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Single Apheresis Session on the 4th Day of Granulocyte Colony-Stimulating Factor Administration Seems Convenient to Collect Enough Peripheral Blood Stem Cells from Healthy Donors

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Keywords
Granulocyte colony-stimulating factor · Healthy donors · Peripheral blood stem cells · Very good mobilizer

Abstract

Background: To minimize adverse events of peripheral blood stem cell (PBSC) collection in healthy donors, it is reasonable to limit the total dose of granulocyte colony-stimulating factor (G-CSF) and/or the number of apheresis days without decreasing of PBSCs yield. Therefore, we have started to collect G-CSF induced PBSCs on day 4 instead of on day 5. So, we retrospectively aimed to investigate the results of this 4-day G-CSF administration. ***Study Design and Methods:*** Seventy-six healthy donors who performed on G-CSF induced PBSCs donation consecutively between January 2020 and July 2022 were included in this study. G-CSF (filgrastim) at 2 × 5 μg/kg/day subcutaneously was applied. Apheresis started on day 4. ***Results:*** Sixty-nine (90.8%) of 76 donors provided enough PBSCs on day 4 apheresis session. Younger age ($p = 0.004$), higher PB CD34+ cell count on the 4th day of G-CSF ($p < 0.001$), and male donor ($p = 0.010$) were correlated with increased amounts of PBSCs yield. Univariate and multivariate logistic regression analyses to predict very good mobilizers (collected PBSCs ≥8 × 10^6/kg after the first apheresis) were performed. In multivariate logistic regression analyses, male sex ($p = 0.004$), PB CD34+ cell count ≥100/μL on the 4th day of G-CSF ($p < 0.001$), and glomerular filtration rate ≥115 mL/min ($p = 0.031$) were found to be independent predicting factors to demonstrate very good mobilizer. ***Conclusion:*** It seems that starting the apheresis on the 4th day of G-CSF administration is effective and to provide minimal G-CSF exposure in healthy donors.

Introduction

The peripheral blood stem cells (PBSCs) are the most frequently used stem cell source nowadays in allogeneic hematopoietic stem cell transplantation (allo-HSCT). Granulocyte colony-stimulating factor (G-CSF) is used for stem cell mobilization in healthy donors, and then PBSCs are collected by apheresis. Although guidelines from the American Society of Blood and Marrow Transplantation (ASBMT) recommends beginning apheresis on the fifth day of G-CSF administration in allogeneic donors, the starting day of apheresis is debatable [1]. Collection centers generally start an apheresis

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Table 1. Schema of G-CSF administration

	Apheresis protocol					
	G-CSF starting day (day 0)	day 1	day 2	day 3	day 4 (first apheresis day)	day 5 (second apheresis day)*
Morning dose	No	Filgrastim 5 μg/kg	Filgrastim 5 μg/kg	Filgrastim 5 μg/kg	Filgrastim 5 μg/kg	Filgrastim 5 μg/kg*
Evening dose	Filgrastim 5 μg/kg	Filgrastim 5 μg/kg	Filgrastim 5 μg/kg	Filgrastim 5 μg/kg	Filgrastim 5 μg/kg*	No

*If enough PBSCs (≥4 × 10^6/kg) were not collected by single apheresis at day 4, it would be done.

procedure between 4 and 6 days of G-CSF administration for PBSCs collection [2].

Hypocalcemia-related problems, vasovagal reactions, thrombocytopenia are major apheresis complications, and rarely severe complications include severe hypertension and cardiac arrest [3–5]. G-CSF-related problems are bone pain, headache, and flu-like symptoms such as malaise, nausea, night sweats, rarely seen severe complications include splenic rupture and cardiovascular events [6–8].

To minimize adverse events of the PBSC collection, it is reasonable to limit the total dose of G-CSF and/or the number of apheresis days without decreasing of PBSCs yield. If possible, to collect enough PBSCs by single apheresis on the 4th day of G-CSF exposure would be an ideal target to protect healthy donors from procedure related complications [9–11]. Therefore, we have started to collect PBSCs on day 4 instead of on day 5 of split dose G-CSF administration from healthy donors for a while in our center, compatible with this strategy.

In this study, we retrospectively aimed to present the results of this new strategy. The primary objective of this study was to determine the allogeneic donors who provided enough collected CD34+ PBSCs per patient kilogram (× 106/kg) on the first day of apheresis (day 4 of G-CSF administration). The secondary objective was to find out the possible factors to impact the collection yield.

