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Can the bone marrow harvest volume be reduced safely in hematopoietic stem cell transplantation with pediatric sibling donors?

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Background
Reduced harvest volumes in pediatric donors appear to have the potential to reduce donor-associated risks while maintaining engraftment in recipients; however, the allowable harvest volume reduction remains undefined.

Methods
We retrospectively analyzed the data pairs of 553 bone marrow (BM) harvests from pediatric (age at harvest <18 yr) sibling donors and clinical outcomes of 553 pediatric (age at infusion <14 yr) transplant-naïve recipients to assess the optimal BM harvest volume needed from pediatric donors to obtain the desired CD34+ cell count ($\geq 3.0\times10^6$ cells per kg of recipient weight), and to study its impact on the clinical outcomes of transplantation in pediatric recipients.

Results
The minimum desired CD34+ cell count of $\geq 3.0\times10^6$ per kg of recipient weight was achieved for 506 (95.3%) of donor-recipient pairs. The median CD34+ cell yield was 6.4×10^6 per kg of recipient weight (range, 1.2–33.8×10^6) in donors younger than 5 years old at harvest, 4.7×10^6 (range, 0.3–28.5×10^6) in donors aged 5–10 years and 2.1×10^6 (range, 0.3–11.3×10^6) in donors older than 10 years ($P<0.001$).

Conclusion
The infused CD34+ cell dose ($\times10^6$ cells/kg of recipient weight) had no impact on GRFS; however, a CD34+ cell dose of $>7\times10^6$ cells/kg of recipient weight did not improve hematopoietic recovery.

Key Words Marrow transplant, Harvest volume, Pediatric donors

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is the only treatment modality that provides a cure or produces long-term survival for a variety of pediatric diseases, including certain hematologic malignancies, bone marrow (BM) failure, hemoglobinopathies, immunodeficiencies, and metabolic disorders [1, 2]. The development of BM, umbilical cord blood, and mobilized peripheral blood as graft sources for hematopoietic stem cell transplantation has occurred [3]. Despite the increased use of mobilized peripheral blood and umbilical cord blood, BM remains the primary graft source in pediatric patients [2]. HLA-matched siblings are considered the best donors for HSCT due to the reduced risk of transplant-related complications and improved clinical outcomes [1, 2, 4]. BM donation is a well-tolerated procedure with few complications, most of which are perioperative events, including anesthesia-related events, postoperative pain, and anemia [5, 6]. Stroncek *et al.* [7] reported a significant correlation between the marrow harvest procedure duration and anesthesia duration with donor pain and fatigue following BM donation; however, BM harvest procedure duration had the highest correlation with post-harvest pain and fatigue.

The National Marrow Donor Program (NMDP) recom-

mends that the collected BM volume not exceed 20 mL/kg of donor body weight to minimize the risk associated with the BM harvesting procedure on the donor and to have a sufficient volume of collected BM; this volume was determined based on adult donors and is suitable for children as well [6]. Furey *et al.* [6] developed a potential algorithm for calculating the BM harvest volume. The proposed algorithm revealed that BM harvest volume required to achieve the desired CD34+ cell count ($\times10^6$ cells/kg of recipient weight) depends on the donor age, and harvesting up to 20 mL/kg of donor weight may not be necessary for all pediatric donors. This may result in a smaller BM harvest volume without pediatric transplant outcome. This algorithm needs large-scale validation.

Age-related changes in human hematopoietic stem/progenitor cells have been reported [8], and Yabe *et al.* [9] confirmed that pediatric donors younger than 5 years provide a larger number of nucleated cells and CD34+ cells relative to their body weight than older pediatric BM donors, which may enable a smaller harvest volume. Collecting a smaller harvest volume would benefit donors by decreasing the anesthesia duration, reducing the risk of blood allotransfusion, decreasing post-procedure pain, and shortening the recovery period [6]. However, Bittencourt *et al.* [10] reported that a CD34+ cell dose of 3×10^6 cells per kg of recipient weight or more from a matched sibling donor improved hematopoietic recovery in pediatric and adult patients.

This retrospective study determined the optimal amount of BM for pediatric recipients from matched sibling donors. Additionally, we examined transplant survival, post-transplant infections in the first 100 days, and Graft-Versus-Host Disease (GVHD) free or relapse-free survival (GRFS) to CD34+ cell dose per kg of recipient weight. We validated the algorithm proposed by Furey *et al.* [6] to determine the BM volume for harvesting 5×10^6 CD34+ cells per kg based on recipient weight (mL/kg of donor weight) [(recipient weight×5.0)÷(donor weight×0.7), (recipient weight×5.0)÷(donor weight×0.36), and (recipient weight×5.0)÷(donor weight×0.3) for corresponding donor age groups <6, 6–12, and >12 yr, respectively].

