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Factors affecting hematopoietic stem cell mobilization and apheresis in allogeneic donors: The role of iron status

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ABSTRACT

Infused CD34 cell count has a significant impact on transplant outcome. In this retrospective study, we aimed to analyze the impact of donor iron parameters on peripheral blood stem cell (PBSC) collection. A total of 303 related donors were included in the study. The mobilization regimen, recombinant G-CSF, was given for four consecutive days. A $CD34^+$ cell count below 2×10^6/kg was defined as mobilization failure which was demonstrated in 23 donors (7.6%). Mobilization failure was more frequent in female donors than male donors (13.7% vs 3.4%). Body mass index, mean corpuscular volume, hemoglobin and ferritin levels were found to be lower in donors with mobilization failure. Body mass index was significantly correlated with PBSC count on the 4th day of G-CSF. Body mass index, male gender, mean corpuscular volume and ferritin levels had significant impact on PBSC count. Although PBSC count was found to be similar between female and male donors, female gender was shown to have an adverse impact on PBSC collection, which may be attributed to lower body weight and concurrent iron deficiency.

1. Introduction

Several reports indicate that infused hematopoietic stem cell (HSC) count is closely associated with transplant outcomes. As early neutrophil and platelet engraftment are considered to have favorable impact on survival, identifying risk factors for HSC mobilization in healthy donors as well as optimal donor selection is essential for successful transplantation [1–3].

Peripheral blood is the preferred stem cell source in allogeneic stem cell transplantation [1,4]. Peripheral blood stem cell (PBSC) harvesting is a non-invasive and painless procedure which provides rapid engraftment and fewer complications when compared to bone marrow harvesting [4]. Several factors, including older age [5–9] and female gender [5,7,10,11] were identified as unfavorable factors for PBSC mobilization. There are a few reports about the relationship between iron status and stem cell mobilization in patients with hematological malignancies [12,13]; however, the impact of donor iron parameters on PBSC mobilization has not been investigated. In this retrospective study, we analyzed the impact of the donor iron profile on PBSC collection in 303 healthy donors.

2. Material and methods

2.1. Donor selection

A total of 303 consecutive healthy PBSC donors were enrolled in this retrospective study. All donors were HLA matched and unrelated donors were excluded from the analysis. Donor characteristics including age, gender, weight and height as well as baseline hemoglobin (Hb), mean corpuscular volume (MCV), white blood cell (WBC), platelet (Plt) and ferritin levels were recorded. Body mass index (BMI) was calculated based on the formula, weight/height^2. The study was approved by Gazi University Ethics Committee.

2.2. Mobilization and apheresis

Recombinant G-CSF was used for four consecutive days as mobilization regimen. A total of 214 donors (83.2%) received filgastrim, while 43 donors (16.8%) were mobilized with lenogastrim at a single daily dose of 10 μg/kg. The first apheresis was performed on the fourth day of G-CSF, if the PBSC > 10/μL. G-CSF administration was continued until optimal CD34 count (4×10^6 $CD34^+$ cells/kg) was obtained or mobilization failure (MF) was observed. Mobilization failure (MF) was the primary endpoint which was defined as failure to reach a minimum CD34 count of 2×10^6 $CD34^+$ cells/kg.

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Table 1
Demographic and anthropometric data.

Donor age (years)	33 (12–73)
Donor gender (*n*)	178 male 125 female
Donor relationship *n* (%)	290 (95%) sibling 5 (1.7%) cousin 4 (1.3%) sun 2 (0.7) mother 2 (0.7) father
Height (cm)	
Donor	167.7 ± 9.2
Recipient	166.4 ± 10.5
Weight (kg)	
Donor	72.2 ± 6.4
Recipient	68.6 ± 13.6
BMI (kg/m^2)	
Donor	25.7 ± 5.4
Recipient	25.3 ± 11.8

Height, weight and BMI were expressed as mean ± standard deviation.

Apheresis was performed 2–5 times of the estimated blood volume using standard continuous-flow blood cells separator (Fresenius Com. Tec, Fresenius As. Tec, Fenval Amicus, Fenval CS3000 plus). CD34$^+$ cell counts in the peripheral blood and in the apheresis product were measured by flow cytometry in accordance with the ISHAGE protocol [14].

2.3. Statistical analysis

Categorical variables were compared with chi-square test. Student-*t* and Mann Whitney *U* tests were used to compare continuous parameters. The relationships between HSC count and certain parameters were tested with simple correlation analysis and regression models. PBSC count, total CD34 product and CD34 product/recipient weight were used for analysis of the factors affecting mobilization. Statistical analysis was performed using SPSS 16. All statistical tests were performed two-sided and *p* value 0.05 was considered as statistically significant.