Methods

Donors and Mobilization Protocol

We included all donors (n = 76) who performed G-CSF induced PBSCs donation between January 2020 and July 2022 at the Apheresis Unit at Akdeniz University Hospital in this study. All donors were mobilized with G-CSF (filgrastim) at 2 × 5 μg/kg/day subcutaneously rounded to the nearest commercial vial dose (300 and 480 μg). G-CSF was administered at 08:00 a.m. in the morning and 08:00 p.m. in the evening as 2 split doses. Mobilization procedure was started with split evening dose of G-CSF on day 0. After the eighth split morning doses of G-CSF on day 4, the blood sample was taken for measuring peripheral blood (PB) CD34+ cell counts. According to our institutional policy, if PB CD34+ cell counts were >10/μL, the collection procedure started 2–4 h later than morning dose of G-CSF on day 4. If the harvested PB CD34+ cell yield was more than ≥4 × 10^6/kg, which is defined PBSCs count for optimal engraftment in allo-HSCT, the procedure was finished. If the harvested PB CD34+ cell target yield was <4 × 10^6/kg after the first apheresis, an additional 2 more doses of G-CSF were administered and a similar apheresis procedure performed on day 5, to achieve the target yield of PBSCs. The donors with the amount of collected PBSCs were ≥8 × 10^6/kg after the first apheresis was defined as a very good mobilizer (at least 2 times more than required PBSCs for optimal engraftment). The apheresis protocol applied to healthy donors that we mentioned above is presented in Table 1.

CD34+ Cell Count Enumeration by Flow Cytometry

CD34+ cell counts both from a donor and collected product were quantified according to ISHAGE protocol by using flow cytometer BD FACSCanto II and BD FACSCanto Software. BD Stem Cell Enumeration kit that consisted of CD45-fluorescein isothiocyanate (clone 2D1) antibody, CD34-phycoerythrin (clone 8G12) antibody, and 7-aminoactinomycin D viability dye was used in the process. Accuracy and sensitivity determination of process was performed by using two-level BD™ Stem Cell Control Kit.

Apheresis Procedure

When collecting PB CD34+ cells, Spectra Optia (Terumo, Blood Cell and Technologies, Lakewood, CO, USA) apheresis machines were used. A bilateral peripheral venous or central line was required for the collection. The donor's total blood volume was processed 2 times at between 50 and 60 mL per minute over 3–6 h. The procedure was finished after processing twice the donor's blood volume. The ratio 1 to 10 (1:10) Acid Citrate Dextrose A was used as an anticoagulant agent in the procedure. 10% calcium gluconate, 20–60 mL, was infused intravenously to the donors during the apheresis as well.

Statistical Analyses

All statistical analyses were performed using SPSS version 22.0 software (Armonk, NY, USA). Descriptive statistics were presented as numbers and percentages for categorical variables and mean ± standard deviation, median (minimum value–maximum value) for continuous variables. Normal distribution of continuous variables was assessed with visual (histograms and probability graphics) and analytic methods (Kolmogorov-Smirnov and Shapiro-Wilk's test). χ^2 tests were used for comparison of categorical variables in independent groups.

Median (min-max) values were presented for data without the normal distribution, and the Mann-Whitney U test was used for comparison analyses between the two groups. The data with normal distribution were presented by mean ± SD, and the independent sample t test was used for comparison analyses between the two groups as well. Among groups that were formed according

Table 2. Characteristics of donors

Variables	Total (n = 76)	Donors with collected adequate PBSCs after first apheresis (N = 69) (90.8%)	Donors with collected adequate PBSCs after second apheresis (N = 7) (9.2%)	p value
Sex, n (%)				0.424[a]
Male	46 (60.5)	43 (62.3)	3 (42.9)	
Female	30 (39.5)	26 (37.7)	4 (57.1)	
Age, years				**0.032**[b]
Median (min-max)	35 (15–68)	34 (15–68)	43 (29–61)	
PB CD34 positive cell count on 4th day of G-CSF administration before apheresis, /μL				<**0.001**[b]
Median (min-max)	94.5 (18–187)	104 (32–187)	44 (18–80)	
Hb, g/dL				0.518[b]
Median (min-max)	14.3 (10.3–17.2)	14.3 (10.3–17.2)	12.1 (10.8–16.4)	
Leukocyte, /μL				0.051[b]
Median (min-max)	46,275 (22,920–100,470)	49,500 (22,920–100,470)	39,390 (31,780–48,930)	
Neutrophil, /μL				0.093[b]
Median (min-max)	37,795 (17,520–86,470)	39,490 (17,520–86,470)	33,520 (26,130–39,870)	
Lymphocyte, /μL				0.161[b]
Median (min-max)	7,400 (2,190–15,100)	7,400 (2,190–15,100)	4,310 (2,950–9,500)	
Monocyte, /μL				0.120[b]
Median (min-max)	6,000 (1,830–13,000)	6,000 (1,830–13,000)	3,950 (2,310–13,000)	
Platelet, /μL				0.398[b]
Median (min-max)	223,500 (146,000–474,000)	224,000 (146,000–474,000)	219,000 (184,000–255,000)	
NLR				0.524[b]
Median (min-max)	5.9 (2.2–14.8)	5.8 (2.2–14.8)	8.4 (3.5–11.1)	
MLR				0.879[b]
Median (min-max)	0.82 (0.22–2.01)	0.82 (0.22–2.01)	0.79 (0.53–1.41)	
PLR				0.212[b]
Median (min-max)	33.7 (13.6–93.2)	33.7 (13.6–93.2)	52.7 (22.4–72.4)	
GFR, mL/min				0.156[b]
Median (min-max)	113.7 (67.3–135.8)	114.5 (67.3–135.9)	105.9 (92.9–116.6)	
CRP, mg/L				0.732[b]
Median (min-max)	1.1 (0.1–24.7)	1.1 (0.1–24.7)	1.1 (0.5–6.5)	
Patient weight, kg				
Median (min-max)	70 (8–110)			
Donor weight, kg				0.202[b]
Median (min-max)	76.5 (51–113)	78 (51–113)	70 (55–95)	
Donor BMI, kg/m^2				0.075[b]
Median (min-max)	26.2 (19.9–38.2)	26.5 (19.9–38.2)	24 (20.5–30)	
Donor BMI, kg/m^2				0.152[c]
18.5–24.9	29 (38.2)	24 (34.8)	5 (71.4)	
25–29.9	32 (42.1)	31 (44.9)	1 (14.3)	
>30	15 (19.7)	14 (20.3)	1 (14.3)	
The amount of PBSCs at first apheresis, ×10^6/kg				<**0.001**[b]
Median (min-max)	7.1 (1.5–26.4)	7.5 (4–26.4)	2.6 (1.5–3.7)	