MATERIAL AND METHODS

Study design

In this retrospective study, we reviewed the medical records of all pediatric sibling donors (age at harvest <18 yr) who had undergone a single BM harvest between January 2007 and December 2017 at our center. The medical records of all consecutive pediatric transplant-naïve recipient-donor pairs were obtained from the computerized patient information management system. Data of the volume of BM harvested relative to donor weight, harvest-related hospitalization days and side effects, transplant characteristics, and outcome-related parameters for recipients were collected. The parents or legal guardians provided written informed consent as per institutional practice.

BM harvest procedure

Multiple punctures were used to harvest BM from the posterior iliac crest under general anesthesia. With each attempt, a volume of approximately 5 mL was aspirated [11, 12]. The target volume was 15–20 mL/kg of recipient weight and did not exceed 20 mL/kg of donor weight [13]. The BM product was processed in a stem cell laboratory, and flow cytometry was used to count CD34+ and CD3+ cells.

Definitions

We used a range of ±5 kg to determine whether the donor's weight was the same, lower, or higher than that of the recipient. Overall survival (OS) was defined as survival with or without the primary disease. However, transplant-related mortality (TRM) was defined as death due to any cause other than relapse of malignant disease. Acute GVHD was defined according to the method described by Glucksberg *et al.* [14]. Chronic GVHD was defined in accordance with the National Institutes of Health guidelines [15]. The time to neutrophil engraftment is the first day of achieving an absolute neutrophil count $\geq0.5\times10^9$/L for three consecutive days from the time of BM infusion. The time to platelet engraftment was the first day of platelet count $>20\times10^9$/L without transfusion for 7 days after BM infusion. The CD34+ cell dose was $\times10^6$ cells/kg of recipient weight. A composite index of GRFS was defined as survival in the absence of grade III–IV acute GVHD, systemic therapy-requiring chronic GVHD, relapse of malignant disease, rejection of the graft in patients with a nonmalignant disorder, or death from any cause during the first year after allogeneic transplantation [16]. Event-free survival (EFS) was defined as survival in the absence of any of the following during follow-up: relapse of primary disease or rejection of the graft (primary and secondary), development of a new malignancy, or death from any cause throughout the course of follow-up.

Infection episodes in recipients

All infections in transplant recipients from day 0 to day 100 post-transplantation were reviewed. Bacteremia was defined as the presence of viable bacteria in the blood. Viremia was defined as a cytomegalovirus load >500 copies/mL, an Epstein-Barr virus load $>1,000$ copies/mL, or an adenovirus load $>1,000$ copies/mL based on PCR results [17]. Definitions of invasive fungal infections were performed as previously described by Satwani *et al.* [18].

Statistical analysis

Descriptive statistics for continuous variables were provided as medians and ranges, and categorical variables were presented as numbers and percentages. We used the chi-square or Fisher's exact test to test the significance of the associations between categorical variables. Independent-sample Mann–Whitney U tests and Kruskal–Wallis tests were used to test the significance of the differences between two and more than two categories of continuous variables, respectively. Spearman's correlation coefficient was used to calculate correlations between continuous variables. Binary

logistic regression was used to test the effects of CD34+ cell dose per kg of recipient weight on GVHD, survival, and GRFS. SPSS Statistics for Windows (version 20.0, IBM Corp., Armonk, NY, USA) was used to analyze the data. $P<0.05$ was considered to be statistically significant.

RESULTS

Recipients' characteristics

We included the data of 531 donor-recipient pairs in this study. The median age of the recipients was 4.4 years, and >50% of them were boys (N=298, 56.1%). Most recipients underwent transplantation for nonmalignant disorders. Table 1 shows demographic and transplant characteristics. The median CD34+ cell dose infused was 7.0×10^6 (1.3–17.7) per kg of recipient weight.

Donor characteristics and BM harvest volume

The study cohort comprised all pediatric (age at harvest <18 yr) sibling donors who underwent a single BM harvest and their recipients. Of the 531 donor-recipients pairs, 276 (52.0%) donors were boys, 146 (27.5%) were younger than 5 years old at harvest, 182 (34.3%) were between 5 and 10 years old, and the remaining 203 (38.2%) were 10 years old or older; the median donor age was 8.4 years (1.0–18.0), 66 (12.4%) donors weighed less than their recipients, 108 (20.3%) had equivalent weights, and 357 (67.2%) weighed more.

The median BM volume harvested per kg of donor weight was significantly higher for lower-weight donors than for equivalent or higher-weight donors (15.7 mL/kg vs. 14.6 mL/kg or 9.0 mL/kg, respectively, $P<0.001$). The median volume of BM harvested per kg of recipient weight was significantly higher in higher-weight donors than in equivalent-weight or lower-weight donors (22.3 mL/kg vs. 14.8 mL or 8.7 mL/kg, respectively, $P<0.001$).

Table 1. Demographic and transplants characteristics of the recipients (N=531).

