HHS Public Access

Author manuscript

Pediatr Transplant. Author manuscript; available in PMC 2021 August 13.

Published in final edited form as:

Pediatr Transplant. 2018 December ; 22(8): e13287. doi:10.1111/petr.13287.

The effect of bone marrow graft composition on pediatric bone marrow transplantation outcomes

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Abstract

Hematopoietic stem cell graft cellular composition has been generally accepted to impact outcomes. Recent studies question this hypothesis. We conducted a single-center retrospective study of sixty-one pediatric BMT recipients for malignant (68%) and nonmalignant diseases (32%) examining effects of graft composition on engraftment, acute GVHD, chronic GVHD, and survival at day 100 and 1 year. Grafts contained a median of 3.63×0^8 TNC/kg (range: $0.031–10.31 \times 10^8$ TNC/kg) and 4.09×10^6 $CD34^+$/kg (range: $0.76–24.15 \times 10^6$ $CD34^+$/kg) with median neutrophil and platelet engraftment times of 17 and 29 days, respectively. A univariate analysis showed a trend for increasing TNC and increasing time to neutrophil engraftment HR: 0.875; CI: 0.075–1.001). Increasing $CD34^+$ counts shortened time to platelet engraftment (HR: 1.085; CI: 1.015–1.161). No significant relationship was found between TNC, $CD34^+$, or $CD3^+$ and acute or chronic GVHD. TNC or $CD34^+$ did not affect day 100, 1-year survival, or 2-year survival. Increasing $CD3^+$ counts demonstrated a negative trend on day 100 survival (HR: 1.108; CI: 1.001–1.036) but not 1-year survival or 2-year survival. These results add additional data questioning the effect of graft composition on outcomes in pediatric BMT patients with important ramifications for the management of donors.

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AUTHORS' CONTRIBUTIONS

V.F, J.J, and M.H: conceived and designed the project; V.F, E.H, E.W, C.K, A.D, and M.H: acquired the data; V.F, A.W, E.GM, and M.H: analyzed and interpreted the data; V.F and M.H: took lead in writing the manuscript and making all revisions.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

1 | INTRODUCTION

The cellular composition of the hematopoietic stem cell graft has generally been accepted to influence transplant outcomes. Typically, a TNC of ≥3.0 × 10^8/kg is preferred, with a desired minimum of ≥2.0 × 10^8/kg.[1,2] The majority of studies have concluded that a higher $CD34^+$ cell count results in faster neutrophil and platelet recovery as well as improved overall survival, both of which correlate with decreased infectious complications and treatment-related mortality.[3–5]

More recent studies, however, have questioned this hypothesis for BM grafts. A NMDP study published in 2010 that included forty-four pediatric patients demonstrated a lack of effect of the composition of unrelated BM donor grafts on outcomes.[6] Recipients underwent myeloablative or reduced intensity preparative regimens with a heterogeneous donor population ranging from 8/8 high-resolution HLA-matched donors up to two or more allele-or antigen-mismatched donors. Subsequently, in 2014, a study of ninety-four pediatric recipients receiving myeloablative preparative regimens with matched sibling donors or matched unrelated donors at a single center showed no impact of total or myeloid $CD34^+$ cell counts on neutrophil engraftment or transplant-related mortality.[7] Given these discrepancies over time, we conducted a retrospective analysis to analyze the impact of graft composition on outcomes for pediatric recipients of unmanipulated BMT.

2 | MATERIALS AND METHODS

This study included sixty-one pediatric patients who underwent unmanipulated allogeneic BMT for both malignant and nonmalignant diseases from July 2007 through December 2014 at our institution. Patient and transplant characteristics are summarized in Table 1. The median age was 7 years (range: 5 months-20 years). Over half of the patients (68%) had a malignancy diagnosis. The majority of patients (84%) received myeloablative preparative regimens, either total body irradiation-based with a dose of 1200 cGy or busulfan-based with targeted pharmacokinetics of an average concentration at steady state of 600–900 ng/mL. The most common reduced intensity regimen was fludarabine, melphalan, and alemtuzumab. Almost half of the donors (46%) were related, and no donors were more than a one allele or antigen mismatch at HLA A, B, C, or DR. All patients received calcineurin inhibitor-based GVHD prophylaxis with most patients (79%) receiving methotrexate as the second agent. Methotrexate dosing was based on the type of donor. Patients with matched family donors received methotrexate at 5 mg/m^2 on days +1, +3, and +6 while all others received 15 mg/m^2 on days +1 followed by 10 mg/m^2 on days +3, +6, and +11 with folinic acid rescue as previously described.[8] Patients did not receive granulocyte colony-stimulating factor.

