Combined Umbilical Cord Blood and Bone Marrow from HLA-Identical Sibling Donors for Hematopoietic Stem Cell Transplantation in Children With Hemoglobinopathies

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Background. It is well established that umbilical cord blood and bone marrow are biologically different stem cell sources. ***Patients and Methods.*** We analyzed the feasibility and outcome of hematopoietic stem cell transplantation (HSCT) in 13 children (median age 5.9 years) with hemoglobinopathies after the co-infusion of cord blood (CB) and bone marrow (BM) from the same human leucocyte antigen (HLA) identical sibling donor. We also compared outcomes of children with co-transplantation to outcomes in children with hemoglobinopathies who had received a BM (n = 21) or CB (n = 22) transplant alone. ***Results.*** Compared to CB transplant (CBT) recipients, the co-transplant group had more rapid neutrophil (17 vs. 25 days, $P = 0.013$) and platelet (29 vs. 48 days, $P = 0.009$) recovery and less transplant related mortality. Patients who received a co-transplant had a lower incidence of ≥grade II acute (0% vs. 26.3%) and chronic (0% vs. 21%) graft versus host disease (GVHD) compared to BM transplant (BMT) recipients ($P = 0.055$ and 0.045, respectively). With a median follow-up of >60 months in each treatment group, the 5-year probability of event free survival (EFS) was 100% in the co-transplant group, 90% after BMT and 86% after CBT ($P = 0.42$). ***Conclusion.*** Co-transplantation of CB and BM from HLA-identical sibling donors appears to be a feasible and effective strategy to further optimize outcomes of HSCT for hemoglobinopathies. Pediatr Blood Cancer

Key words: bone marrow transplant; cord blood transplant; hemoglobinopathy; sickle cell disease; thalassemia

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) remains the only curative treatment option for severe hemoglobinopathies. Immunological rejection of the donor graft and complications of graft-versus-host disease (GVHD) are the primary barriers to a successful outcome of HSCT. While these complications occur most commonly after alternative donor or human leucocyte antigen (HLA)-mismatched donor HSCT, patients undergoing HLA-identical sibling HSCT also are at risk. A 5–10% incidence of rejection and 15–20% risk of acute GVHD >grade II has been described after HLA identical sibling donor HSCT for hemoglobinopathies [1–5]. Factors that influence graft rejection include the intensity of the conditioning regimen, drugs used for GVHD prophylaxis and cell dose infused, particularly after cord blood transplantation (CBT) [6,7]. In addition, biological features of the hematopoietic stem cell (HSC) source itself appear to influence the risk of developing these complications, especially the lower risk of acute GVHD observed after CBT [8–11].

The use of cord blood (CB) for HSCT in non-malignant disorders evolved in part as a method to mitigate the risk of GVHD —a complication of allogeneic HSCT that generates no benefit for non-malignant disorders. Initial efforts focused on collecting and banking CB from sibling donors [12]. The success of this approach was suggested by initial results of sibling donor CBT for hemoglobin disorders reported one decade ago in which the 2-year disease-free survival in thalassemia and sickle cell disease was 79% and 90%, respectively and probability of developing acute GVHD and chronic GVHD was 6% and 11% [8], respectively. However, the benefit of protection from GVHD was balanced by slower kinetics of neutrophil and platelet engraftment after CBT compared to bone marrow (BM) grafts [13,14]. This was illustrated in a series of 78 related CBT cases in which the median time to neutrophil and platelet engraftment was 35 days and 53 days, respectively in patients receiving $<3.7 \times 10^7$ total nucleated cells/kg (TNC/kg). In contrast, in patients who received a higher cell dose, the median time to neutrophil and platelet engraftment was 25 days and 45 days, respectively [15]. This suggests that, similar to unrelated donor CBT [16], the nucleated cell dose is an important predictor of outcome in the related donor setting also.

Hence, we considered the utility of supplementing a sibling CB unit with a BM harvest from the same sibling donor, when the CB cell dose was judged insufficient for engraftment. The assessment of this approach included measuring the rates of graft rejection and GVHD, as there was concern that the biological characteristics of BM might increase the risk and severity of GVHD compared to that observed when using CB alone [17]. In balance, it was anticipated that more rapid engraftment from BM derived progenitor cells might reduce the prolonged neutropenia and thrombocytopenia period associated with CBT leading to reduction in transplant related mortality (TRM) and might also reduce the risk of primary graft failure. Here, we report the results of a retrospective study to assess the feasibility, engraftment, GVHD risk and outcomes after co-transplantation of HLA-identical sibling donor CB and BM as the stem cell source in children undergoing HSCT for

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Conflict of interest: M.C.W. is medical director of ViaCord Cord Blood Bank Processing Laboratory; all other authors claim no conflict of interest.