Variables of interest	Observations
Age at infusion, year, median (range)	4.4 (0.04–17.4)
Male, sex, N (%)	298 (56.1%)
Malignant and pre-malignant disease, N (%)	125 (23.5%)
Acute lymphoblastic leukemia	53 (42.4%)
Acute myelogenous leukemia	45 (36.0%)
Chronic myeloid leukemia	7 (5.6%)
Myelodysplastic syndromes	8 (6.4%)
Other leukemia	7 (5.6%)
Hodgkin's disease	1 (0.8%)
Non-Hodgkin's lymphoma	4 (3.2%)
Nonmalignant disease, N (%)	406 (76.5%)
Immunodeficiency	159 (39.2%)
Hemoglobinopathies	108 (26.6%)
Bone marrow failure including severe aplastic anemia	86 (21.2%)
Histiocytic disorders	29 (7.1%)
Metabolic disorders	19 (4.7%)
D isorders related to platelet defects	5 (1.2%)
Conditioning regimen, N (%)	
No conditioning	67 (12.6%)
Myeloablative	451 (84.9%)
Reduced intensity	13 (2.4%)

Hemoglobin levels and Packed Red Blood Cells (PRBC) transfusion

The median hemoglobin (Hb) levels before harvest were significantly lower in lower-weight donors than in equivalent or higher-weight donors (115.0 vs. 119.0 or 125.0 g/L, respectively, $P<0.001$), and the same was true for post-harvest levels (lower weight: 97.0 vs. equivalent weight: 97.0 vs. higher weight: 110.0 g/L, $P<0.001$). Additionally, the median decrease in hemoglobin levels after harvest was significantly different among the three donor weight groups (lower weight: 20.0 vs. equivalent weight: 21.0 vs. higher weight: 16.0 g/L, $P<0.001$).

Given the indication for PRBC transfusion, including symptomatic anemia (hypotension not responding to fluid boluses, tachycardia) or Hb below 80 g/L, 47 (8.9%) donors required allogeneic PRBC transfusions post-harvest. The need for PRBC transfusion for donors was significantly associated with their age group at harvest ($P<0.001$); donors younger than 5 years old had the highest association with the need for PRBC transfusion (N=30, 20.5%), followed by donors between 5 and 10 years (N=13, 7.1%), and then those ≥10 years old (N=4, 2.0%). The median BM harvest volume relative to donor weight (kg) among donors requiring PRBC transfusion was 17.0 mL/kg (8.1–21.8 mL/kg), while that among those donors who did not require PRBC transfusion was 10.2 mL/kg (1.4–21.0, $P<0.001$).

For post-harvest PRBC-transfused donors, the median pre- and post-harvest hemoglobin concentrations were 112.0 g/L (91–143 g/L) and 89.0 g/L (75.0–128.0 g/L), respectively, with a median decrease in the hemoglobin level of 26.0 g/L (2.0–62.0 g/L). The median pre-harvest hemoglobin concentration, post-harvest hemoglobin concentration, and decrease in the hemoglobin level were 124 g/L (91.0–169.0 g/L), 106.0 g/L (66.0–152.0 g/L) and 18.0 g/L (0.0–51.0 g/L), respectively, for donors who did not require post-harvest PRBC transfusion ($P<0.001$, <0.001, and 0.007, respectively). The need for PRBC transfusion was also significantly associated with donor weight at harvest ($P<0.001$); 17 (25.8%) lower-weight donors, 23 (21.3%) equivalent-weight donors, and 7 (2.0%) higher-weight donors required PRBC transfusion.

CD34+ cell yield (10^6 cells per kg of recipient weight) relative to donor age

The CD34+ cell yield per kilogram of recipient weight was significantly higher in younger donors. In particular, the median CD34+ cell yield was 6.4×10^6 ($1.2–33.8\times10^6$) in donors younger than 5 years old at harvest, while the yields were 4.7×10^6 ($0.3–28.5\times10^6$) for those aged 5–10 years and

2.1×10^6 ($0.3–11.3\times10^6$) for those older than 10 years of age ($P<0.001$).

CD34+ cell yield (10^6 cells per kg of recipient weight) relative to harvest volume

Harvested BM was able to satisfy the minimum CD34+ cell dose of $\geq3.0\times10^6$ per kg of recipient weight [19] in 506 (95.3%) paired recipients. In this subgroup of 506 donor-recipient pairs, the median CD34+ cell yield ($\times10^6$ per kg of recipient weight) was higher for the youngest donor group (8.0 vs. 7.5 vs. 7.0, P=0.350) and donors who weighed more than the corresponding recipient (lower weight: 5.1 vs. equivalent weight: 6.4 vs. higher weight: 8.1, $P<0.001$, Table 2). A weak negative correlation between the BM harvest volume (mL/kg) per donor weight and CD34+ cell yield $\times10^6$ per kg of recipient weight was observed (Spearman's rho=-0.154, $P<0.001$). Fig. 1 shows a scatterplot depicting ANC recovery with respect to the total volume harvested per donor weight and CD34+ cell dose $\times10^6$ per kg of recipient weight.

