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Challenge to Predict Mobilized Peripheral Blood Stem Cells on the Fourth Day of Granulocyte Colony-Stimulating Factor Treatment in Healthy Donors: Predictive Value of Basal CD34^{+} Cell and Platelet Counts

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A longitudinal, prospective, observational, single-center cohort study on healthy donors was designed to identify predictors of CD34^{+} cell mobilization on day 4 after granulocyte colony-stimulating factor (G-CSF) administration. As potential predictors of mobilization, age, sex, body weight, height, blood volume, WBC count, peripheral blood (PB) mononuclear cell count, platelet (Plt) count, and hematocrit and hemoglobin levels were considered. Two different evaluations of CD34^{+} cell counts were determined for each donor: baseline (before G-CSF administration) and in PB on day 4 after G-CSF administration. One hundred twenty-two consecutive healthy donors with a median age of 47.5 years were enrolled. The median value of CD34^{+} on day 4 was 43 cells/μL (interquartile range, 23 to 68), and 81.1% of donors had $\geq$20 cells/μL. Basal WBC count, Plt count, and CD34^{+} were significantly higher for the subjects with CD34^{+} levels over median values on day 4. A multivariate quartile regression analysis, adjusted by sex, age, basal CD34^{+}, and basal Plt count, showed a progressively stronger relationship between baseline CD34^{+} and Plt levels and the CD34^{+} levels on day 4. The basal CD34^{+} cut-off level to predict the levels of CD34^{+} on day 4 was either $\leq$2 cells/μL or $\geq$3 cells/μL and that of basal Plt count was $\leq$229 $\times$ 10^{9}/L or $\geq$230 $\times$ 10^{9}/L, respectively, to determine whether mobilization therapy should or should not be attempted. PB stem cell mobilization with G-CSF was highly effective on day 4, and herein we describe a model for predicting the probability of performing PB stem cell collection after a short course of G-CSF.

INTRODUCTION

In recent years the number of allogeneic stem cell transplantations (AlloSCTs) has progressively increased worldwide [1,2]. Mobilized peripheral blood (PB) stem cells (PBSCs), compared with bone marrow grafts, have a higher stem cell content, lead to faster engraftment [3,4], and are more convenient for collection. As a consequence, the use of PBSC grafts has significantly increased. Initially, there were concerns about mobilization in the allogeneic setting due to normal donor toxicity [5,6] and the risk for graft-versus-host disease in the recipient [7], but studies have demonstrated granulocyte colony-stimulating factor (G-CSF) to be well tolerated by healthy donors (HDs) without any effects on long-term survival [8].

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Filgrastim and lenograstim are growth factors of choice for mobilizing PBSCs in HDs [9]. Recent evidence supports the safety and efficacy of G-CSF biosimilars for PBSC mobilization in the AlloSCT setting [10,11]. The recommended G-CSF dose is 10 mg/kg/body weight (BW) daily [12], by subcutaneous injection, in daily or twice-daily dosing [13]. Many donor factors such as age, sex, weight, body mass index, and blood cell counts have been reported as factors influencing mobilization performance [14-18].

Most centers start the collection of PBSCs on the fifth day after administration based on well-known $CD34^+$ cell kinetic studies, indicating that the maximal increase in PB $CD34^+$ cell levels is achieved after 5 days of G-CSF administration [19,20]. Nevertheless, several studies have reported that an adequate number of $CD34^+$ cells may be collected on day 4 of G-CSF treatment [21-25] and that the proportion of the more immature ($CD34^+$/$CD38^-$) subpopulation is greater than that observed after a longer administration of G-CSF [26]. Thus, we can avoid 1 more day of G-CSF exposure and its potential side effects. In consideration of the above, monitoring of $CD34^+$ cell counts would help to tailor the beginning of the collection procedure, with the first PBSC collection performed in HDs exhibiting at least 20×10^6/L $CD34^+$ cells in the PB.