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hemoglobinopathies and compare their outcomes to patients who had received CB or BM grafts alone.

PATIENTS AND METHODS

Data Collection

Consecutive patients treated by HSCT between June 1998 and July 2009 in the Oakland Sibling Cord Blood Bank registry, Memorial Sloan Kettering Cancer Center (MSKCC), University of California, San Francisco (UCSF), and Nationwide Children's Hospital (NCH) databases were analyzed retrospectively. Inclusion criteria for the study were: age 0–18 years, diagnosis of transfusion dependent thalassemia or severe sickle cell disease, recipient of an HLA-identical sibling donor HSCT with BM, CB or combined CB and BM (co-transplant) as the graft source with a myeloablative conditioning regimen. Patients receiving co-transplantation or CB alone were transplanted at multiple institutions (Oakland Children's Hospital, UCSF, MSKCC, and NCH were the major institutions that performed these HSCT) and data were reported to the Oakland Sibling Cord Blood Bank registry or MSKCC database. Hence these institutions were also selected to report data on BMT to limit the center bias. Outcomes data on consecutive patients receiving BM transplantation (BMT) for hemoglobinopathies during the same time period (to limit bias due to period effect) was collected from these institutional databases. The collected data included relevant pre-and post-transplant clinical demographics, engraftment, GVHD, and overall outcomes. The study variables for each patient were confirmed by institutional PIs through personal communications. The retrospective study was approved by the local institutional review board of the participating institutions.

Transplant

In all patients receiving co-transplant or BMT, the donor-recipient histocompatibility was determined by intermediate or high resolution HLA typing of HLA-A, B and high resolution typing of DRB1 loci. CB selection was based on intermediate or high resolution for HLA-A and B loci and DNA typing for DRB1. Donor chimerism was analyzed by quantitative polymerase chain reaction (PCR) analysis for microsatellite DNA markers in whole blood. Fluorescent in situ hybridization (FISH) for Y chromosome or PCR of the amelogenin marker for Y chromosome was used in sex-mismatched transplants at local institutions.

Endpoints

Neutrophil engraftment was defined as the first of three consecutive days that the absolute neutrophil count (ANC) $>0.5 \times 10^9$/L. Platelet engraftment was defined as the first day of unsupported and sustained platelet count of $>20 \times 10^9$/L for seven consecutive days. Graft failure was defined as the absence of donor chimerism with aplasia, autologous hematological recovery or receipt of a second HSCT with conditioning. GVHD was graded according to the established CIBMTR criteria [18]. TRM was defined as death due to any cause related to the transplant procedure.

Statistical Analysis

All the variables were summarized by descriptive statistics. For continuous variables, the mean or median and standard deviations were provided. The frequency and percentage were estimated for categorical variables. Clinical parameters (engraftment, incidence of acute GVHD $\geq$grade 2, any evidence of chronic GVHD and TRM) were compared among co-transplant, BMT, and CBT groups. For continuous variables, a one-way AVONA or a one-way layout non-parametric method, where appropriate, was used to test for differences among the three groups (BM, CB, and co-transplant); whereas the Chi-square test was used for categorical data. When the ANOVA test was significant, a post-hoc test Tukey pairwise comparison was performed to detect significant differences between pairs. Holm's procedure was used to correct for multiple comparisons when indicated. Type I error was strongly controlled at $\alpha = 0.05$ for single comparisons and with adjustment for multiple comparisons. The data were analyzed using the statistical software SAS version 9.2 (SAS Institute, Cary, NC).

Deaths due to any cause and graft failure were considered events; while these were also considered as competing events for GVHD. Patients who were alive without an event were censored at the last follow-up. The 5-year probabilities of EFS were estimated by using Kaplan–Meier method and expressed as percentage $\pm$ standard error.

RESULTS

Patients, Disease and Treatment Regimens

Thirteen subjects with hemoglobinopathy received an HLA-identical sibling donor HSCT utilizing combined CB and BM grafts as the source of HSC (co-transplant), while 21 and 25 children had received BMT or CBT alone, respectively (Table I). The median age at HSCT was similar in the three groups. Majority of the children had received a myeloablative preparative regimen of busulfan (14–16 mg/kg), cyclophosphamide (200 mg/kg), and anti-thymocyte globulin (ATGAM, 90 mg/kg). Cyclosporine (CSA) and standard short course methotrexate (15 mg/m^2, 10 mg/m^2, 10 mg/m^2, 10 mg/m^2 on Day +1, +3, +6, and +11, respectively) was administered most frequently for GVHD prophylaxis after BM alone and co-transplantation, while CSA + mycophenolate mofetil (MMF) was the most common GVHD prophylaxis administered after CBT.