Hematopoietic cell recovery in recipients

The median time for neutrophil engraftment was 16 days (10–37), and platelet recovery was 29 days (6–148 days). No correlation was found between the infused CD34+ cell dose ($\times10^6$ per kg of recipient weight) and platelet recovery time (Spearman's rho=0.02, P=0.686). However, the infused CD34+ cell dose ($\times10^6$ per kg of recipient weight) was negatively correlated with ANC recovery time (Spearman's rho=-0.105, P=0.024). However, the malignant and nonmalignant subgroups differed in transplant analysis. The median time for neutrophil engraftment for recipients with the malignant disease was 17 days (10–49 days) compared with 15 days (10–51 days) for recipients with a nonmalignant disorder ($P<0.001$). The median time to platelet engraftment for recipients with the malignant disease was 26 days (15–110 days) versus 31 days (6–148 days) for recipients with the nonmalignant disorders (P=0.002).

Among all recipients, ANC engraftment by day +28 was observed in 450 (84.7%), whereas 72 (13.6%) did not achieve this milestone, and late ANC engraftment (beyond day +28) was recorded in 9 (1.7%). The median CD34+ cell dose infused ($\times10^6$ per kg of recipient weight) was higher in the recipients who achieved ANC engraftment by day +28 than in those who achieved engraftment after day +28 [6.8×10^6, (1.5–17.22) vs. 6.5×10^6, (1.3–11.0), P=0.239]. However, the infused CD34+ cell dose ($\times10^6$ per kg of recipient weight) was significantly lower in those who achieved ANC engraftment by day +28 than in those who did not achieve this milestone [6.8×10^6, (1.3–17.2) vs. 9.0×10^6, (3.0–17.7), $P<0.001$]. We classified ANC engraftment according to the CD34+ cell dose infused ($\times10^6$ per kg of recipient weight): $<4\times10^6$ (N=58), 4 to $<7\times10^6$ (N=203), 7 to $<10\times10^6$ (N=167), and 10×10^6 and above (N=103). The highest decline in the ANC engraftment rate of 14.9% relative to the CD34+ cell dose ($\times10^6$ per kg of recipient weight) infused was observed in the 7 to $<10\times10^6$ category, which ranged from 84.45 to 71.8%. A lower cell dose correlated with ANC engraftment ($P<0.001$; Fig. 2).

Infections during the first 100 days post-infusion

Infections during the first 100 days after transplantation were recorded in 168 (31.6 %) recipients. Of the 247 isolates, 156 (63.2%) were bacterial, 51 (20.6%) were viral, 38 (15.4%) were fungal, and 2 (0.8%) were parasitic. No significant association between the CD34+ cell dose infused ($\times10^6$ per kg of recipient weight) and the incidence of infection was found (odds ratio, 0.982; 95% CI, 0.939–1.03; P=0.443).

Survival and GRFS

With a median follow-up time of 64.3 months (95% CI, 58.5–70.0 mo) and 100 mortality events, the 3- and 5-year cumulative OS probabilities for our cohort were 81.7±1.7% (events=95) and 80.6±1.8% (events=99), respectively. The 1-year TRM was 15.6% (N=82). The infused CD34+ cell ($\times10^6$ per kg of recipient weight) and TNC (per kg of recipient

Table 2. Harvest volume (mL) per donor age (yr) and weight (kg) for minimum CD34+ cell dose of $\geq3.0\times10^6$ per kg of recipient weight (N=506) with respect to actual CD34+ cell yield $\times10^6$ per kg of recipient weight.

	Harvests meeting minimum CD34+ dose required and CD34+ cell yield (per kg recipient)		Harvest volume (mL/kg donor)	CD34+ cell yield (per kg donor)
	N=506	Median (range)	Median (range)[a]	Median (range)[a]
Donor age, years (N)				
Less than 5 (N=146)	140 (95.9%)	8.0 (3.3–30.7)[b]	12.8 (3.8–21.8)	6.5 (1.2–33.8)
5–10 (N=182)	179 (98.4%)	7.5 (3.1–31.9)[b]	11.6 (1.7–21.2)	4.7 (0.3–28.5)
10 and above (N=203)	187 (92.1%)	7.0 (3.0–28.0)[b]	8.1 (1.4–20.1)	2.2 (0.3–11.3)
Donor's weight (kg) compared to Rec (N)				
Lower (N=66)	60 (90.9%)	5.1 (3.2–15.8)[a]	15.6 (8.3–20.7)	9.4 (4.5–33.8)
Equivalent within ±5 kg (N=108)	104 (96.3%)	6.4 (3.6–24.8)[a]	14.2 (5.8–21.8)	6.5 (3.2–16.6)
Higher (N=357)	342 (95.8%)	8.1 (3.0–31.9)[a]	9.0 (1.4–19.0)	2.9 (0.3–28.5)

[a] $P<0.001$. [b] P=0.350.