Table 2 (continued)

Variables	Total (n = 76)	Donors with collected adequate PBSCs after first apheresis (N = 69) (90.8%)	Donors with collected adequate PBSCs after second apheresis (N = 7) (9.2%)	p value
The volume of PBSCs at first apheresis, mL				0.221[b]
Median (min-max)	280 (150–550)	280 (150–550)	300 (262–400)	
The amount of PBSCs at the end of apheresis procedures, $\times 10^6$/kg				**0.001**[b]
Median (min-max)	7.1 (4–26.4)	7.5 (4–26.4)	5 (4.5–6.7)	

G-CSF, granulocyte colony-stimulating factor; Hb, hemoglobin; NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; GFR, glomerular filtration rate; CRP, C-reactive protein; BMI, body mass index; PBSCs, peripheral blood stem cells. [a]Fisher exact χ^2 test. [b]Mann-Whitney U test. [c]Pearson χ^2 test.

to the amount of collected CD34+ PBSCs after the first apheresis ($\geq 8 \times 10^6$/kg vs. $<8 \times 10^6$/kg), whether there was a statistically significant cutoff value for the variables found to be significant in the comparison analyses, was evaluated by ROC analysis.

The ROC analyses were applied to determine the optimal predictive cutoff values for the amount of collected CD34+ PBSCs after the first apheresis. The area under the ROC curve (AUC) results were considered excellent for AUC values between 0.9–1, good for AUC values between 0.8–0.9, fair for AUC values between 0.7–0.8, poor for AUC values between 0.6–0.7 and failed for AUC values between 0.5–0.6 [12, 13]. Variables that may be independent association factors for the amount of collected CD34+ PBSCs after the first apheresis were evaluated by multivariate logistic regression analysis. Variables with $p < 0.05$ as determined by univariate analysis were entered into multivariate logistic regression analysis. $p < 0.05$ was considered to be statistically significant.

Results

Characteristics of PBSCs Donors

Over the study period, a total of 83 apheresis procedures were done with 76 healthy donors, of whom 30 (39.5%) and 46 (60.5%) were females and males, respectively. The median age of donors was 35 years old. The median hemoglobin (Hb), leukocyte, neutrophil, and thrombocyte count of donors before apheresis on the first collection day were 14.3 g/dL, 46,275/μL, 37,795/μL, and 223,500/μL, respectively. Moreover, neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio, and platelet-to-lymphocyte ratio, which were calculated according to the first collection day complete blood count before apheresis, were 5.9, 0.82 and 33.7, respectively.

The PB CD34+ cell measurements were a median of 94.5/μL (range 18–187) on the 4th day and 44/μL (range 15–98) on the 5th day of G-CSF administration. The median basal C-reactive protein was 1.1 mg/L. The median value of glomerular filtration rate (GFR), which was calculated automatically by the system for every individual according to serum creatinine, age, and sex, was 114.2 mL/min. The median weight of patients who would be infused PBSCs was 70 kg, and the median weight of donors was 76.5 kg. Donors were divided into 3 subgroups according to body mass index (BMI); 29 (38.2%) donors were in the BMI <25 kg/m^2 (underweight and normal) group, 32 (42.1%) donors were in the BMI = 25–29.9 kg/m^2 (overweight) group, and 15 (19.7%) donors were in the BMI >30 kg/m^2 (obesity) group. The median BMI was 26.2 kg/m^2 as well. Donor characteristics were presented in Table 2.