BM grafts from family donors were collected by harvest according to our institutional standard operating procedure and the Foundation for the Accreditation of Cellular Therapy standards. Unrelated donor BM grafts were collected and couriered from the corresponding collection center following NMDP standards. Marrow processing included red blood cell depletion for twenty patients (33%) and plasma depletion for twenty-nine patients (48%) and was performed at our institution. Red blood cell depletions were performed using the COBE2991 (Terumo BCT) in manual mode according to the manufacturer's instructions.

For TNC determination, the product bag was weighed, and the weight of the bag was subtracted to give the weight of the product in grams. The gram weight of the product was divided by the specific gravity factor of 1.058 for the product to yield the volume of the product in mL. This final product volume was then multiplied by the white blood cell count performed on an automated hematology analyzer. The BM stem cell product was analyzed using commercially available Beckman Coulter (BC) Stem-Kit™ reagents (Miami, FL) and BC Navios (Brea, CA) flow cytometers.

Study end-points included neutrophil engraftment, platelet engraftment, day 100 survival, 1-year survival, 2-year survival, acute GVHD, and chronic GVHD. Neutrophil engraftment was defined as the first day of an absolute neutrophil count of 0.5×10^9/L or higher for three consecutive days and platelet engraftment as the first day with a platelet count of 20×10^9/L or higher at least 7 days following a platelet transfusion. Day 100, 1-year survival, and 2-year survival were measured from the day of transplant and included death from any cause. GVHD was defined per the Glucksberg scale for acute GVHD and chronic GVHD per the chronic GVHD NIH consensus guidelines.[9]

Cox proportional hazard regression models were used with HR and 95% CI to determine the effect of graft composition on engraftment and survival. Logistic regression models were used to determine the relationship of graft composition to acute and chronic GVHD with OR and 95% CI.

3 | RESULTS

The median and range of total nucleated, $CD34^+$, and $CD3^+$ cell counts of the marrow products are reported in Table 2. Notably, four (7%) transplants were performed with a TNC below 1.0×10^8/kg. All grafts with a TNC below 1.0×10^8/kg were red blood cell-depleted. Three of the four were matched-related donor grafts. Two recipients had severe aplastic anemia, one patient had sickle cell disease, and one patient had secondary MDS. Ten transplants (16%) were performed with a TNC between 1.0 and 2.0×10^8/kg. A total of three (5%) patients received a $CD34^+$ cell dose below 1.0×10^6/kg, with two of these patients being part of the group of four patients with a TNC below 1.0×10^8/kg. Additionally, three patients (5%) received a $CD34^+$ cell dose between 1.0 and 2.0×10^6/kg.

The impact of graft composition on engraftment and survival is detailed in Table 3. Sixty of sixty-one (98%) patients achieved neutrophil engraftment despite fourteen patients (23%) receiving a TNC dose below 2.0×10^8 TNC/kg. The patient who did not engraft had a cell dose of $>5.0 \times 10^8$ TNC/kg but died of progressive disease on day +40. The median time to neutrophil engraftment was 17 days, with the longest time to engraftment extending to 45 days. Univariate analysis showed a trend for a relationship between increasing TNC and increasing time to neutrophil engraftment (HR: 0.875; CI: 0.075–1.001) such that for every 1.0×10^8/kg unit increase in TNC, there was a 12.5% decrease in the rate of neutrophil engraftment. No effect was identified with $CD34^+$ and $CD3^+$ doses on neutrophil engraftment (HR: 1.040; CI: 0.961–1.124 and HR: 0.992; CI: 0.976–1.009, respectively).

Fifty-two patients (85%) achieved platelet engraftment with a median time of 29 days. The nine patients who did not achieve platelet engraftment died from either disease relapse,[4] sinusoidal obstruction syndrome,[2] infections,[2] or GVHD.[1] There was no correlation between TNC or $CD3^+$ and platelet engraftment (HR: 0.979; CI: 0.842–1.137 and HR: 1.007; CI: 0.990–1.024, respectively). An association was seen between increasing $CD34^+$ counts and shorter time to platelet engraftment (HR: 1.085; CI: 1.015–1.161), such that for every 1.0 $\times 10^6$/kg unit increase in $CD34^+$ counts, there was an 8.5% increase in the rate of platelet engraftment. There was no secondary graft failure in our cohort.

Eighteen patients (30%) were defined as having grade two or higher acute GVHD. Nine patients had grade two, eight patients had grade three, and one patient had grade 4 GVHD. Eight patients (18%) of the forty-five patients alive after day 100 developed chronic GVHD. Six of these patients had extensive chronic GVHD, and two patients had limited chronic GVHD. No significant relationship was found between TNC, $CD34^+$, or $CD3^+$ and acute or chronic GVHD, respectively. These results are displayed in Table 4.