3. Results

A total of 303 donors [median age: 33 (12–73) years; M/F: 178/125] were enrolled in this study. Donor and recipient characteristics are shown in Table 1. The target PBSC count (>10/μL) was obtained in 247 (81.5) and 51 (16.8%) donors, on the fourth and fifth days of G-CSF, respectively. In 4 donors target PBSC count was obtained on the sixth day, however in 1 donor we could not obtain optimum PBSC. Mean PBSC count was found to be 30.2 ± 21.2/μL on the fourth day of G-CSF.

Mobilization failure was determined in 23 donors (7.6%). Target HSC count was obtained after the first apheresis for all 191 donors (63%). Hematopoietic stem cell count in first and total apheresis product was $2.7 \pm 2.3 \times 10^6$/kg and $4.8 \pm 2.3 \times 10^6$/kg based on recipient weight, respectively. Comparison of donor characteristics based on mobilization failure is presented in Table 2. Mobilization failure was more frequent in female donors (13.7% vs 3.4%). Mean PBSCs were not different between male and female (27.4 ± 19.1 vs 31.2 ± 21.3; $p > 0.05$). Mean total CD34 product and CD34 product/weight-recipient were higher in male than female donors (366.5 ± 187.1 vs 279.9 ± 142.2; $p < 0.001$ and 5.2 ± 2.4 vs 4.1 ± 1.9; $p < 0.001$). Mean BMI, Hb, MCV and ferritin levels were lower in donors with mobilization failure. Weight comparison in donors according to recipient was more prominent in donors with mobilization failure (−4.6 ± 25.6 vs +8.6 ± 26.7; $p = 0.02$). Age, WBC and Plt counts were not different between two groups. Mean ferritin level was significantly lower in female donors when compared to male donors (29.9 ± 35.2 vs 80.5 ± 86.7; $p = 0.001$).

Univariate linear regression analysis indicated that BMI (OR: 3.61, $p < 0.001$) had a significant impact on PBSC count which was obtained on the fourth day of G-CSF, however age, WBC, Plt, Hb and ferritin levels had no significant impact on PBSC count ($p > 0.05$). On second linear regression models, BMI (OR: 2.1, $p < 0.001$), gender (OR: 1.56, $p = 0.02$), ferritin (OR: 1.45, $p = 0.03$) and MCV (OR: 1.42, $p = 0.04$) had an impact on HSC count in the total apheresis product. Age, WBC, Plt and Hb levels did not show any significant impact on HSC count of the product ($p > 0.05$). In multivariate analysis, BMI ($p = 0.01$), gender ($p = 0.03$) and ferritin levels ($p = 0.04$) were independent predictive factors for HSC count of total apheresis product.

4. Discussion

In this study, prevalence of MF was 7.6% which was reported to be 2–40% in previous studies. Target HSC count was obtained at the end of the first apheresis in 191 donors (63%) compatible with previous reports (60–80%) [6–8,10,15–17]. This wide range may be attributed to small sample size and lack of standardization including definitions of MF and target HSC count. In a study by Suzuya et al., MF, which was found to be 8.4% in 59 related donors, was defined as PBSC less than 20/μL on the fifth G-CSF day [6]. Rubia et al. described MF as CD34$^+$ cell less than 4×10^6/kg in first apheresis product which was reported to be 40% [16]. Prevalence of MF was 2% in a study by Ings et al. and they collect >4×10^6/kg HSC in first apheresis product in 63% of donors [7]. Our results were compatible with the literature.

Body mass index, Hb, MCV and ferritin levels were lower in donors with MF when compared to cases without MF. Median age was not different between two groups. Mobilization failure was more frequent in female donors, however this difference lost significance when donor body weight was included in the analysis. In addition, median PBSC count was not different in female and male donors. Body mass index had a significant impact on the PBSC count of the fourth G-CSF day. It is well known that donor BMI is an important factor affecting HSC yield [18,19]. Adipose tissue is a source of HSC, and the other explanation of the close relationship between HSCT yield and donor's BMI is that we use actual body weight for G-CSF dose. Donors with higher BMI may be mobilized with overdose G-CSF compared to donors with ideal body weight. Another result of the present study is the donor weight discrepancy according to recipient reported lower among poor mobilizer donors. Donor BMI should be considered especially for the patients with alternative donor option. Furthermore, BMI, gender, and ferritin represented an independent impact on HSC count of the apheresis product in multivariate models.

A number of studies demonstrated a relationship between advanced age [5–11,15–17] and MF in HSC donors. In general, younger age is considered to be a favorable factor for successful collection. Although advanced age may lead to lower HSC counts and increase the risk of apheresis complications, there are some studies that did not confirm the correlation of age and HSC count [11,20,21]. However, the results are conflicting, as pediatric and adult donors were included in the same analysis and cutoff value for age subgroups were different in most of the studies [9,15,16]. In our study, mean age was not found to be different between cases with or without MF.