Recently, our clinical study described a graphic algorithm (independent of age, sex, blood volume [BV], and BW) by which it is possible to predict $CD34^+$ mobilization on day 5 using baseline $CD34^+$ values [27]. Herein we designed a longitudinal, prospective, observational, single-center cohort study aimed at testing whether the baseline PB $CD34^+$ cell count is also able to predict the effectiveness of allogeneic PBSC mobilization as soon as day 4.

METHODS

The trial was performed in accordance with the ethical guidelines of our institution, and informed consent was obtained from each participant. This study was a longitudinal, prospective, observational, single-center cohort study. One hundred twenty-two consecutive siblings, white HDs, were referred to the Transplant Program of the Grande Ospedale Metropolitano "Bianchi-Melacrino-Morelli" of Reggio Calabria (Italy), in which 111 (91%) were from the cohort of the previous study [27] and 11 (9%) were new cases. HDs received a subcutaneous injection of G-CSF, lenograstim, once daily in the morning at a dose of 10 μg/kg BW. The day of pretreatment evaluation and the first day of G-CSF administration were conventionally considered as day 0 and day 1, respectively. Prophylaxis with paracetamol was administered to prevent potential side effects of G-CSF.

Somatic characteristics (sex, age, BW, height), estimates of total BV, and basal complete blood count from all HDs were collected. BV was calculated using the Nadler method [28], based on a person's height, BW, and sex. Complete blood count consisted of WBC count, PB mononuclear cell count, hematocrit, platelet (Plt) count, hemoglobin, and basal $CD34^+$ cell concentrations. Complete blood count was carried out no later than 1 week before starting G-CSF. PB mononuclear cells were defined as lymphocytes plus monocyte cells. Two different determinations of $CD34^+$ cells were obtained from each donor: baseline (before G-CSF administration) and in PB on the morning of day 4 (after G-CSF administration).

Moreover, the evaluation of the HDs included a detailed medical history, physical assessment, electrocardiogram and echocardiography, ultrasound examination of the upper abdomen, clinical chemistry, urinalysis, infectious disease markers, thrombophilia screening, ABO, Rh typing, and a pregnancy test in women of childbearing age (urine or serum). To mobilize PBSCs, HDs had to meet the following criteria: not on treatment with acetylsalicylic acid or antiaggregating agents, anticoagulants, angiotensin-converting enzyme inhibitors, or lithium; no splenomegaly; negative personal history of coagulation disorders or history of iritis or active autoimmune diseases; no chronic cardiovascular and respiratory disease; not a carrier of the sickle cell trait; and not currently pregnant or breastfeeding. Written informed consent of donors was obtained after a detailed description of the potential side effects and risks of G-CSF.

Immunophenotyping Analysis

Circulating $CD34^+$ cell concentrations in PB were determined by flow cytometry according to the International Society for Hematotherapy and Graft Engineering single-platform method [29]. Single-platform, microbead-based cell counting procedures were also accomplished using either flow count beads (Beckman Coulter, Miami, FL) or TruCount tubes (BD Biosciences, San Jose, CA). Absolute $CD34^+$ cell levels were calculated by dividing the total number of validated relevant cell events by the number of microbead events; this value was then multiplied by the known concentration of microbeads. The percentage of $CD34^+$ cells was obtained using logical gate interpolating nucleated WBCs and CD45 positive-to-dimly positive events as the cell denominator. Ungated events were acquired up to a minimum of at least 500 $CD34^+$ events, and up to at least 75,000 $CD45^+$ events were included in the sample list mode file or after 7 minutes had elapsed. In addition, nuclear staining with 7-aminoactinomycin D was used to exclude dead or apoptotic cells [30]. The laboratory subscribes to an external quality assessment service program (UK Neqas) [31] to exclude that different techniques influenced the results.

Endpoints

The primary endpoints of this study were to identify the relationship between baseline and day 4 $CD34^+$ cell concentrations and to assess the predictive value of baseline $CD34^+$ levels for $CD34^+$ mobilization on day 4, taking into account standard somatometric/demographic variables (sex and age) and baseline laboratory characteristics.

