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EVALUATION OF PREDICTABILITY OF BONE MARROW CELL CELLULARITY FROM RELATED AND UNRELATED DONORS

Simone Pereira Lermontov[1], Cecilia Ferreira da Silva Borges[1], Simone Maradei Pereira[1], Marta Colares Nogueira[1], Luís Fernando da Silva Bouzas[1], Simone Carreiro Brasil[1], Cláudia Valeria Ramos Ribeiro[1], Karen Wagner de Souza[1], Pedro Felipe Couto Vieira[1], Carla Cristina Pedrosa de Lira de Morais[1], Leonardo Javier Arcuri[1]

1 Instituto Nacional de Câncer

Correspondence to: Simone Pereira Lermontov (simone.lermontov@inca.gov.br)

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ABSTRACT

Introduction: For successful hematopoietic stem cell transplantation, a minimum number of total nucleated cells should be obtained (TNC) during the bone marrow (BM) harvest procedure. Objectives: Evaluate the predictability of the BM TNC collected from an interim sample collected during the procedure and the factors related to high-cellular harvest. Methodology: This is a retrospective observational study including BM donors and recipient from 2017 to 2019. The final TNC was based on an interim quantification of TNC and was compared with the actual final TNC obtained. Results: 81 donors were included and interim TNC of 53 donors were available. Based on linear regression, a significant correlation was found between the volume of BM collected and the interim and final TNC (n=53; R2=0.83; P<0.001). The relationship between donor and recipient weight significantly influenced the collection yield. There was also a positive correlation between the volume of BM collected and the interim and final count of TNC (n=53; r=0.88; p<0.001). The difference in donor and recipient weight also had a positive correlation (p<0.02). Conclusion: Our results showed that interim TNC quantification can help to achieve a better performance during the procedure, allowing real-time re-estimation of the volume needed to be harvested.

Keywords: Hematopoietic Stem Cell Transplantation; Bone Marrow Harvest; Donors.

INTRODUCTION

Stem cells are probably the best-known cell type and has been used for over 50 years, mainly in diseases related to the hematopoietic and immune systems. Over the last decades, the method of collecting these cells has been refined, incorporating multiple steps to ensure the safety of donors and the best results for recipients[1].

A minimum number of total nucleated cells (TNC) during bone marrow (BM) collection procedure is necessary for a successful hematopoietic stem cell transplantation (HSCT). Some authors[2–4] state that the minimum TNC should be between 3 and $5x10^8$/kg and that values below $2x10^8$/kg are considered inadequate. Other studies[5,6] suggest that a higher dose of TNC may improve overall survival and reduce transplant-related mortality. There is a trend towards incorporating $CD34^+$ counts in the bone marrow harvest routine; some authors suggest that the minimum $CD34^+$ count should be between 2 and 4 x 10^6/kg for BM[2,7,8].

To improve the harvested TNC and $CD34^+$, there are mainly two possibilities: (1) to increase the harvested volume or (2) to increase the TNC concentration. The target volume of BM collection is empirically based on recipient weight, with a target of 15 to 20 mL/kg (usually limited to 20 mL/kg of the donor's weight). Although this method is widely used, a significant percentage (27%-50%) of recipients receive a relatively low dose of TNC ($<2.4x10^8$/kg)[6]. To obtain a higher dose of TNC, clinicians generally tend to collect as much BM as possible through multiple small-volumes BM aspirates and/or by using fenestrated needles. The former strategy can prolong anesthesia time, increase the number of puncture sites and increase blood loss in donors[5,6,9].

A large volume of BM can also be harmful for recipients, due to the risk of fluid overload. The most effective and safest way to reach the target dose is to increase the amount of TNC collected[6,10]. Therefore, this study aimed to evaluate the predictability of the total cellularity of the collected bone marrow from an interim sample collected during the procedure and to evaluate factors related to a bone marrow collection with a total nucleated cells (TNC) greater than $4x10^8$/kg of recipient's weight.

METHODOLOGY

This is a retrospective observational study, including related and unrelated hematopoietic stem cell transplant (HCT) donors from a public hospital in the Rio de Janeiro, Brazil. The data collection was from 2017 to 2019. The stem cell collection procedure was performed in the operating room, under general anesthesia, and the bone marrow was aspirated from the posterior iliac crests, bilaterally, using a needle with lateral holes and fenestrated (Argon Medical Devices). On average, 3 to 5 ml were aspirated per puncture in each iliac crest to avoid sample dilution.

The BM collection teams consisted of two physicians and two experienced nurses, two nursing technicians, and an anesthesiologist. The average time for each collection was estimated at 90 minutes, and the maximum time was 120 minutes. The targeted final collected BM volume was estimated by 20 mL per kg of the recipient's body weight, with a maximum amount of 20 mL per kg of the donor's body weight.