The Collection Yield of the 4-Day Administration of G-CSF

All donors provided enough PB CD34+ cell counts to start the collection procedure on the 4th day of G-CSF administration. While 69 (90.8%) of 76 donors achieved targeted PBSCs at the end of the first apheresis day, second apheresis was needed in 7 (9.2%) donors. When added to the second apheresis day results (5th day of G-CSF administration) to the first apheresis day (4th day of G-CSF) results, those 7 (9.2%) donors reached the targeted PBSCs ($\geq 4 \times 10^6$/kg). Meanwhile, only 1 donor provided $\geq 4 \times 10^6$/kg PBSCs on the 5th day apheresis session among 7 donors. The median number of PBSCs and the median volume of PBSCs that was collected at first apheresis were 7.1×10^6/kg and 280 mL. Furthermore, the median number of PBSCs, both collected after first and second apheresis, was 7.1×10^6/kg as shown in Table 2.

The Comparison of the Single Apheresis and Double Apheresis Groups

There was no significant difference between the 2 groups in terms of sex, Hb levels, leukocyte, neutrophil, lymphocyte, monocyte, and platelet counts, neutrophil-to-lymphocyte ratio, monocyte-to-lymphocyte ratio, platelet-to-lymphocyte ratio, GFR, C-reactive protein, donor weight, donor BMI, and the volume of collected PBSCs. The median age was younger in the single apheresis group than the double apheresis group (34 vs. 43 years old, p = 0.032). Also, the median count of CD34+ cells in PB on the 4th day of G-CSF administration before the first apheresis

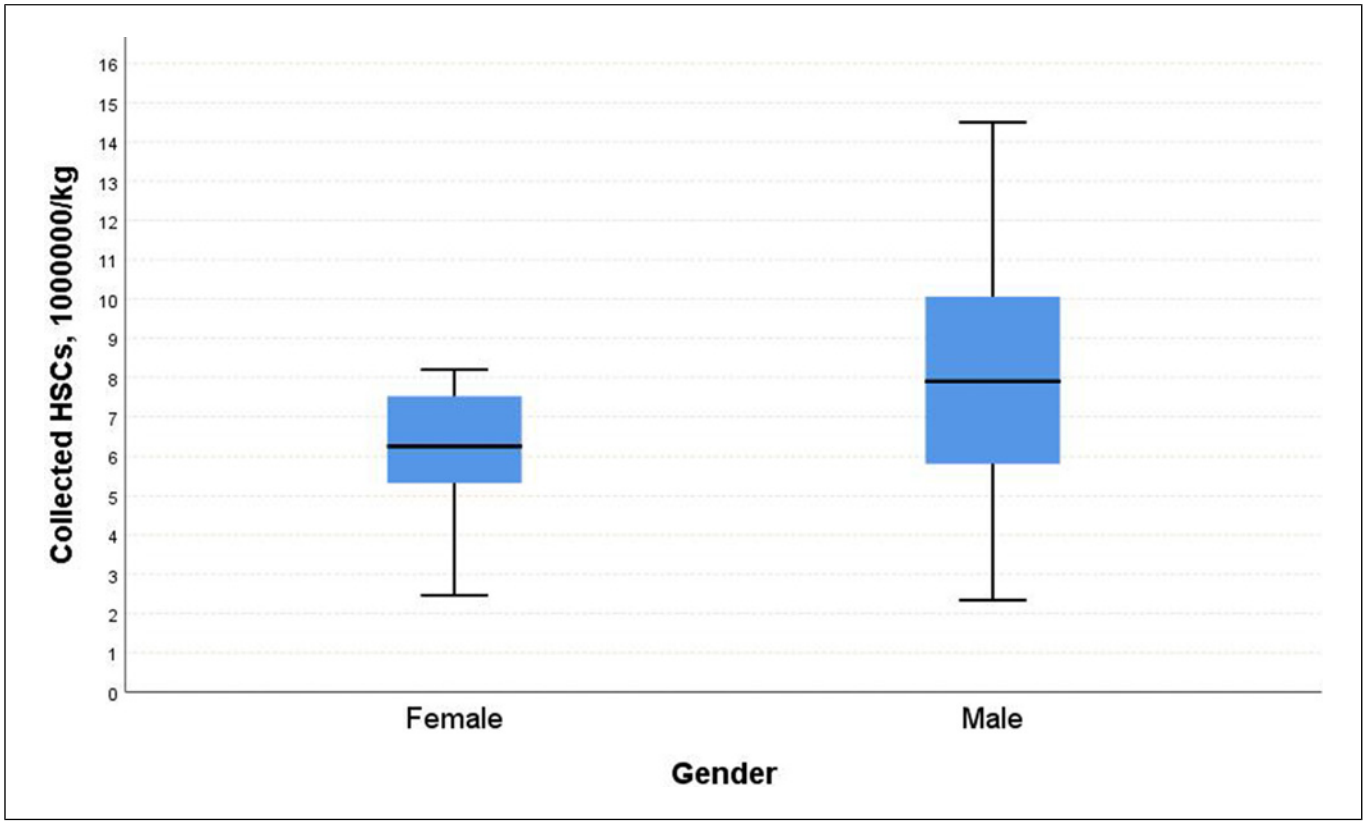

Fig. 1. Median counts of collected PBSCs after the first apheresis (7.9 vs. 6.3 × 10^6/kg, $p = 0.010$).

was significantly higher in the single apheresis group compared with the double apheresis group (104 vs. 44/μL, $p < 0.001$). The median number of collected PBSCs after the first apheresis session was higher in the single apheresis group than the double apheresis group (7.5 vs. 2.6 × 10^6/kg, $p < 0.001$) as well. Although it was achieved that to collect enough PBSCs for allo-HSCT after the second apheresis in the double apheresis group, the median amount of PBSCs at the end of apheresis procedures were still significantly lower in the double apheresis group compared with the single apheresis group (5 vs. 7.5 × 10^6/kg, $p = 0.001$) as presented in Table 2.