Statistical Analysis

Data are expressed as median and interquartile range or percent frequency, as appropriate. The Mann-Whitney test was used to identify variables associated with PBSC $CD34^+$ mobilization on day 4 after administration of G-CSF. To analyze the distribution of $CD34^+$ on day 4 by different levels of basal $CD34^+$, a graphic approach of cumulative distribution was used. To estimate the simultaneous effects of covariates significantly associated with cell mobilization on day 4, multivariate quantile regression models were adopted. In such models we always introduced age and sex, irrespective of their significant or nonsignificant association with the outcome variable.

The quantile model used was as follows:

$$q = q\,q + q, \quad \in 122; \ \in 4$$

where n is the sample size of 122, k is the number of independent variables considered, W is a vector containing n observations of CD34 on day 4 (the dependent variable), β is a vector containing coefficients to be estimated, ε is a classic error term, q is the specified quantile of CD34 at day 4, and X is a $122 \times K$ matrix of the independent variables.

To compare observed and predicted (ie, model-derived) estimates, a graphic approach plotting observed and predicted median values of $CD34^+$ levels on day 4 was used based on associations with median values of covariates (Plts and basal CD34 $P < .05$) resulting from the multivariate quantile regression. As a measure of uncertainty for each point estimate, the 95% confidence interval of the predicted median value was determined. Data were analyzed using STATA/IC 13.1 for Windows (Stata Corp., College Station, TX).

RESULTS

Table 1 shows the donor characteristics for the overall sample and for subjects divided according to the median $CD34^+$ value on day 4 (43 cells/μL; interquartile range, 23 to 68). Of note, 81.1% of donors had $\geq$20 cells/μL. Table 1 also shows that basal WBC counts, Plt counts, and $CD34^+$ levels were significantly higher for subjects with $CD34^+$ levels over median values on day 4.

In age- and sex-adjusted multivariate models we only included as additional covariates all variables that were significantly associated with $CD34^+$ counts on day 4. Thus, 2 multivariate quartile regression analyses were performed: the first adjusted by age, sex, basal $CD34^+$, and Plt count and the second by sex, age, basal $CD34^+$, and basal WBC count. In the first model (Table 2) both basal $CD34^+$ and basal Plt counts significantly predicted (P = .00 for both variables) the study outcome. In detail, this model shows a progressively stronger relationship between baseline $CD34^+$ and $CD34^+$ levels on day 4 (such an effect being significant from the median baseline quantile of $CD34^+$ onward). Remarkably, a relationship was also found with basal Plt levels, with this association being significant from the first quartile of basal Plt levels or greater. These results clearly indicated that, independently, basal $CD34^+$ and Plt levels were positively associated with $CD34^+$ on day 4 holding constant age and sex. An age- and sex-adjusted

Table 1
Donor Characteristics Stratified by CD34+ Median Values on Day 4

		CD34 at Day 4		
	All	Below Median	Above Median	*P*
Sex, % men	54.9	54.1	55.7	.86
Age, yr	47.5 (36-55)	52 (36-57)	43 (35-52)	.15
Weight, kg	72.5 (60-82)	71 (60-80)	75 (62-82)	.43
Height, m	1.7 (1.6-1.8)	1.7 (1.6-1.8)	1.7 (1.6-1.7)	.72
BV, L	5 (4.2-5.7)	4.9 (4.2-5.6)	5.2 (4.3-5.7)	.49
Baseline WBC count, $\times 10^9$/L	6.7 (5.4-7.6)	6.1 (5.4-7.1)	7 (6-8.1)	.005
Baseline hemoglobin, g/dL	14.2 (13.2-15.1)	14 (13-14.9)	14.6 (13.3-15.3)	.17
Baseline HCT, %	42.1 (39.4-44.8)	42 (39-44.2)	42.4 (40.2-45)	.55
Baseline platelets, $\times 10^9$/L	230 (202-257)	210 (190-234)	245 (221-271)	<.001
Baseline CD34+, cells/μL	2 (2-4)	2 (1-3)	3 (2-4)	<.001

Values are expressed in median (range). HCT indicate the hematocrit.

multivariate quartile regression analysis, including WBC count instead of Plt counts as a predictor, did not provide statistically significant results (P = .07) for median quantile values (Table 3).