The aspirated BM was homogenized with an anticoagulant solution of heparin 5000UI/mL and 0,9% saline. The final concentration was 100 UI/ml (heparin and saline solution). The total volume of the solution used was 10% of the estimated volume for each BM collected. Closed-system (BioAccess®) collection bags and filters were used. The total volume of the harvested BM was obtained by subtracting the volume of anticoagulant from the volume of the mix.

Based on the study by Wang and Cols[6], our bone marrow transplant team in 2017 instituted an intermediate collection of TNCs when half of the total target volume (20 kg/mL of the recipient's weight) was reached. A 2 mL sample of the concentrate was withdrawn from the BM collection bag and placed in an EDTA tube. This sample was sent at the end of the procedure with the collection bag to the lab for leukometry analysis. The collection team was not aware of the interim count during the procedure.

$$Estimated\ final\ TNC = Interim\ TNC \times \frac{Final\ collected\ volume}{Interim\ collected\ volume}$$

To analyze the correlation between final and intermediate TNC, we included all donors with interim and final quantification (Spearman correlation). For analysis of factors associated with the amount of TNC collected, we included all adult donors and recipients. We did not include children, who are usually underweight, to avoid a biased results. For the analysis of factors associated with the amount of TNC collected, linear regression (TNC as a continuous variable) and logistic regression (TNC > $4.0x10^8$/kg being the outcome) were performed. We considered statistically significant two-sided P-values less than 0.05. Analyzes were performed using the R statistical program, version 4.1.0.

The study was approved by the local Research Ethics Committee and all donors signed an informed consent form, following the ethical precepts of research with human beings.

RESULTS

The characteristics of the 81 donors was separated in two groups (Table I). Group A included the donors with a TNC count ≥ $4x10^8$/kg and group B with a TNC count $<4x10^8$/kg. In both groups, there was a male predominance, the mean age of the groups was equivalent. Unrelated donors had a higher TNC count than related ones (P = 0.126).

The harvested volume was between 6.9 and 22.7 mL/kg, with a median of 15.4 (receiver weight), when recipients were heavier than donors. All harvested bone marrow had <20mL/kg of the donors' weight, following the standards of the National Marrow Donor Program (NMDP). The collected BM volumes

also did not have a statistical significance (P = 0.436) when group A (CNT ≥ 4 x10^8/kg) was compared with group B (CNT <4 x10^8/kg).

Regarding ABO compatibility, 66.7% of compatible donors had a TNC count ≥ 4x10^8/Kg, while 68.8% of non-compatible donors had a TNC count <4x10^8/Kg (P= 0.792). Among the 81 included donors, 53 had interim TNC quantification by the half-time of the collection. Based on linear regression, a significant correlation was found between the volume of BM collected and the intermediate and final count of TNC (n = 53; R2 = 0.83; P <0.001; Figure 1).

The relationship between donor and recipient weight significantly influenced the collection yield; only 7.7% of BM collections from donors who weighed less than their recipients achieved a TNC count ≥ 4×10^8/kg, compared with 84% of collections from donors heavier than their recipients (P<0.003). Figure 2 show that the weight difference between the donor and recipient achieved a positive relationship with the number of TNC. Donors weighing less than the recipient's weight had a lower count of 4x10^8/Kg TNC. Figure 3 shows a positive but weak correlation between the measurement of TNC and $CD34^+$ Cells (n = 53; R2 = 0.44).

DISCUSSION

Our results showed a positive correlation between the volume of bone marrow collection and the intermediate and final count of total nucleated cells (n = 53; r = 0.88; p <0.001). This suggests that if the intermediate count is implemented during the procedure in the operating room, it may contribute to achieving ideal cellularity (TNC ≥ 4x10^8/kg) in donors with lower-than-expected interim counts, in addition to saving donors from possible risks and side effects in those with higher-than-expected interim counts.

Other authors[6] who have implemented the interim quantification of TNC also found a positive correlation between interim and final total counts (r= 0.8774; p<0.001), corroborating our results and demonstrating that this strategy can be effective. However, we know that other issues can also influence the achievement of good cellularity, such as, the selection of the ideal donor (younger men and donor who weigh more than their recipients) as well as experienced centers and operators[3,5,9–12].

In our study, we did not find a significant correlation between TNC and donor gender (P = 0.722), despite the predominance of males. This brings us to other factors that can also influence BM collection, such as age; however, we didn't find a correlation between age and the TNC of donors (p=.094). A study[13] carried out in a cohort of donors from the National Marrow Donor Program identified a significant decrease in the quality of BM collections over time associated with older ages, male gender, and race, reinforcing the importance of care in donor selection. Unfortunately, data regarding donor race were incomplete, and thus comparisons of this variable were not possible.

