Brief report

Comparison between peripheral blood progenitor cell collection on the 4th or 5th day of granulocyte colony-stimulating factor treatment in allogeneic stem cell donors: implications for hematopoietic progenitor cell apheresis guidelines

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INTRODUCTION

The collection of hematopoietic progenitor cells by apheresis, designated hematopoietic progenitor cells apheresis (HPC-A), is the most frequently used source of $CD34^+$ cells for hematopoietic stem cell transplantation (HSCT)[1]. The biggest advantage of HPC-A, compared to bone marrow harvest, is represented by the large number of $CD34^+$ cells that can be obtained, which leads to a more rapid engraftment in the allogeneic setting[2]. However, in order to reach the optimal target $CD34^+$ cell dose (>4×10^6/kg recipient bodyweight)[3] to perform a transplant, the donor must receive granulocyte colony-stimulating factor (G-CSF) to mobilise progenitor cells from the bone marrow[4]. National and international guidelines recommend the first apheresis on the 4th or 5th day of G-CSF treatment (10 µg/kg, divided into two daily injections) when the $CD34^+$cell concentration in peripheral blood reaches its maximum[4-6]. Unfortunately, this treatment is associated with side effects such as bone and muscle pain[7], nausea[7], headaches[8], and (more rarely) splenic rupture[9], myocardial infarction and thrombotic events, which appear to be directly dependent on the administered dose of G-CSF[10].

In transfusion medicine, donor protection is one of the main tasks[11]. In fact, the stem cell donor is a healthy person who agrees to take drugs and to undergo invasive medical procedures purely out of solidarity with others. Therefore, mobilisation protocols should be regularly reviewed[12] and, if necessary, updated in order to ensure the best and safest procedures for donors.

Nowadays, to reduce the final dose of G-CSF and, consequently, the incidence of any side effects, many centres perform apheresis on the 4th day of treatment, if the number of $CD34^+$ cells is adequate (≥20/µL). However, only a few studies, performed on a limited number of donors, have compared apheresis on the 4th or 5th day of G-CSF treatment in terms of target $CD34^+$ cell dose (>4×10^6/kg/patient), purity of the collected cells (reduced content of white blood cells [WBC], granulocytes and platelets [PLT]), and donor safety[13]. Thus, in this retrospective study, our primary end point was to investigate whether HPC-A on the 4th day after the first dose of G-CSF might lead to an adequate harvesting of stem cells, reducing G-CSF exposure.

MATERIALS AND METHODS

Donors

Ten Italian transplant centres belonging to the Italian Society of Hemapheresis and Cellular Manipulation (SIdEM) joined the study (see Online Supplementary Appendix).

A total of 840 healthy donors (age >18 years), related or matched unrelated (MUD), who underwent apheresis between 2005 and 2015 were included in the study; donor characteristics are shown in **Table I**. After HPC mobilisation by G-CSF, they were subjected to apheresis on the 4th or 5th day of treatment, based on the achievement of the threshold of ≥20/μL $CD34^+$ cells in peripheral blood. Lenograstim or filgrastim were used indifferently in donors recruited for the study as assessment of their different effects on stem cell mobilisation was not one of the study endpoints. To reduce the bias associated with the use of different substances, donors were selected so that the percentages of subjects treated with the same substance were similar in donors who started HPC-A on the 5th day of treatment with G-CSF and in donors who started on the 4th day.

Donors who discontinued the treatment with G-CSF were excluded from the study. Donors who failed to mobilise HPCs (not achieving a peripheral $CD34^+$ cell count ≥20/μL) were also excluded, since the study covers the decade (2005-2015) preceding the approval of Plerixafor in poorly mobilising allogeneic stem cell donors (AIFA Official Gazette 2 August 2017).