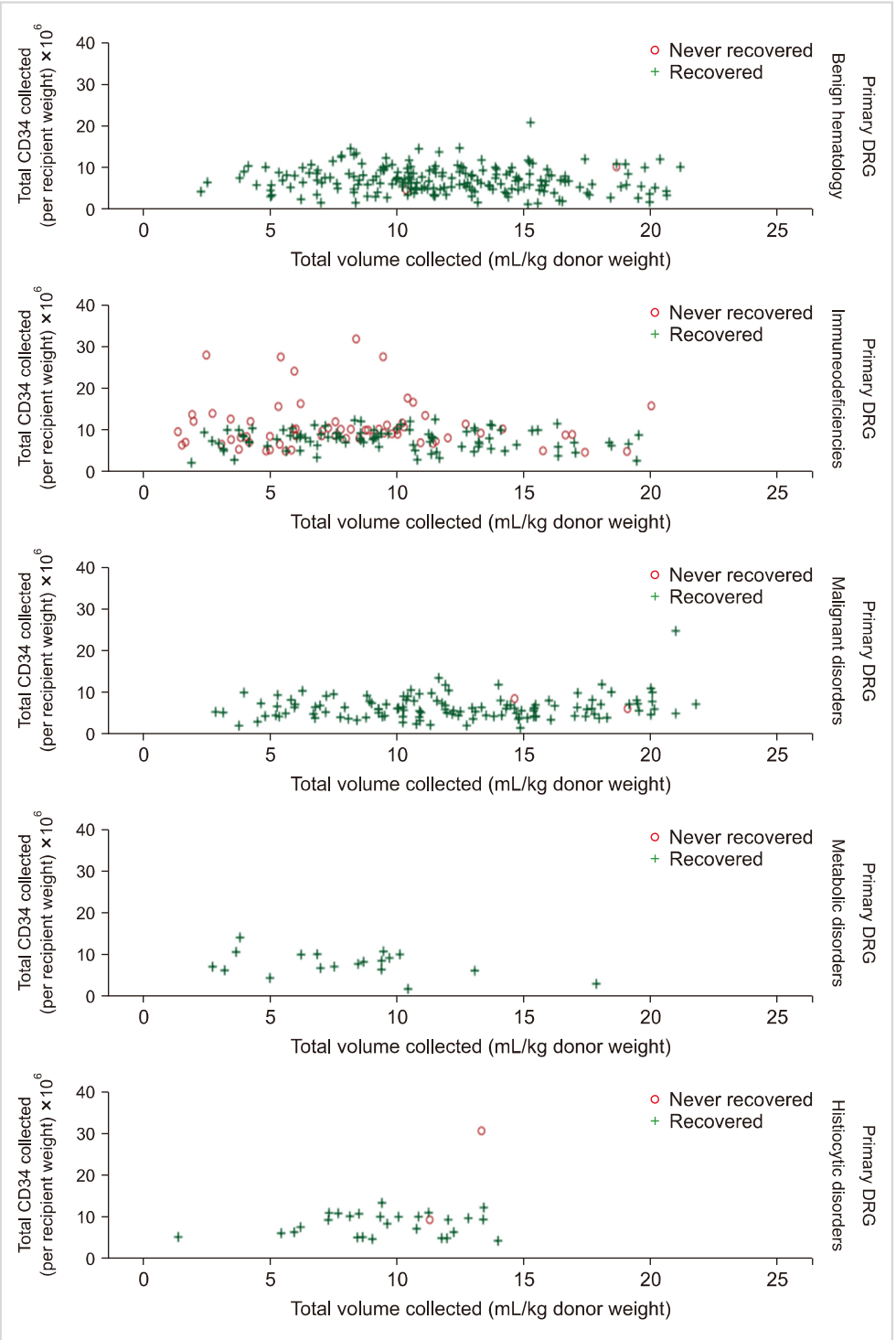

Fig. 1. Harvest volume mL/kg per donor weight by CD34+ cell dose ($\times 10^6$ cells per kg of recipient weight).

weight) doses were not associated with overall mortality (P=0.589 and P=0.991, respectively). The GRFS rate was 73.8% (139 events) during the follow-up period. Last follow-up EFS was 73.8% (119 events). The infused CD34+ cell dose ($\times 10^6$ per kg of recipient weight) was not significantly associated with GRFS (P=0.829). The incidence of overall acute GVHD (grades I–IV) was 23.9% (N=127), of which grade III–IV events accounted for 24.4% (N=31). Additionally, the CD34+ infused cell dose ($\times 10^6$ per kg of recipient weight) was not found to be associated with the incidence of acute GVHD, severe acute GVHD (grades III–IV only), or chronic GVHD (P=0.541, 0.645, and 0.833, respectively). Based on chimerism studies, our recipients day +100 engraftment rate was 97.7% (N=508); 9 (1.7%) had primary graft failure, and 3 (0.6%) had secondary graft failure. We could not evaluate 11 (2.1%) recipient's engraftment status on day +100 because they died early.