Figures 1 and 2 show that the basal CD34+ cut-off levels able to predict CD34+ on day 4 were $\leq$2 cells/μL and $\geq$3 cells/μL, respectively, whereas CD34 levels associated with basal Plt levels were $\leq 229 \times 10^9$/L and $\geq 230 \times 10^9$/L, respectively. It is interesting to note that the observed median CD34+ values always fell within the 95% confidence interval of each point estimate for both predictors, indicating the observed and predicted values overlapped.

Figure 3A shows the univariate distribution of basal CD34+ values identified by the multivariate analysis using the cumulative distribution CD34+ on day 4. Specifically, 50% of donors with basal CD34+ $\leq$ 2 cells/μL on day 4 had no more than 30 cells/μL (first perpendicular line), whereas 50% of donors with basal values $\geq$ 3 cells/μL on day 4 had values of 56 cells/μL (second perpendicular line).

Figure 3B shows the univariate distribution of basal Plt values identified by multivariate analysis using the cumulative distribution of CD34+ on day 4. Specifically, 50% of donors with basal Plt counts $\leq 229 \times 10^9$/L on day 4 had no more than 27 cells/μL (first perpendicular line), whereas 50% of donors with basal values $\geq 230 \times 10^9$/L on day 4 showed values below 53 cells/μL (second perpendicular line).

DISCUSSION

Currently, mobilized PBSC are the preferred and major source of stem and progenitor cells harvested for AlloSCT because of the higher yield of these cells, faster engraftment, and decreased procedural risks compared with harvested bone marrow cells. However, G-CSF treatment, which is required to mobilize PBSCs, can lead to side effects such as bone and muscle pain, nausea, headaches, and febrile symptoms. More rare but severe complications of G-CSF include splenic rupture, myocardial infarction, pulmonary microembolism, and thrombotic events [32-35]. Mobilization failure is 1 of the most common problems observed during PBSC collection. The failure rate ranges between 2% and 5% in HDs. Many donor characteristics have been identified as possible predictors of good PBSC mobilization: age, sex, weight, body mass index, blood cell counts, and PBSC concentration before G-CSF administration. G-CSF dose, brand type (ie, lenograstim versus filgrastim), and administration schedule have also been reported as factors influencing mobilization performance. The purpose of this study was to identify clinically significant factors that could predict the effectiveness of CD34+ cell mobilization (and not collection) with special focus on the value of the basal CD34+. The factors we considered (such as weight, sex, age, WBCs, and Plts) are individual characteristics that cannot be changed.

The success of AlloSCT depends on harvesting an adequate number of cells and accurate prediction of when to commence apheresis. In the allogeneic setting, CD34+ cell count helps in predicting the yield of apheresis and therefore in tailoring the duration of the procedure. Donors may undergo the first collection on the fourth to fifth days of G-CSF administration, provided that at least 20×10^6/μL CD34+ cells are counted in the PB [12]. Commonly, the apheresis procedure is performed on day 5 after initiation of G-CSF administration because at that time the concentration of stem cells in the PB reaches a maximum [36].

Table 2
Multivariate Analysis of Basal CD34+ and Plt Levels Based on CD34+ Mobilization Levels on Day 4 after G-CSF Administration*

	Quantile regression		
	.25	.50	.75
Dependent variable: total CD34+/kg cell count on day 4			
Basal CD34+ levels	**1.1**	**6.6**	**9.8**
95% confidence interval	−6.2 to 10.3	1.2-12.12	1.9-17.7
P	.57	.02	.01
Basal Plt levels	.2	.2	.3
95% confidence interval	**.1-.3**	**.1-.3**	**.1-.4**
P	.00	.00	.01

Data are regression coefficients, 95% confidence intervals, and *P* values. Each regression coefficient indicates the increase for each quantile of total CD34+/kg cell count on day 4 for each unitary increase in basal CD34+ and Plt levels (see Statistical Analysis for further details).