Factors That Associated with the Count of Collected PBSCs in the Whole Population

A negative correlation was found between the age of the donor and the collected PBSCs. It was observed that while the age of donor decreased, the amount of collected PBSCs increased (Spearman correlation test, $r = -0.323$, $p = 0.004$). There was a positive, strong correlation between the PB CD34+ cell count on the 4th day of G-CSF administration before apheresis and the amount of collected PBSCs. More CD34+ cells in the PB were associated with increased amounts of harvested PBSCs ($r = 0.720$, $p < 0.001$).

Furthermore, the median numbers of harvested PBSCs on the 4th day of G-CSF was significantly higher in male donors than in female donors as presented in Figure 1 (7.9 vs. 6.3 × 10^6/kg, $p = 0.010$). In addition to those, increased Hb ($r = 0.281$, $p = 0.014$), higher leukocyte ($r = 0.334$, $p = 0.003$), and neutrophil ($r = 0.302$, $p = 0.008$) counts were associated with the more collected PBSCs. The increased GFR ($r = 0.280$, $p = 0.014$) and donor weight ($r = 0.242$, $p = 0.035$) were other indicators to show higher collection yield as well. There was not any other factor that had a correlation with the collected PBSCs.

The Comparison of Very Good Mobilizers versus Others

We compared the very good mobilizers and the other donors to predict the variables that have the potential role of successfully harvested PBSCs. There were 29 (38.2%) donors in the very good mobilizers group and 47 (61.8%) in the other group.

There was a significant male predominance in very good mobilizers when compared with the other group ($p = 0.017$). Similarly, the younger donor age ($p = 0.007$) was found in the very good mobilizers group. The median of the PB CD34+ cell count on the 4th day of G-CSF before apheresis ($p < 0.001$), Hb ($p = 0.007$), leukocyte ($p = 0.011$), neutrophil ($p = 0.019$), and GFR ($p = 0.017$) were also found significantly higher in the very good mobilizers group than the other group, respectively.

Table 3. The comparison of possible variables between very good mobilizer group and other group

Variables	The collected PBSCs after first apheresis (<8 × 10^6/kg) (*n* = 47) (61.8%)	The collected PBSCs after first apheresis (≥8 × 10^6/kg) (very good mobilizers) (*n* = 29) (38.2%)	*p* value
Sex, *n* (%)			**0.017**[a]
Male	23 (48.9)	23 (79.3)	
Female	24 (51.1)	6 (20.7)	
Age, years			**0.007**[b]
Median (min-max)	38 (19–68)	29 (15–60)	
PB CD34 positive cell count on 4th day of G-CSF administration before apheresis, /μL			<**0.001**[b]
Median (min-max)	69 (18–174)	128 (60–187)	
Hb, g/dL			**0.007**[c]
Mean±SD	13.7±1.6	14.6±1.3	
Leukocyte, /μL			**0.011**[b]
Median (min-max)	41,680 (22,920–84,750)	51,520 (29,520–100,470)	
Neutrophil, /μL			**0.019**[b]
Median (min-max)	34,880 (17,520–71,750)	42,560 (24,340–86,470)	
Lymphocyte, /μL			0.525[b]
Median (min-max)	8,000 (2,190–12,100)	7,300 (3,730–15,100)	
Monocyte, /μL			0.351[b]
Median (min-max)	5,630 (1,830–13000)	6,000 (1,920–13000)	
Platelet, /μL			0.700[b]
Median (min-max)	219,000 (146,000–474,000)	224,000 (146,000–318,000)	
NLR			0.877[b]
Median (min-max)	5.5 (2.2–14.4)	6.4 (2.7–14.8)	
MLR			
Median (min-max)	0.81 (0.22–2.01)	0.89 (0.29–2)	0.589[b]
PLR			0.383[b]
Median (min-max)	36.1 (15.5–93.2)	30.3 (13.6–62.9)	
GFR, mL/min			**0.017**[b]
Median (min-max)	110 (67.3–135.9)	116.8 (86.1–131.2)	
CRP, mg/L			0.847[b]
Median (min-max)	1.1 (0.1–24.7)	1.1 (0.1–8.6)	
Donor weight, kg			0.069[c]
Mean±SD	75.7±14.1	81.9±14.4	
Donor BMI, kg/m^2			0.630[c]
Mean±SD	26.1±4.1	26.6±3.8	
Donor BMI, kg/m^2			0.558[d]
18.5–24.9	20 (42.6)	9 (31)	
25–29.9	19 (40.4)	13 (44.8)	
>30	8 (17)	7 (24.2)	

G-CSF, granulocyte colony-stimulating factor; Hb, hemoglobin; NLR, neutrophil-to-lymphocyte ratio; MLR, monocyte-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; GFR, glomerular filtration rate; CRP, C-reactive protein; BMI, body mass index; HSCs, hematopoietic stem cells; SD, standard deviation. [a]Continuity correction χ^2 test. [b]Mann-Whitney U test. [c]Independent samples *t* test. [d]Pearson χ^2 test.