Comparison of the harvest volume with the NMDP Guidelines

The BM volume harvested from the sibling donors at our institution was within ±2.0 mL/kg of donor weight, as specified in the NMDP guidelines of up to 20 mL/kg of donor weight. However, compared with the optimum harvest volume calculated according to the formula proposed by Furey *et al.* [6], our harvest volume was equivalent (within ±2.0 mL/kg of donor weight) in 149 (28.1%) donors, lower in 43 (8.1%) donors, and higher in the remaining 339 (63.8%) donors. Upon subdividing the donors harvested higher than that proposed by Furey *et al.* [6] into three categories and then analyzing for ANC engraftment, we observed that subsequent increases above ±2.0 mL/kg of equivalent harvest volume, when infused in recipient pairs, did not produce more favorable results in terms of overall ANC recovery (P=0.001; Fig. 3, Table 3) and non-primed donors (P=0.017; Table 3).

DISCUSSION

This is among the most extensive retrospective single-center studies to date, reporting and analyzing the relationship between BM harvest volume and infused CD34+ cell doses ($\times10^6$ per kg of recipient weight) in the pediatric population. In this study, several important observations were made. A CD34+ cell count of $>7\times10^6$ per kg of recipient weight was not associated with improved hematopoietic recovery. Harvesting Large volumes may result in an unnecessarily high CD34+ cell count, and the volume of BM harvested

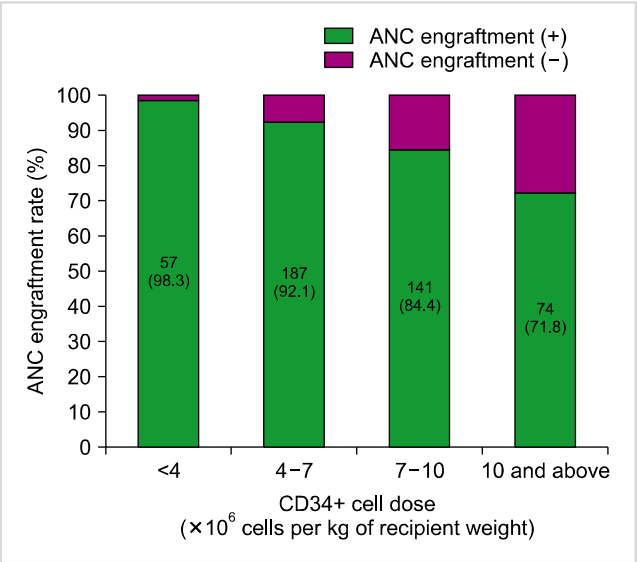

Fig. 2. CD34+ cell dose ($\times10^6$ cells per kg of recipient weight) by ANC engraftment.

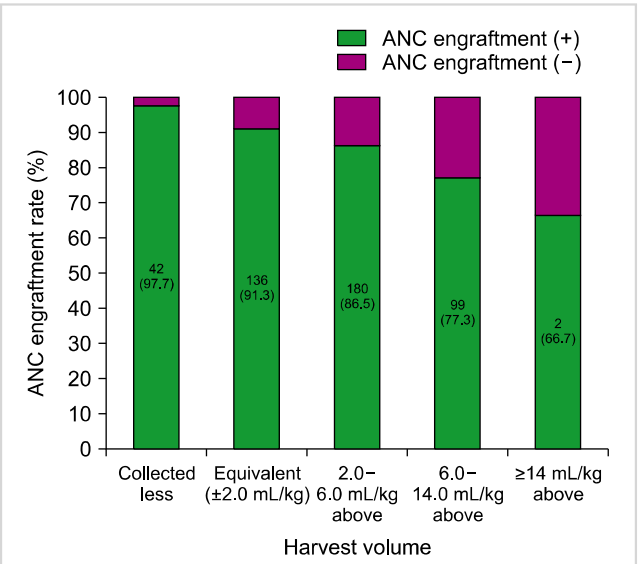

Fig. 3. Volume harvested by ANC engraftment rate compared with proposed optimal harvest volume quantification by Fury *et al.* [6].

Table 3. Harvest volume (mL/kg per donor weight) by ANC engraftment.

	ANC recovery (-)(N=72)	ANC recovery (+)(N=459)	Total (N=531)	*P*
Harvest volume per donor weight (N=531)				0.001
Less than equivalent	1 (2.3%)	42 (97.7%)	43 (8.1%)	
Equivalent[a]	13 (8.7%)	136 (91.3%)	149 (28.1%)	
2–6 mL above equivalent	28 (13.5%)	180 (86.5%)	208 (39.2%)	
6–14 mL above equivalent	29 (22.7%)	99 (77.3%)	128 (24.1%)	
≥14 mL above equivalent	1 (33.3%)	2 (66.7%)	3 (0.6%)	
Harvest volume per donor weight (non-primed only)	N=64	N=264	N=328	0.017
Less than equivalent	0 (0.0%)	8 (100.0%)	8 (2.4%)	
Equivalent[a]	12 (13.2%)	79 (86.8%)	91 (27.7%)	
2–6 mL above equivalent	25 (17.7%)	116 (82.3%)	141 (43.0%)	
6–14 mL above equivalent	27 (31.4%)	59 (68.6%)	86 (26.2%)	
≥14 mL above equivalent	0 (0.0%)	2 (100.0%)	2 (0.6%)	

[a] ±2.0 mL/kg calculated as proposed by Furey *et al.* [6].

can be safely reduced for younger donors. The proposed Furey *et al.* [6] algorithm may provide guidelines for determining the BM harvest volume required to obtain the desired CD34+ cell dose ($\times 10^6$ per kg of recipient weight), resulting in a safe reduction in the harvested volume in pediatric donors.