* Model adjusted by age and sex

Table 3
Multivariate Analysis of Basal CD34+ and WBC Count Levels based on CD34+ Mobilization Levels on Day 4 after G-CSF Administration*

	Quantile Regression		
	.25	.50	.75
Dependent variable: total CD34+/kg cell count on day 4			
Basal CD34+ levels	.5	7.6	8.9
95% confidence interval	−6.2 to 7.2	3.7-11.4	2.0-15.8
P	.82	.00	.01
Basal WBC count levels	4.8	5.0	7.1
95% confidence interval	.0-9.4	−.4 to 10.4	2.2-12.6
P	.05	.07	.00

Each regression coefficient indicates how much increases in different quantiles the dependent variable (total CD34+/kg cell count on day 4) for each unitary increase in basal CD34+ and WBC levels (see Statistical Analysis for more detail).

* Model adjusted by age and sex.

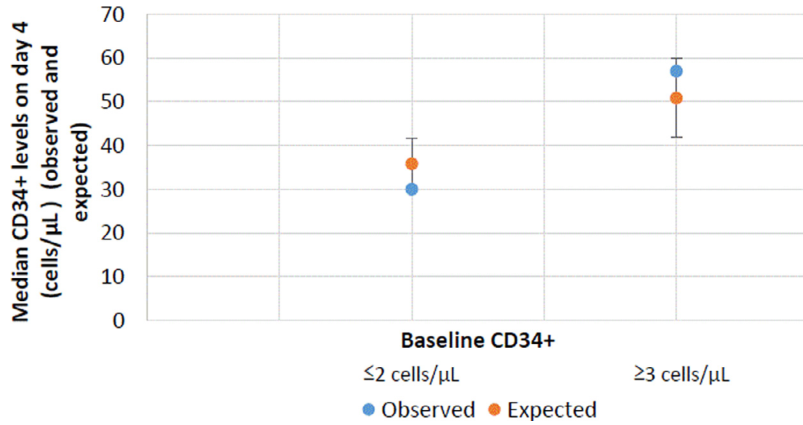

Figure 1. Scatter plot of observed and predicted median values of CD34+ levels on day 4 (with 95% confidence intervals on model data) of the baseline CD34+ level (≤2 versus ≥3 cells/μL) adjusted by age, sex, and baseline Plt levels.

Nevertheless, several studies have clearly demonstrated that PBSC mobilization with G-CSF is highly effective even on the fourth day of G-CSF. We showed that 81.1% of donors in our series had $20 \times 10^6/\mu$L CD34+ cells on the fourth day, and an early start of the collection would potentially avoid G-CSF–related side effects and complications.

Moreover, we present a model for predicting "good HDs mobilizers" on day 4. Only 2 variables, basal CD34+ and Plt counts before G-CSF administration, are required for evaluation in this model. All information relative to these variables can be readily collected, justifying the examination of the feasibility of this model in clinical practice. Correlations between each of these 2 variables and the CD34+ cell count on day 4 have never been reported previously. Our approach is unique in that we evaluated variables in combination and used the variables to build a model for predicting candidates who would be suitable for beginning apheresis after a short G-CSF treatment. This model makes it possible to determine the probability of achieving a sufficient number of CD34+ cells on day 4 and planning of collection. Doctors could also use this model to select the more suitable donors from among donor candidates for a particular recipient. Furthermore, the detection of poor mobilizers before G-CSF administration would help to identify candidates who should be indicated for a mobilizing agent other than G-CSF. The use of an additional mobilizing agent such as AMD3100 in predicted poor mobilizers would reduce the probability of failed PBSC collection from HDs.

Future studies will evaluate the combination of the 2 baseline predictors to have an estimation of CD34+ levels on day 4. Unfortunately, this is not possible at this stage because we need a larger sample size. We are scheduling a multicenter study to generate an online calculator to allow the combination of the 2 predictors and the estimation of CD34+ levels on day 4 as the output variable.