There was not any other variable that was statistically different between the 2 groups as shown in Table 3.

The variables that were significantly different as a result of comparison between 2 groups, such as age, PB CD34+ cell count on the 4th day of G-CSF, Hb, leukocyte, neutrophil, and GFR applied ROC analysis to find predictable cutoff values that show very good mobilizers. The results of ROC analyses revealed that only PB CD34+ cell

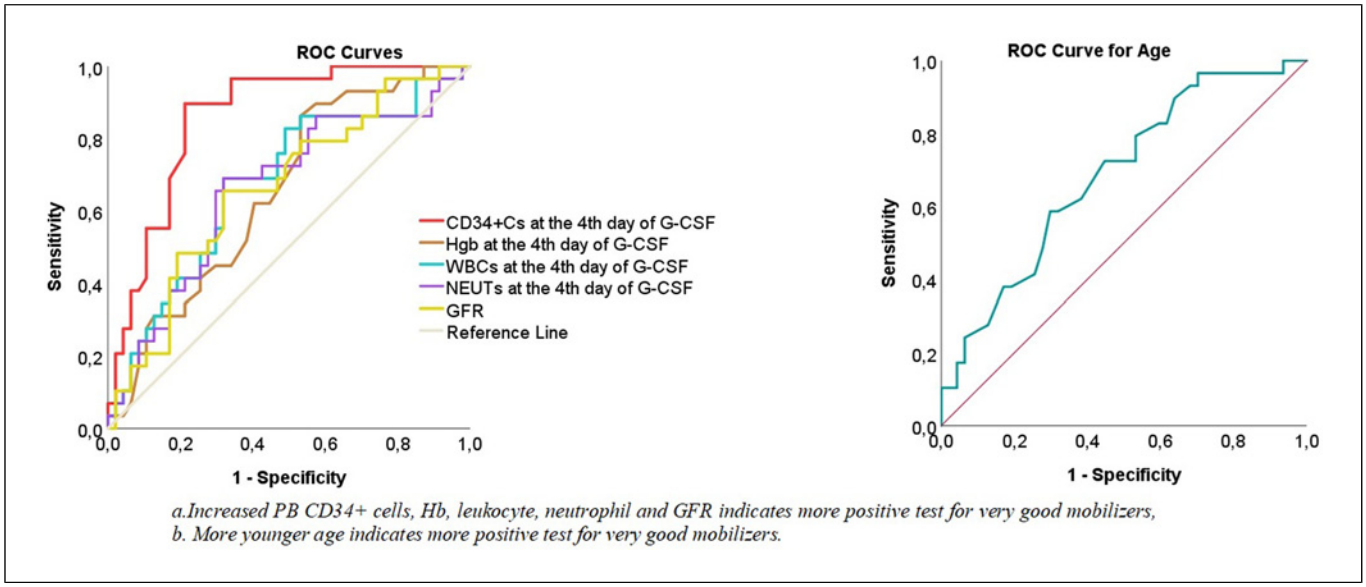

Fig. 2. Receiver operating characteristic analysis of the following patient characteristics: the PB CD34+ cells, hemoglobin (Hb), leukocyte, neutrophil, glomerular filtration rate (GFR) (a) and age (b) (dependent variable = collected PBSCs at first apheresis, ≥8 × 10^6/kg).

Table 4. The ROC analysis results of PB CD34+ cell values to predict the collection of very good mobilizer (≥8 × 10^6/kg)

Parametre	AUC (95% CI)	*p* value	Cutoff	+LHR	Sensitivity, %	Specificity, %	PPV	NPV
PB CD34+ cell count on the 4th day of G-CSF administration before apheresis, /μL	0.859 (775–0.942)	<**0.001**	≥75	2.4	97	60	60	97
			≥100	4.2	90	79	72	93

+LHR, positive likelihood ratio; PPV, positive predictive value; NPV, negative predictive value.

count was more predictive for collected PBSCs (AUC = 0.859, 95% CI = 0.775–0.942; $p < 0.001$) and the others had weak AUC values (<0.7) (Fig. 2). Because of that, the cutoff values could not be calculated by ROC analyses for Hb, leukocyte, neutrophil, GFR, and age. The median or closer to median of variables was used in univariate and multivariate logistic regression analyses. For PB CD34+ cell count, ≥100/μL instead of ≥75/μL was better to predict very good mobilizer as shown in Table 4. So, ≥100/μL was used in both logistic regression analyses.