BM donation is a safe and well-tolerated procedure. In a prospective study conducted by the European Group for Blood and Marrow Transplantation Pediatric Diseases Working Party, the risk of blood transfusion after BM harvest was associated with donors younger than 4 years and a BM harvest volume exceeding 20 mL/kg [2]. In our study, the need for allogeneic blood transfusions was significantly associated with age at harvest, especially in donors younger than 5 years old and donors with disproportionately low weight relative to the recipient's weight. This may lead to careful consideration of the maximum volume of BM that can be harvested from young children.

The impact of infused CD34+ cell dose ($\times 10^6$ per kg of recipient weight) on clinical outcomes in children undergoing allogeneic HSCT, mainly with unrelated donors, has been reported. PBSCT and UD BMT showed that infusion of a higher CD34+ cell dose ($\times 10^6$ per kg of recipient weight) is associated with improved patient outcomes [20, 21]. A few studies have reported the outcome of CD34+ cell doses from a matched sibling donor, and a recommended dose of $\geq 3\times 10^6$ CD34+ cells per kg of recipient weight was associated with hematopoietic reconstitution [6, 10]. We found that the infused CD34+ cell dose ($\times 10^6$ per kg of recipient weight) was not associated with recipient OS, EFS, or GRFS. We did not observe any correlation between the infused CD34+ cell dose ($\times 10^6$ per kg of recipient weight) and platelet recovery time or the incidence of infections in the first 100 days. Our data showed that a CD34+ cell dose of $>7\times 10^6$ per kg recipient weight did not improve hematopoietic recovery in children with an HLA-matched sibling donor. As previously reported [9, 19, 22], our data confirm that a CD34+ cell dose ($\times 10^6$ per kg of recipient weight) is significantly related to donor age; donors younger than 5 years yielded higher concentrations of CD34+ cells, suggesting that the harvested volume can be reduced safely without a negative impact on the CD34+ cell count [6, 9].

Increasing collected BM volume boosts CD34+ cell yield. However, increasing the collected volume can increase anesthesia time, puncture sites, and blood loss [23]. Our data showed that in BM harvested at volumes up to 20 mL/kg of donor weight, CD34+ cell doses $\geq 3\times 10^6$ per kg of recipient weight were collected from 506 (95.3%) donors.

Few studies demonstrated a positive correlation between the total harvested BM and TNC number [12, 24]. However, we found no positive correlations between the total BM volume and TNC number or CD34+ cell count ($\times 10^6$ per kg of recipient weight), suggesting that the collected BM volume can be decreased in pediatric donors, especially in those below 5 years.

Using our data, we validated the algorithm proposed by Furey *et al.* [6] regarding the optimal dose of CD34+ cells ($\times 10^6$ per kg of recipient weight) and BM volume. We demonstrated that incremental increases in harvested volume did not provide more favorable results in ANC recovery, further emphasizing that BM harvesting at volumes up to 20 mL/kg of a child donor weight might not be necessary for optimal clinical outcomes in every pediatric recipient.

In conclusion, this is the largest retrospective study to report and analyze the relationship between marrow harvest volume and CD34+ cell dose ($\times 10^6$ per kg of recipient weight) in the pediatric population from a single transplant center. Unmanipulated CD34+ cell dose $>7\times 10^6$ per kg of recipient weight did not improve hematopoietic recovery. It was not correlated with platelet recovery time, infectious toxicity, GVHD incidence, mortality, or GRFS. Small BM can safely be harvested in younger donors. The proposed algorithm could determine the BM harvest volume needed to obtain the desired CD34+ cell dose, reducing the harvested volume safely in pediatric donors. A larger observational study is needed to determine the ideal minimum CD34+ cells dose for pediatric sibling allogeneic HSCT.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Behfar M, Faghihi-Kashani S, Hosseini AS, Ghavamzadeh A, Hamidieh AA. Long-term safety of short-term administration of filgrastim (rhG-CSF) and leukophresis procedure in healthy children: application of peripheral blood stem cell collection in pediatric donors. Biol Blood Marrow Transplant 2018;24:866-70.
2. Styczynski J, Balduzzi A, Gil L, et al. Risk of complications during hematopoietic stem cell collection in pediatric sibling donors: a prospective European Group for Blood and Marrow Transplantation Pediatric Diseases Working Party study. Blood 2012;119:2935-42.
3. Panch SR, Szymanski J, Savani BN, Stroncek DF. Sources of hematopoietic stem and progenitor cells and methods to optimize yields for clinical cell therapy. Biol Blood Marrow Transplant 2017;23:1241-9.
4. American Academy of Pediatrics. Committee on Bioethics. Children as hematopoietic stem cell donors. Pediatrics 2010;125:392-404.
5. Deotare U, Al-Dawsari G, Couban S, Lipton JH. G-CSF-primed bone marrow as a source of stem cells for allografting: revisiting the concept. Bone Marrow Transplant 2015;50:1150-6.
6. Furey A, Rastogi S, Prince R, et al. Bone marrow harvest in pediatric sibling donors: role of granulocyte colony-stimulating factor priming and $CD34^+$ cell dose. Biol Blood Marrow Transplant 2018;24:324-9.
7. Stroncek DF, Holland PV, Bartch G, et al. Experiences of the first 493 unrelated marrow donors in the National Marrow Donor Program. Blood 1993;81:1940-6.