In a previous study [27] our group identified the relationship between baseline and day 5 CD34+ cell concentrations using different statistical approaches and assessed the value of baseline CD34+ levels for predicting CD34+ mobilization on day 5 compared with that provided by standard somatometric/demographic variables (sex, age, BW, BV, and height) and baseline laboratory characteristics. This study showed that baseline PB CD34+ cell levels predicted the effectiveness of allogeneic PBSC mobilization in HDs independently of age, sex, BV, and BW. Our algorithm could be used to maximize PBSC collection efficiency and minimize resource utilization because the precollection CD34 cell count remained the most accurate and reliable predictor of mobilization. The current study provides a graphic algorithm (independent of age, sex, BV, and BW) by

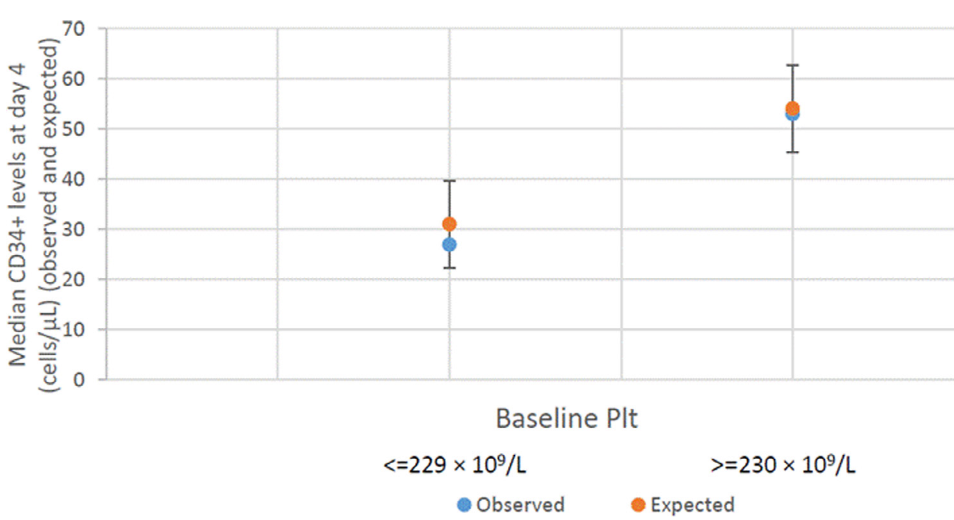

Figure 2. Scatter plot of observed and predicted median values of CD34+ cell counts on day 4 (with 95% confidence levels on model data) and basal Plt levels (≤229 versus ≥230×10^9/L) adjusted by age, sex, and baseline CD34+ levels (cells/μL).