Table I - *Characteristics of donors enrolled in the study*

Donors' characteristics	First apheresis		p value
	Day 4	Day 5	
Number of donors	253	587	
Age (years)	45 (16-73)	45 (17-74)	0.81
Sex (M/F)	141/112	306/281	0.34
Donor's weight (kg)	74 (45-121)	71 (42-150)	0.06
Recipient's weight (kg)	75 (3-144)	70 (4-130)	0.002
$CD34^+$ cells pre/mL	67,947 (11,590-388,360)	86,942 (9,860-505,692)	0.0001
WBCs pre ($\times10^9$/L)	40.11 (17.76-94)	42.52 (13.07-90)	0.0336
MNCs pre ($\times10^9$/L)	5.15 (0.5-11.1)	5.8 (1.31-76.4)	<0.001
PLTs pre ($\times10^9$/L)	216 (122-503)	228 (77-449)	0.0014

M: male; F: female; WBC: white blood cells; MNC: mononuclear cells; PLT: platelets.

The following clinical data were collected for each enrolled donor and stored in a computerised database:

- donor characteristics (age, weight, sex, G-CSF schedule, day of the first apheresis, hematological parameters before mobilisation procedure);
- parameters of apheresis (total processed volume, duration of the procedure, manufacturer and model of the device used, adverse events);
- graft cell composition (WBCs, mononuclear cells [MNCs], hematocrit [Ht], PLTs, $CD3^+$, $CD4^+$, $CD8^+$, $CD19^+$, $CD34^+$ cells).

Written informed consent was obtained from each donor. The study was performed in accordance with the principles outlined in the Declaration of Helsinki and approved by the Ethical Committee of the "G. d'Annunzio" University of Chieti-Pescara on 11th November 2016.

Mobilisation procedure

All donors were treated with subcutaneous injections of 10 μg/kg of G-CSF, divided into two daily doses, for 4 or 5 days. The first apheresis was performed on the 4th day (after 7 G-CSF injections) or day 5 (after 9 G-CSF injections).

Collection of hematopoietic progenitor cells

All collections were performed via peripheral venous access using the COBE Spectra system (Terumo, Tokyo, Japan), CS-3000 Blood Cell Separator (Baxter International, Deerfield, IL, USA), COM.TEC or AMICUS systems (Fresenius Kabi, Bad Homburg vor der Höhe, Germany) according to the manufacturers' recommendations. To reduce the bias associated with the use of different devices, donors were selected so that the percentages of subjects undergoing apheresis with the same devices were similar in donors who started HPC-A on the 5th day of treatment with G-CSF and those who started on the 4th day. The number of cycles and the volume of blood processed were adjusted to achieve a target $CD34^+$ cell dose of 4×10^6/kg recipient bodyweight.

Flow cytometry

White blood cells and $CD34^+$ cells were counted in pre- and post-apheresis blood samples, as well as in the

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product, by flow cytometry, using the FACSLyric™ System (BD Biosciences, Franklin Lakes, NJ, USA). For identification of $CD34^+$ and $CD45^+$cells, a Stem Cell Enumeration Kit (BD Biosciences Cat# 344563, RRID:AB 2868793) was used, whereas T, B lymphocytes and natural killer cells were identified using the BD Multitest™ 6-colour TBNK Reagent (#644611; BD Biosciences), according to the manufacturers' recommendations. All procedures were regularly subjected to internal and external quality controls (IQC/EQC).

Calculation of variables

The $CD34^+$cell collection efficiency (CE), the proportion of $CD34^+$ cells passing through the separator that is harvested, was calculated as reported by Flommersfeld *et al.*[13].

Statistical analysis

Descriptive data are given as median, quartiles or mean and range. Comparison between groups was performed by non-parametric tests: χ^2, Fisher's exact, Mantel-Haenszel, Wilcoxon signed-rank, Mann-Whitney U, Kruskal-Wallis and Spearman's rank correlation coefficient. To identify the factors influencing the achievement of the target dose, a univariate logistic regression analysis was performed, whereas a multivariate logistic model was carried out to study the influence of collection timing on the incidence of the second procedure. All statistical tests were evaluated at α=0.05, using SAS (version 9.3) (SAS Institute, Cary, NC, USA; RRID:SCR_008567) or R software environment (version 4.0.3; RRID:SCR_001905 [R Foundation for Statistical Computing, Vienna, Austria]).

