD34+ cell counts or by a harvest of fewer than 1×10⁶ CD34+ cells/kg.

PBSC mobilization was unsuccessful in 138 patients (Table 1). After a 7-day resting period, a once daily subcutaneous administration of G-CSF at the dose of 10 μg/kg/d for 5 to 6 days was offered to all these patients. On the fifth and the sixth days of G-CSF administration, PB CD34+ cells were counted. If the count exceeded 10/μL, leukapheresis was initiated on the fifth or sixth day. Cumulative chemotherapy-induced toxicity to the bone marrow was calculated for each patient using a scoring system devised by Drake *et al.*[6] Data were compared using the χ^2 test or Fisher's exact test when indicated. Paired sample data were compared using Wilcoxon's signed rank test. Mobilization was successful in 90 patients (65.2%) from whom a median of 3.5×10⁶ CD34+ cells/kg (range 2.5 to 28) were harvested with a median of 2 leukaphereses (range, 1-4). Mobi-

Table 1. Patients' characteristics.

N. of patients	138
Sex (male/female)	68/70
Median age, years (range)	49 (16-68)
Diagnosis, N. of patients	
Non-Hodgkin's lymphoma	48
Hodgkin's disease	16
Multiple myeloma	28
Acute leukemia	27
Solid tumor	19
Previous therapy scoring system*	
Median Pre PBSC harvesting score (range)	50 (0-270)
Median n. of drugs used (range)	3 (0-9)
Median n. of drug exposure to toxicity factor 4 (range)	0 (2-27)
Median n. of cycles of chemotherapy (range)	6 (0-64)
Previous extensive radiotherapy (n. of patients)	21
Median time elapsed between last cycle of CT prior to the mobilization regimen by CT plus G-CSF (months, range)	0 (0-48)
Mobilization regimen	
Cyclophosphamide (120 mg/kg) + G-CSF (5 μg/kg/d)	79
DHAP + G-CSF (5 μg/kg/d)	9
CHOP-regimen + G-CSF (5 μg/kg/d)	10
Anthracycline + Arac + G-CSF (5 μg/kg/d)	26
Others + G-CSF (5 μg/kg/d)	14

**Chemotherapy scoring system by Drake et al.;[6] CT: chemotherapy.*