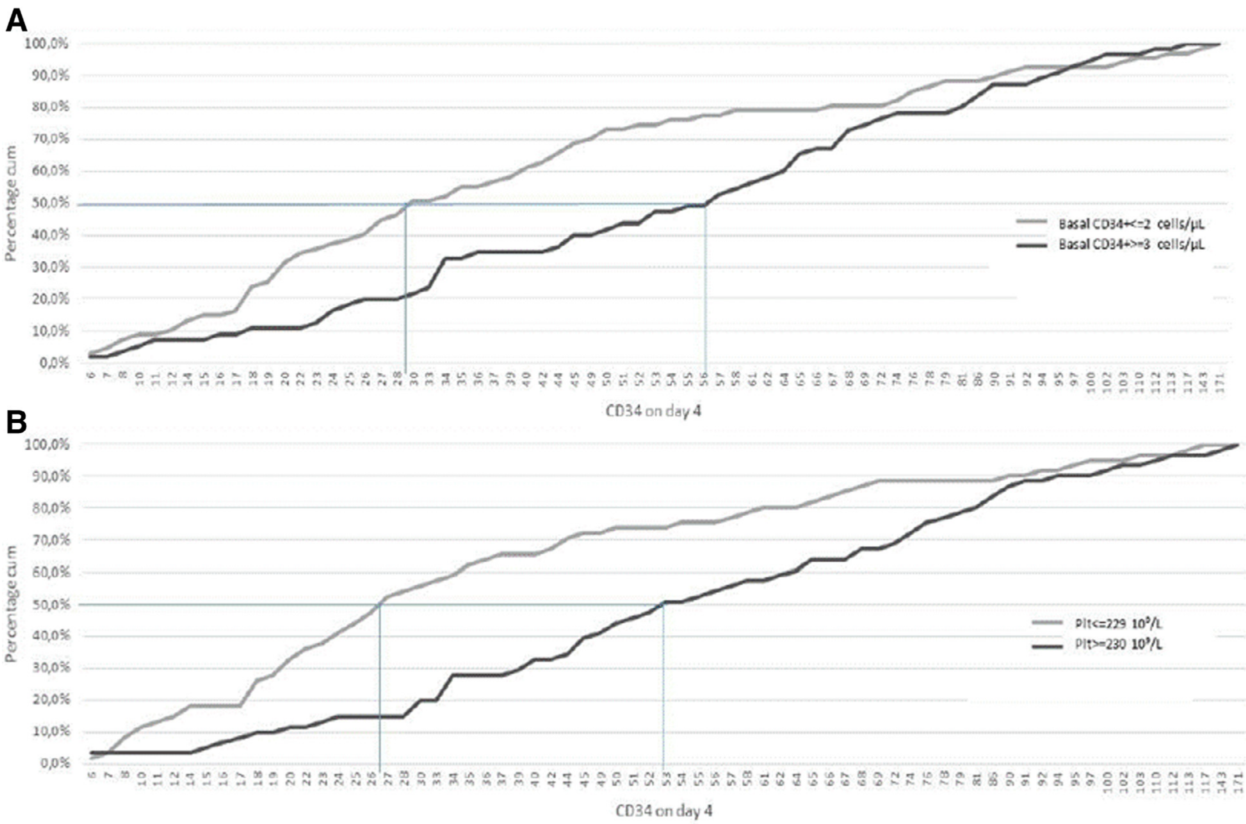

Figure 3. Cumulative distribution of CD34⁺ levels on day 4 based on baseline CD34⁺ (A) and platelets (B) categories.

which it is possible to predict CD34⁺ mobilization not only on day 5 but also on day 4 using baseline CD34⁺ values. Moreover, the algorithm appears to be more robust and more strongly correlated with the basal Plt count. The Plt count before PBSC mobilization has repeatedly been identified as a risk factor for poor mobilization in the autologous transplant setting, and the definition of a baseline Plt concentration [37,38] threshold may be used as a predictive factor of successful mobilization with plerixafor [39]. No such data exist for allogeneic donors.

Our algorithm is extremely easy to use because it relies only on baseline CD34⁺ values and the initial Plt count. In this way it is possible to select HDs who can undergo the apheresis procedure on day 4. It is possible to identify poor mobilizers who could potentially be switched to plerixafor in the allogeneic setting. It must be kept in mind that an important goal is good compliance of the HDs; thusm any medical procedure able to improve this compliance is to be pursued.

We believe the results of this study are interesting and could represent an instrument to be used in clinical practice. There are some limitations in the model developed in this study. First, the results are essentially based on laboratory tests that might suffer from variability in the patient and operator. However, the study results (mainly those reported in Figures 1 and 2) indicate that accuracy is substantially preserved (the observed values of CD34⁺ levels on day 4 fall within the 95% confidence interval of the predicted estimates), and this is also true for precision (the 95% confidence intervals are rather narrow). In a statistical sense, the 95% confidence interval means that if a series of identical studies were carried out repeatedly on different samples from the same population and a 95% confidence interval for the sample estimate is calculated in each study, then, in the long run, 95% of these confidence intervals would include the population value. Thus, the narrower the 95% confidence interval (as those we found in our study), the better the predicted estimate. In any case, the model was constructed using a relatively low sample size, and for this reason an external validation with a larger and more representative cohort is required to provide further support for the practical usefulness of this model. Such an external validation will allow the development of a multidimensional graphic or tabular algorithm through which clinicians can easily predict the degree of mobilized PBSCs on day 4 in HDs. Second, our study protocol did not contemplate a cost-effectiveness analysis, an issue that demands to be formally investigated in a future study.

In conclusion, G-CSF can be highly effective in HDs on day 4 to mobilize a sufficient number of stem cells. Herein we have developed a model for predicting the probability of performing PBSC collection after a short course of G-CSF.

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