Univariate and Multivariate Logistic Regression Analyses to Predict Very Good Mobilizers (Donors Who Provided ≥8 × 10^6/kg PBSCs at First Apheresis on Day 4)

Univariate analysis revealed that male sex ($p = 0.011$), age <35 years ($p = 0.046$), PB CD34+ cell count ≥100/μL on the 4th day of G-CSF ($p < 0.001$), leukocyte ≥50,000/mm^3 ($p = 0.013$), and GFR ≥115 mL/min ($p = 0.009$) were variables that significantly associated with being very good mobilizer. Furthermore, although ≥14 g/dL Hb had a tendency to higher collected HSCs, it did not reach statistical significance as presented in Table 5.

Owing to the powerful relationship between GFR and age ($r = -0.721$, $p < 0.001$), those 2 variables were included separately in multivariate models so as not to disrupt model compliance. When multivariate logistic regression analysis was performed by using variables that were significant ($p < 0.05$) in the univariate logistic regression analysis, male sex ($p = 0.011$) and PB CD34+ cell count ≥100/μL on the 4th day of G-CSF ($p < 0.001$) were independent associated factors to show very good mobilizer in model A as shown in Table 5. Similarly, male sex ($p = 0.004$), PB CD34+ cell count ≥100/μL on the 4th day of G-CSF ($p < 0.001$), and GFR ≥115 mL/min ($p = 0.031$) were found to be independent predicting factors to demonstrate very good mobilizer in model B (Table 5).

Discussion

Our goal in this study was to share the results of the apheresis procedure when collecting PBSCs from healthy donors in our center in recent years. The lack of the control arm and retrospective design were the main weaknesses of

Table 5. Univariate and multivariate analyses for predicting very good mobilizer (≥8 × 10^6/kg PBSCs)

Variables	Dependent variable: The collected PBSCs after first apheresis (≥8 × 10^6/kg) (very good mobilizers)								
	univariate logistic regression			multivariate logistic regression model a			multivariate logistic regression model b		
	OR	95% CI	*p* value	OR	95% CI	*p* value	OR	95% CI	*p* value
Model				multivariate logistic regression model a			multivariate logistic regression model b		
Male (ref: female)	4	1.38–11.61	**0.011**	24.32	2.07–286.49	**0.011**	50.84	3.57–723.62	**0.004**
Age <35 years (ref: ≥35)	2.64	1.01–6.84	**0.046**	0.95	0.88–1.03	0.214	–	–	–
PB CD34+ cell ≥100/μL (day 4) (ref: <100)	32.7	8.03–128.01	<**0.001**	141.48	12.25–1,633.71	<**0.001**	240.23	14.28–4,041.2	<**0.001**
Hb ≥14 mg/dL (ref: <14)	2.52	0.93–6.81	0.069	1.82	0.26–12.61	0.541	1.78	0.22–14.57	0.589
Leukocyte ≥50,000/μL (ref: <50,000)	4.63	1.39–15.43	**0.013**	1.19	0.19–7.72	0.850	0.68	0.09–5.34	0.711
GFR ≥115 mL/min (ref: <115)	3.68	1.39–9.76	**0.009**	–	–	–	8.18	1.21–55.34	**0.031**

Due to strong relationship between the leukocyte and the neutrophil (Spearman correlation test, $r = 0.990$, $p < 0.001$), only leukocyte included into the analyses. Owing to powerful relation between the GFR and the age (Spearman correlation test, $r = -0.721$, $p < 0.001$), those 2 variables included separately in multivariate models for not to disrupt model compliance (model a and b). OR, odds ratio; CI, confidence interval; PBSCs, peripheral blood stem cells; PB, peripheral blood; Hb, hemoglobin; GFR, glomerular filtration rate.

our study. We consider that the most important finding was to achieve enough PBSCs on the 4th day of G-CSF administration by single apheresis in more than 90% donors. In addition, younger age, male gender, higher PB CD34+ cell count before first apheresis, increased donor weight and higher Hb, leukocyte, neutrophil, GFR were correlated with more harvested PBSCs yields.

Although ASBMT recommends the starting day of PBSC collection is the 5th day of G-CSF, the starting day is not yet standard for healthy donors [1, 2]. Newell et al. showed that if the PB CD34+ cell >40/μL on day 4 of G-CSF, donor apheresis was performed and adequate PBSCs (>4 × 10^6/kg) were harvested from 49.5% of donors on day 4 by single apheresis [9]. In another study, Kimura et al. reported that starting PBSC apheresis on day 4 was as effective as starting on day 5 in 109 healthy allogeneic donors [14]. Von Oostrum et al. indicated that successful PBSCs harvests (>5 × 10^6/kg CD34+ cell) on day 4 could have been achieved at least in part of donors, especially if used split doses G-CSF [11]. Flommersfeld et al. [10] compared the day 4 and day 5 apheresis day results. They found both of them were similar. The day 4 single collection was enough to provide a target cell yield that was >5 × 10^6/kg CD34+ cell in 77.7% of donors. In the present study, our results are compatible with the literature that we mentioned above. We achieved sufficient collected PBSCs on day 4 by single apheresis in most of the donors, and the collection of PBSCs was completed in the rest of donors on day 5 with the second apheresis session. Thus, we think that the beginning of the PBSCs harvest on day 4 instead of 5 is more feasible.