RESULTS

Donors who started the HPC-A on the 5th day of treatment with G-CSF and those who started on the 4th day were comparable regarding donor demographics and bodyweight; the only variables that differed significantly were: 1) the recipient's weight, which was higher in the group who started apheresis on the 4th day; and 2) MNC, WBC and $CD34^+$ cell counts, which were higher in the group who started apheresis on day 5 (due to the two additional G-CSF injections).

Concerning the primary endpoint as to whether HPC-A on the 4th day after the first dose of G-CSF might lead to an adequate harvesting of stem cells, there was no difference between the two groups, since the target $CD34^+$ cell dose (>4×10^6/kg recipient bodyweight) was reached in 81.97% of donors starting collection on the 4th day and in 81.62% of donors starting collection on the 5th day (p=0.91). However, since the percentage of donors who underwent a second collection procedure (20%, 50 out of 253) was higher in donors who started apheresis on the 4th day, compared to donors who started apheresis on day 5 (12%, 71 out of 587) (p=0.004), we constructed a multivariate logistic model to study any possible influence of collection timing on the incidence of a second procedure. The model revealed that there was no association between the first day of apheresis and the incidence of a second procedure, whereas this was significantly associated with the recipient's weight (**Table II**), which was higher for donors who started collection procedures on the 4th day (**Table I**).

Next, analysing the data from donors who had undergone two collection procedures, we found that 94% of donors who started apheresis on the 4th day reached the target dose compared to 83% of those starting collection procedures on day 5 (p=0.064). A possible explanation for this could be that if those who started apheresis on the 4th day did not reach the target dose, a second collection could still be made at a time when their cell counts were in a positive trend phase, while those who started on day 5 had to undergo the second procedure in a downtrend phase.

We then considered the efficiency of the collection procedures, i.e., the proportion of $CD34^+$ cells harvested/volume processed, at a given $CD34^+$ cell count in the peripheral blood. Data analysis showed that, among donors undergoing a single procedure, collection procedure efficiency was 56% for those who underwent apheresis on the 4th day compared to only 49% for those who underwent apheresis on day 5 (p<0.001). Furthermore, considering the donors who underwent two procedures, we found that 47% of those who started collection on the 4th day achieved an efficiency >50 *vs* only 30% of those who started the collection on day 5 (p<0.0001). This was

Table II - *Multivariate logistic model to evaluate the influence of the collection day on the incidence of the second procedure*

Response variable: *number of procedures* Probability of the model: *2 procedures*		
Variable	**OR**	**p-value**
Collection day	3.039	0.0810
MNC pre ($\times10^9$/L)	0.945	0.0103
$CD34^+$ cells pre/mL	0.998	0.0021
Recipient's weight	1.075	<0.0001

OR: Odds ratio; MNC: mononuclear cells.

probably due to the fact that those who began collection on day 5 had higher WBC cell counts than those who underwent apheresis on the 4th day, and these variables are inversely correlated with collection efficiency[14].

Important differences between the two groups of donors were also found regarding the purity of the product (**Table III**). In fact, despite a very similar content of CD34+ cells, contamination by neutrophils (17 *vs* 45%) (p<0.001), PLTs (1,850×10⁹/L *vs* 2,023×10⁹/L) (p<0.0013), and by RBC (Ht 4 *vs* 6%) (p<0.001) was significantly lower in products collected on the 4th day than in those collected on day 5. This improved purity makes the product more suitable for subsequent manipulations and safer for the recipient[15].

Since there is evidence that female donors mobilise lower numbers of CD34+ cells, we investigated if there was any association between sex and the first day of apheresis[16,17]. The data showed that there was no association (p=0.3372) between sex and the first day of apheresis, therefore, the distribution of the sex variable is homogeneous between donors who started apheresis on the 4th day (112 [44.27%] females and 141 [55.73%] males) and those who started apheresis on day 5 (281 [47.87%] females and 306 [52.13%] males). In addition, we found no association between donor age[16,17] and first day of apheresis (p=0.811), since the age distribution of the donors is comparable between those who started apheresis on the 4th day (median 45; range 16-73) and those who started on day 5 (median 45; range 17-74).