8. Kuranda K, Vargaftig J, de la Rochere P, et al. Age-related changes in human hematopoietic stem/progenitor cells. Aging Cell 2011;10:542-6.
9. Yabe M, Morimoto T, Shimizu T, et al. Feasibility of marrow harvesting from pediatric sibling donors without hematopoietic growth factors and allotransfusion. Bone Marrow Transplant 2014;49:921-6.
10. Bittencourt H, Rocha V, Chevret S, et al. Association of CD34 cell dose with hematopoietic recovery, infections, and other outcomes after HLA-identical sibling bone marrow transplantation. Blood 2002;99:2726-33.
11. Lannert H, Able T, Becker S, et al. Optimizing BM harvesting from normal adult donors. Bone Marrow Transplant 2008;42:443-7.
12. Lisenko K, Stadtherr P, Bruckner T, et al. Bone marrow harvesting of allogeneic donors in an outpatient setting: a single-center experience. Biol Blood Marrow Transplant 2016;22:470-4.
13. Frangoul H, Nemecek ER, Billheimer D, et al. A prospective study of G-CSF primed bone marrow as a stem-cell source for allogeneic bone marrow transplantation in children: a Pediatric Blood and Marrow Transplant Consortium (PBMTC) study. Blood 2007; 110:4584-7.
14. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 1974;18:295-304.
15. Jagasia MH, Greinix HT, Arora M, et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in chronic graft-versus-host disease: I. The 2014 Diagnosis and Staging Working Group report. Biol Blood Marrow Transplant 2015;21:389-401, e1.
16. Holtan SG, DeFor TE, Lazaryan A, et al. Composite end point of graft-versus-host disease-free, relapse-free survival after allogeneic hematopoietic cell transplantation. Blood 2015;125: 1333-8.
17. Rustia E, Violago L, Jin Z, et al. Risk factors and utility of a risk-based algorithm for monitoring cytomegalovirus, Epstein-Barr virus, and adenovirus infections in pediatric recipients after allogeneic hematopoietic cell transplantation. Biol Blood Marrow Transplant 2016;22:1646-53.
18. Satwani P, Baldinger L, Freedman J, et al. Incidence of viral and fungal infections following busulfan-based reduced-intensity versus myeloablative conditioning in pediatric allogeneic stem cell transplantation recipients. Biol Blood Marrow Transplant 2009;15:1587-95.
19. Ince EU, Ileri T, Dogu F, et al. The impact of donor age and sex on the nucleated cell count and CD34 count in healthy bone marrow donors. Pediatr Transplant 2015;19:385-90.
20. Kałwak K, Porwolik J, Mielcarek M, et al. Higher CD34(+) and CD3(+) cell doses in the graft promote long-term survival, and have no impact on the incidence of severe acute or chronic graft-versus-host disease after in vivo T cell-depleted unrelated donor hematopoietic stem cell transplantation in children. Biol Blood Marrow Transplant 2010;16:1388-401.
21. Lee JW, Kim SK, Jang PS, et al. Impact of CD34+ cell dose in children who receive unrelated PBSCT with in vivo T-cell depletion for hematologic malignancies. Bone Marrow Transplant 2015;50:68-73.
22. Pulsipher MA, Levine JE, Hayashi RJ, et al. Safety and efficacy of allogeneic PBSC collection in normal pediatric donors: the pediatric blood and marrow transplant consortium experience (PBMTC) 1996-2003. Bone Marrow Transplant 2005;35:361-7.
23. Wang TF, Chu SC, Chen SH, et al. The effect of different harvest strategies on the nucleated cell yields of bone marrow collection. Biol Blood Marrow Transplant 2011;17:351-5.
24. Shaughnessy PJ, Kissack B, Bickford DJ, et al. Correlation of CD34+ cell counts with volume of bone marrow collected for allogeneic bone marrow harvests. J Hematother Stem Cell Res 2000;9:891-4.