Female donors were shown to have higher risk of MF compared to males in several studies [5,7,10,11]. This increased risk was attributed to the higher blood volume of male donors due to higher body mass. In our study, PBSC counts on the fourth day of G-CSF were similar between male and female donors; however,

Table 2
Comparison of donor characteristics based on mobilization failure.

	With MF ($n=23$)	Without MF ($n=280$)	p
Age (year)	35.0 ± 13.4	30.9 ± 11.1	>0.05
Gender (male/female) (n)	6/17 (3.4%/13.7%)	172/108 (96.6%/86.3%)	0.001
BMI (kg/m^2)	23.4 ± 4.0	26.0 ± 5.5	0.02
Donor weight discrepancy according to recipient (%)	−4.6 ± 25.6	+8.6 ± 26.7	0.02
Hb (g/dl)	13.1 ± 1.7	14.4 ± 1.6	0.001
WBC ($\times 10^3/\mu L$)	6.9 ± 1.6	7.5 ± 2.4	>0.05
Plt ($\times 10^3/\mu L$)	249.1 ± 81.9	258.2 ± 63.9	>0.05
Ferritin (ng/ml)	22.4 ± 17.0	61.5 ± 76.2	0.03
MCV (fl)	82.0 ± 9.1	86.1 ± 7.7	0.01

Continuous variables were presented as mean ± standard deviation.
BMI: body mass index, Hb: hemoglobin, WBC: white blood cell, Plt: platelet, MCV: mean corpuscular volume.

the HSC count of the apheresis product was significantly higher in males.

Although some studies indicated that iron overload (IO) may lead to an increased risk of transplantation related mortality [22–24], the impact of iron metabolism on HSC mobilization has not been clarified. The association of iron status and stem cell mobilization in autologous HSCT was investigated in several studies [12,13]. Park et al. reported that transfusional IO is a poor predictive factor for HSC mobilization in 51 patients with hematological malignancies [13]. Another study from our center, which was performed in 118 patients with hematological malignancies, showed that ferritin levels were higher in cases with MF [12]. Adverse impact of IO on HSC mobilization may be attributed to the defective function of bone marrow adhesion molecules, HSCs and cell interaction via increased levels of reactive oxygen radicals secondary to iron toxicity. Iron overload is a common problem in patients with hematological malignancies due to chronic inflammation, ineffective hematopoiesis and transfusion load. The role of iron metabolism in HSC mobilization of healthy donors remains to be a novel area to investigate. In our study, iron deficiency was an adverse factor for HSC mobilization in allogeneic donors. To the best of our knowledge this is the first study investigating the relationship between iron parameters and HSC mobilization in donors. Kamezaki et al. [25] presented a female donor with iron deficiency anemia who was treated with intravenous iron therapy one month before HSC apheresis. The authors observed MF and higher red blood cell contamination with lower PBSC in the product. As the sedimentation features of the new produced red blood cells within a centrifugal separation chamber were similar to those of mononuclear cells, HSC collection was inadequate. In another study, Pornprasertsud et al. [26] showed a correlation between hematocrit and CD34$^+$ cell count in the product. As G-CSF administration has an inhibitory effect on erythropoiesis, anemia may get worse after G-CSF injection in iron-deficient donors. As a result, increased secretion of EPO may lead to extreme differentiation of stem cells to erythroid progenitor cells leading to low HSC counts. As this study did not reveal ferritin and EPO levels, the results require further confirmation. In our study, hemoglobin, MCV, and ferritin levels were lower in donors with MF. Poglajen et al. [27] also showed that increased red cell distribution is related to mobilization failure in patients with chronic heart failure. Authors pointed out that higher RDW was associated with lover MCV, chronic inflammation – malnutrition and bone marrow dysfunction. Furthermore, ferritin and MCV had an independent impact on HSC count in our analysis. Based on our results, iron deficiency with/without anemia may have adversely affected the HSC count, without a significant impact on PBSC count. Thus, iron deficiency should be treated before the apheresis procedure, which should be performed when the erythrocyte volume is within the normal range. If the procedure is urgent, apheresis procedure should be closely monitored to maintain appropriate flow rate, time and blood volume in order to protect the product from contamination with red blood cells of buffy coat.

In conclusion, higher BMI and male gender are favorable factors for a successful HSC collection. Although PBSC count was similar between males and females, lower blood volume and body weight as well as concurrent iron deficiency may have adversely impact the apheresis outcome. The adverse impact of iron deficiency on HSC collection should be confirmed with further studies.

Authors' contribution

Zübeyde Nur Özkurt: the conception and design of the study, analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, final approval of the version to be submitted.

Leyla Batmaz: the conception and design of the study, interpretation of data, revising it critically for important intellectual content, final approval of the version to be submitted.

Zeynep Arzu Yeğin: the conception and design of the study, interpretation of data, revising it critically for important intellectual content, final approval of the version to be submitted.

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