Finally, no differences were found in the incidence of side effects between donors who underwent apheresis on the 4th day or day 5 (p=0.326). However, the correct evaluation of side effects is very difficult to ascertain in this type of study due to the high diversity in the number and type of possible side effects, the different strategies for collecting information in centres participating in the study, and, above all, the different perceptions of the donors; these factors could all compromise the interpretation of the data.

Table III - *Purity of apheresis products collected on the 4th or the 5th day of treatment with granulocyte colony-stimulating factor*

Graft cell composition	First apheresis		p value
	Day 4	Day 5	
WBC apheresis product (×10⁹)	55 (14-208)	69 (6-410)	<0.001
CD34+/µL apheresis product	1,180 (14-4,580)	1,445 (61-14,240)	0.004
CD34+ apheresis product (×10⁹)	0.38 (0-1.82)	0.39 (0.02-1.3)	0.765
CD3 (%)	40 (3-88)	25 (2-89)	<0.001
CD4 (%)	26 (2-57)	15 (0.1-64)	<0.001
CD8 (%)	13 (1-45)	9 (1-53)	<0.001
CD19 (%)	7 (1-21)	5 (0-66)	<0.001
NK (%)	6 (0.1-75)	3 (0.4-28)	<0.001
MNC apheresis product (×10⁹)	41.23 (0.64-95.1)	32.5 (0-102.4)	<0.001
MNC (%)	83 (34-98)	55 (0-96)	<0.001
Neutrophils (%)	17 (2-66)	45 (4-100)	<0.001
Ht (%)	4 (1-17)	6 (0.2-17)	<0.001
PLTs apheresis product (×10⁹/L)	1,850 (249-6,895)	2,023 (162-8,582)	0.0013

WBC: white blood cells; MNC: mononuclear cells; PLTs: platelets.

DISCUSSION

The major finding of our study is the demonstration that both donors who start HPC collection on the 5th day of treatment with G-CSF and those who start on the 4th day reach the target dose of CD34+ cells, without any statistically significant differences between the two groups. In addition, donors who started collection on the 4th day did not have a significantly higher risk of undergoing a second procedure.

In donors who started the collection on the 4th day, the possibility of a second apheresis when the number of CD34+ cells was still increasing would have made it easier to collect a very large number of cells, which is necessary, for example, in the case of high recipient weight, subsequent manipulation of the product, or haploidentical transplant[18,19].

Furthermore, if the donor is found to be a poor mobiliser, carrying out the first apheresis on the 4th day would make it possible to perform a rescue therapy with Plerixafor immediately (between the 4th and 5th day and not between the 5th and 6th, as reported by the current guidelines)[20], thus increasing the probability of reaching the target cell dose.

CONCLUSIONS

In conclusion, our data demonstrated that the target dose of CD34+ cells can be reached on the 4th day of G-CSF treatment without any significant differences to results from collection on day 5. The donor characteristics may also play a role in the difference in timing of the mobilisation procedure. On this basis, in collaboration with the Italian

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Society of Hemapheresis and Cellular Manipulation, we plan to perform a large multicentre prospective study to confirm our findings.

AUTHORSHIP CONTRIBUTIONS

CP, OI and MDI performed data analysis and revised the paper; CP, OI, LA, FMD, SG, MTM, MM, AO, CSa, LS, TT, MV and PA supervised donor enrollment, and collected and provided donors' data; RG carried out flow cytometry analyses; CP and CS wrote the paper; PA conceived, designed and supervised the study; all Authors have approved the final version of the manuscript.

Keywords: *apheresis, hematopoietic stem cell transplantation, hematopoietic progenitor cell mobilisation, G-CSF, donor safety.*

The Authors declare no conflicts of interest.

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