There was a statistical positive correlation between donor and recipient weight (p<0.02); 84.6% of donors with a weight higher than recipients reached an ideal cellularity (>4x10^8/kg). Among the donors who weighed equal or less than the recipients, only 15.4% achieved this ideal cellularity. A study[10] with 110 unrelated donors revealed a significant impact on the discrepancy between donor and recipient weights on the amount of TNC collected: only 18% of collections from donors who were lighter than their recipient achieved an ideal TNC/kg. Whenever possible, donors with equivalent weight to or greater than the recipient should be selected. When this is not possible, the transplant center should consider the possibility of using peripheral blood stem cells instead of BM[5,9,10].

As expected, the correlation between $CD34^+$ and TNC at the end of BM collection was positive, but weak, preventing us from stating an increase in the number of $CD34^+$ cells with increasing number of TNC. In our study the volume collected did not influence TNC (P=0.436); smaller volumes had a higher TNC when compared with longer ones. Other authors[2,13,14] suggest that large volumes and longer collection times may result in a lower chance of obtaining the target dose of TNC. One hypothesis is that the low-volume bone marrow comes with less peripheral blood contamination, reducing the dilution of the final product[4,10].

In summary, our results showed a positive correlation between the TNCs in the middle and the TNCs at the end of bone marrow collection, which reminds us of the importance of quantifying these cells during the harvest period, possibly decreasing the procedure time and the risks for the donor or increasing the amount of BM harvested from donors that can tolerate larger volumes. However, we must carry on further research to assess at which point the collection can be safely interrupted for the donor, with enough number of TNC for the recipient to engraft.

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TABLE 1. Donor Characteristics and Results of BM Harvesting

Characteristic	Group A CNT ≥ 4 x108	Group B CNT <4 x108	P value
Total, n	45	36	
Age			0.094
Yr, median (SD)*	34 (11.5)	38.8 (13.6)	
Sex, n (%)			0.722
Male	28 (62.2)	21 (58.3)	
Female	17 (37.8)	15 (41.7)	
Types of donars n (%)			0.126
Related	15 (33.3)	20 (55.6)	
Unrelated	23 (51.1)	13 (36.1)	
haploidentical	7 (15.6)	3 (8.3)	
BM harvest volume (mL) n (%)			0.436
mean(SD)	1006.4 (371.3)	1066.6 (306.5)	
164 – 998	15	12	
1000 -1259	18	15	
1318 - 1548	11	9	
ABO compatibility n (%)			0.792
ABO match	26 (66.7)	22 (68.8)	
ABO major mismatch	6 (15.4)	6 (18.8)	
ABO minor mismatch	7 (17.9)	4 (12.5)	
Weight difference n (%)			0.003
Recipient heavier	3 (7.7)	6 (17.1)	
Equal weight, up to 5 kg	3 (7.7)	12 (34.3)	
Donor heavier	33 (84.6)	17 (48.6)	

*SD- Standard deviation

FIG.1- Correlation between midway and final BM cell density (n = 53; r = 0.83; P<0.001).

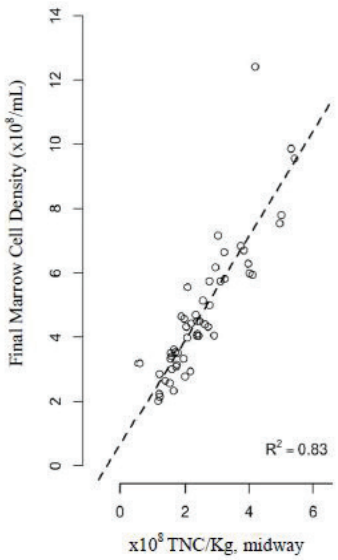

FIG.2 - Correlation between the difference weight of the donor and the recipient and the number of total nucleated cells.

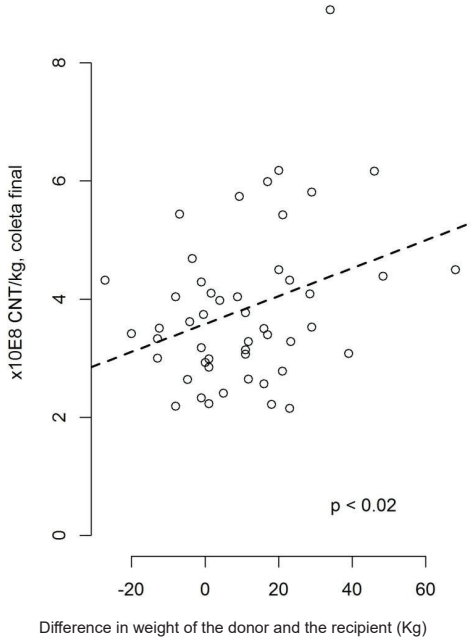

FIG.3 - Correlation between CD34+ and CNT at final of CTH collection.