Forty-five (74%) patients survived to day 100 and forty (66%) survived beyond 1 year from transplant with 37 (61%) patients alive at least 2 years after transplant. The majority of patients who died before day 100 died from relapse, along with two patients from VOD, two patients from acute GVHD, and two patients from infection. For patients who survived to day 100, all deaths prior to 1 year were from relapse except one death from infection and one death from acute GVHD. The three additional deaths after 1 year and prior to 2 years after transplant were due to chronic GVHD (two) and infection (one). Among the combined group of nineteen LCH/HLH/nonmalignant patients, three patients died before day 100 (GVHD, VOD, and infection), with two additional patients dying from infection before and after 1 year, respectively. As shown in Table 3, TNC or $CD34^+$ did not statistically affect either day 100, 1-year survival, or 2-year survival. However, increasing $CD3^+$ counts did demonstrate a negative trend on 100-day survival (HR: 1.108; CI: 1.001–1.036) such that for every 1.0 $\times 10^6$/kg unit increase in $CD3^+$, there was a 1.8% increased risk of death. No effect was seen with 1-year or 2-year survival.

4 | DISCUSSION

The cellular composition of a graft has been accepted to have major implications on transplant outcomes, although there is not a consensus on the exact relationship between composition and outcomes. Common practice is that a larger cell dose is generally preferred. One recent study noted that increasing nucleated cell doses led to faster neutrophil engraftment.[10] A study of 1054 adult patients with AML or MDS who underwent reduced intensity or nonmyeloablative PBSC transplants demonstrated improved outcomes with $CD34^+$ doses of $>4 \times 10^6$/kg and $>6 \times 10^6$/kg for matched sibling and unrelated donors, respectively.[11] Another adult study with AML patients and reduced intensity conditioning with genoidentical PBSC grafts demonstrated improved survival with a higher cell dose only for patients transplanted in CR2 or beyond.[12] Prior to both of these studies, another adult study demonstrated better outcomes with BM grafts with $>2.7 \times 10^8$/kg TNC compared to lower dose BM grafts or PBSC grafts.[13] However, a recent combined adult and pediatric NMDP study and a pediatric-only Austrian study have both concluded that

no relationship exists between cell doses and transplant outcomes including engraftment, mortality, and survival.[6,7] Only one of these studies, however, included pediatric patients with nonmalignant diseases.[6] More recently, a combined adult and pediatric Italian study noted that higher cell doses led to improved outcomes for recipients with nonmalignant diseases and lower cell doses led to higher relapse risks in recipients with early malignant diseases.[14]

Given the changing landscape and potential paradigm shift related to cell doses, the aim of our retrospective study was to analyze the data from our institution on pediatric patients with both malignant and nonmalignant diseases to determine whether graft composition affected transplant outcomes. While our study included less than one hundred patients, our sixty-one patients compare favorably with the forty-four pediatric patients included in the NMDP study and the ninety-four patients included in the Austrian pediatric study.

No relationship was found between TNC and $CD34^+$ and day 100, 1-year survival, or 2-year survival. Interestingly, our data found an unexpected relationship between TNC and neutrophil engraftment such that a larger TNC cell dose resulted in longer time to engraftment. Given our overall prompt median neutrophil engraftment time of 17 days, however, the statistical significance does not translate into meaningful deleterious clinical outcomes. Rather, it allows us to further examine the concept of “more is better” for unmanipulated BM products in pediatric recipients.

Taken together with the recent NMDP study and Austrian pediatric study, our data have significant implications related to cell doses requests and management of donors, particularly minor pediatric donors. As demonstrated by the data above at our institution, almost half of the donors used in our pediatric transplants were related sibling donors and their safety needs to be of equal importance as patients with disease.[15–17] Collecting larger cell doses often requires longer procedural time, causes increased pain, and an increased chance of requiring a transfusion postprocedure. One study demonstrated that when the collection volume was limited to less than 15 mL/kg of donor body weight, fewer transfusions (35% vs 71%) were required postprocedure and almost all transplants resulted in engraftment.[18]

The recent combined adult and pediatric Italian study included one hundred and sixteen patients with nonmalignant disease, and TNC dose did exhibit a positive relationship between increased TNC cell dose and both overall survival and event-free survival.[14] Our study included fifteen patients with nonmalignant disease and sample size precluded a separate subset analysis for these patients. Strikingly though, all four of our patients with TNC $<1.0 \times 10^8$/kg experienced full engraftment, and three of these four patients had nonmalignant diseases (two with severe aplastic anemia and one with sickle cell disease).

Our study did demonstrate that increasing $CD34^+$ cell dose shortened time to platelet engraftment. This is similar to data regarding PBSC transplants,[19] but this relationship was not seen in the recent NMDP marrow study or the recent Austrian pediatric study.[6,7] More importantly, $CD34^+$ cell dose was not related to day 100 survival, 1-year survival, or 2-year survival. Additionally, we found that increased $CD3^+$ cell dose negatively affected day 100

survival. Presumably, this relationship would be through a higher risk of acute GVHD, but our data did not link higher $CD3^+$ cell doses to acute GVHD. This may have been related to the relatively small number of patients in our study with acute GVHD.