Anderlini et al. [15] compared the day 4 and 5 apheresis results, and they found the second apheresis procedure was more needed in the day 4 collection group to obtain a target stem cell yield (4 × 10^6/kg). Although they used higher doses of G-CSF (12 μg/kg/day) and had similar target PBSCs amount, it is interesting that the day 4 collection was less effective than day 5, conversely to our study. It could be based on using different apheresis devices. Because they used COBE Spectra, and we used Spectra Optia which has been reported a better collection results than COBE Spectra [16–18]. There are other studies in the literature that compared day 4 and 5 collections, but pretty higher dose G-CSF (16 or 24 μg/kg/day) was used in those, and different collection devices that had relatively old technology were used. So, it is difficult to compare those with our study [19, 20]. Once again, we would like to emphasize that even if necessary more than just one apheresis, day 4 seems to be better than day 5 for starting the first apheresis.

There are studies that have investigated the impact of possible factors on PBSCs collection yield in the literature. Those factors include gender, donor age, donor weight, donor BMI, G-CSF dose, divided G-CSF dose, leukocyte count on the first apheresis day, baseline leukocyte, and platelet count. There are conflicting results related to those factors. For instance, increased donor age has a negative influence on harvested PBSCs yield in some studies. On the other hand, it has no impact in different studies. This example might be conceivable for all those factors that we mentioned above [2, 21–24].

In our study, the most correlated factor to predict more harvested PBSCs yield was PB CD34+ cell count on the 4th day of G-CSF before apheresis. This is consistent with previous studies by Grigg and colleagues and De la Rubia and colleagues [25, 26]. The second important factor that correlated with increased PBSCs yield in our study was younger age or vice versa. Suzuya and colleagues indicated the most important predictor for a showing successful PBSCs mobilization was younger age as well [24]. Anderlini et al. [15] found that age >55 years was associated with poor PBSCs mobilization. Similarly, De la Rubia and colleagues reported that the donor age ≥38 years was correlated with a lower amount of collected PBSCs yield [23]. Although there is still an uncertainty why the threshold of age was variable between different studies or how age is influenced PBSCs yield, age associated bone marrow hematopoietic dysfunction might be one of the reasons [27].

The suggested amount of infused PBSCs range is 4–8 × 10^6/kg in allo-HSCT [1]. While we generally infuse 4–5 × 10^6/kg PBSCs per allo-HSCT, the other transplantation center might use higher count of PBSCs. Thus, if we collect PBSCs as much as possible in high quantities, this would provide flexibility for transplant physicians during the transplantation. On the other hand, our priority has to be donor safety, so, to provide enough PBSCs from healthy donors with minimal G-CSF exposure and apheresis cycle should be an ideal approach. With this point of view, we separated donors as very good mobilizers and others to determine independent factors related to predict very good mobilizers. PB CD34+ cell ≥100/μL on the 4th day of G-CSF, male gender and GFR ≥115 mL/min were significantly associated with a very good mobilizer in multivariate regression analysis.

Neither PB CD34+ cell count on the 4th day of G-CSF nor male sex are surprising for predicting very good mobilizer. The interesting one is to continue the significant impact of GFR at the end of the multivariate analyses. GFR is based on gender and age. To clarify whether the impact of GFR was real or originated from gender and age, we considered all possible effects of these 2 variables on GFR in statistical analyses. To the best of our knowledge, this is the first study to show the effect of GFR on G-CSF induced PBSCs collection in healthy donors to date. Our study is relatively small size, so, we think that further large-scale studies will elucidate the impact of GFR on PBSCs collection.

In conclusion, our study shows that more than 90% of healthy donors provide enough PBSCs yield with single apheresis on day 4. It seems that starting the apheresis on the 4th day of G-CSF administration is appropriate in terms of efficient PBSCs collection and minimal G-CSF exposure in healthy donors. This study also demonstrates PB CD34+ cell count on the 4th day of G-CSF, male sex, and GFR are independent factors to show the very good mobilizer donor. Further prospective studies will clarify the results of the present study and/or any other factor associated with G-CSF induced PBSCs collection from healthy donors.

Statement of Ethics

This retrospective and non-interventional study was reviewed and approved by the Institutional Ethics Board of Akdeniz University School of Medicine (Approval No. 01.11.2023/24). The study was conducted in accordance with the Declaration of Helsinki. Written informed consent from the parent/legal guardian of participants was not required for this retrospective study in accordance with local/national guidelines.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest.

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Author Contributions

O.K.Y. designed the study conception, collected data, and wrote the manuscript D.Y. performed the statistical analyses. The remaining authors collected data, reviewed the manuscript, contributed edits, and provided patient care.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author (O.K.Y.).

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