Our study adds to the growing body of literature that questions the effect of the cellular composition of unmanipulated BM grafts in pediatric patients with malignant diseases. Our study is retrospective with a heterogeneous patient population; however, these results may have potentially important ramifications regarding the management of donors, especially pediatric minor donors. Additional data are particularly needed in the area of nonmalignant transplants and in the setting of reduced intensity conditioning regimens. Pediatric transplant cooperative groups have an opportunity to further investigate these areas in a multicenter fashion. This is a key area in order to optimally balance the safety of donors with the outcomes for recipients.

ACKNOWLEDGMENTS

Supported in part by the Biostatistics Shared Resource, Hollings Cancer Center, and Medical University of South Carolina (P30 CA138313).

Abbreviations:

aGHVD	acute graft vs host disease
AML	acute myeloid leukemia
BM	bone marrow
BMT	bone marrow transplant
cGVHD	chronic graft vs host disease
CI	confidence interval
CR2	2nd complete remission
GVHD	graft vs host disease
HLA	human leukocyte antigen
HLH	hemophagocytic lymphohistiocytosis
HR	hazard ratio
LCH	langerhans cell histiocytosis
MDS	myelodysplastic syndrome
NIH	National Institute of Health
NMDP	National Marrow Donor Program
OR	odds ratio

PBSC	peripheral blood stem cell
TNC	total nucleated cell count
VOD	veno-occlusive disease

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TABLE 1

Patient characteristics

Total N = 61	**N (%)**
Diagnosis	
Leukemia, Lymphoma, MDS	42 (68)
LCH/HLH	4 (7)
Nonmalignant	15 (25)
Preparative regimen	
TBI-based	23 (38)
Busulfan-based	28 (46)
RIC or Cy/ATG	10 (16)
Disease status	
CRI/untreated	23 (38)
CR2	11 (18)
CR3 or refractory	10 (16)
Nonmalignant	17 (28)
GVHD prophylaxis	
CNI plus MTX	48 (79)
CNI plus CellCept	9 (15)
CNI plus steroids	4 (6)
HLA	
10/10 related	25 (41)
<10/10 related	3 (5)
10/10 unrelated	21 (34)
<10/10 unrelated	12 (20)
ABO mismatch	
No mismatch	36 (59)
Major mismatch	13 (21)
Minor mismatch	10 (16)
Bidirectional mismatch	2 (3)
Sex mismatch	
None	32 (53)
Female donor, male recipient	21 (34)
Male donor, female recipient	8 (13)
CMV status	
Donor (−), recipient (−)	10 (16)
Donor (−), recipient (+)	24 (40)
Donor (+), recipient (−)	5 (8)
Donor (+), recipient (+)	22 (36)
GVHD	
Acute	18 (30)
Chronic	8 (13)

TABLE 2

Graft characteristics

	Median	**Range**
TNC	3.63×10^8 TNC/kg	$0.031–10.31 \times 10^8$/kg
CD34+	4.09×10^6 CD34 + /kg	$0.76–24.15 \times 10^6$/kg
CD3+	3.43×10^6/kg	$0.0017–110.7 \times 10^6$/kg

TABLE 3

Impact of graft composition on engraftment and survival

	TNC Unit 10^8	CD34+ Unit 10^6	CD3+ Unit 10^6
Neutrophil engraftment			
HR (95% CI)	0.875 (0.765, 1.001)	1.04 (0.961, 1.124)	0.992 (0.976, 1.009)
Platelet engraftment			
HR (95% CI)	0.979 (0.842, 1.137)	1.085 (1.015, 1.161)	1.007 (0.990, 1.024)
Day 100 Survival			
HR (95% CI)	1.008 (0.809, 1.255)	0.979 (0.860, 1.115)	1.018 (1.001, 1.036)
1-year survival			
HR (95% CI)	0.997 (0.825, 1.206)	0.936 (0.818, 1.070)	1.014 (0.998, 1.030)
2-year survival			
HR (95% CI)	1.017 (0.857, 1.207)	0.94 (0.833, 1.060)	1.013 (0.997, 1.028)

TABLE 4

Impact of graft composition on GVHD

	TNC Unit 10^8	**$CD34^+$ Unit 10^6**	**$CD3^+$ Unit 10^6**
Acute GVHD			
OR (95% CI)	1.117 (0.882, 1.416)	0.979 (0.846, 1.133)	1.004 (0.979, 1.030)
Chronic GVHD			
OR (95% CI)	1.205 (0.889, 1.632)	1.053 (0.895, 1.239)	1.004 (0.970, 1.039)