Rationale and Feasibility of Combined Grafts

An insufficient pre-thaw cell dose ($<2 \times 10^5$ CD34+ viable cells/kg recipient weight) in the stored CB unit to ensure donor engraftment was the rationale for a co-transplant. The median UCB CD34+ dose was 1.1×10^5/kg of recipient body weight (range 0.1–4.4×10^5/kg) and therefore, BM was co-infused to augment the cell dose. The median co-infused BM cell dose was 1.8×10^8/kg (range 0.9×10^8 to 2.6×10^8 TNC/kg) recipient weight. The majority of co-infusions were performed back to back on the same day, except for one patient who received donor BM infusion 2 days after the CB infusion. There were no adverse events or infusion reactions after the co-infusion.

Engraftment and Outcomes After Co-Transplantation

All 13 patients who received co-transplantation of CB and BM had durable engraftment of donor cells. There were no major infections during the initial HSCT course in the co-transplant group. No TRM was reported and all the patients are transfusion independent or asymptomatic after HSCT. Long-term grafts are stable and majority of patients have 100% donor chimerism, while 3 (18%) patients have stable mixed chimerism (range: 62–91% donor cells).

TABLE I. Patient and Transplant Characteristics

	CB + BM	BMT	CBT	*P*-value
Total number of patients	13	21	22	
Age (years) (median)	5.9 (2.2–13)	6.9 (0.8–14.5)	5.2 (1.8–11.7)	0.36
Diagnoses				
Thalassemia	8	7	16	0.15
Sickle cell disease	5	14	6	0.23
Conditioning regimens				
Bu (14–16 mg/kg); CY 200 mg/kg; ±ATGAM	10	18	20	
Flu; Bu (14 mg/kg); Campath	2	3		
Flu; Melphalan (180 mg/m^2), ATG	1		2	
Median UCB dose CD34$^+$/kg (range)	1.1×10^5/kg (0.1–4.4)	—	2.5×10^5/kg (0.2–6)	
Median BM dose TNC/kg (range)	1.8×10^8/kg (0.9–2.6)	4.6×10^8/kg (1.4–14.4)	—	
GVHD prophylaxis				
CSP + MTX	11	19	1	
CSP + Pred			2	
CSP	2			
MMF + CSP		2	19	
Median follow-up				
months (range)	66 (33–91)	70 (26–162)	76 (45–120)	NS

Comparison to CB and BM Transplantation

The outcomes of co-transplantation patients with sickle cell disease or thalassemia were compared to children with a hemoglobinopathy who received BMT or CBT alone (Table II). The median time to neutrophil and platelet engraftment in the co-transplant group was 17 days (range: 12–27 days) and 29 days (range: 18–90), respectively. Compared to the recipients of CB alone grafts, the combined graft recipients experienced more rapid neutrophil and platelet recovery. There was no graft rejection after CB or co-transplant grafts, but 9.5% of those treated by BMT had graft rejection, including two primary graft failures. One patient with graft rejection after BMT had received a sub-optimal cell dose (1.5×10^8 TNC/kg recipient weight) and no obvious cause for rejection was identified in the other patient with thalassemia. One patient with graft failure is alive and well after a second HSCT using the same sibling donor, while the other patient had autologous recovery.

None of the co-transplanted patients developed acute GVHD ≥grade 2 and no chronic GVHD was observed during long-term follow-up. Similarly, the cumulative incidence of acute and chronic GVHD was very low after CBT (5% and 0%, respectively). In contrast, there was a trend towards a higher incidence of acute and chronic GVHD in the evaluable patients who received BMT (Table II).

There was no TRM in the patients who received co-transplantation (Table II). All the patients who received co-transplant survive with a median follow-up of 66 months (range: 33–91 months). No deaths were reported in the BMT group, while two deaths (9%) were reported early after CBT due to infection and pulmonary complications. This difference in TRM was not statistically significant in the treatment groups. One late death was reported in the CBT group due to seizures. The 5-year Kaplan–Meier probabilities of EFS in all three groups are shown in Figure 1.

DISCUSSION

The outcomes after HLA-ID sibling donor CBT and BMT for the treatment of non-malignant disorders are similar, especially when cell dose parameters are adhered to for selection of CB units [6]. Unfortunately, the minimum stem cell dose for ensuring engraftment after CBT for non-malignant disorders is largely unknown. Nevertheless, recent data suggest that a CB nucleated cell dose of $>3.5 \times 10^7$/kg of recipient body weight may be necessary to ensure

TABLE II. Comparison of HSCT Outcomes for Hemoglobinopathies After Co-Transplant, BM, and CB Grafts

	Co-transplant (N = 13)	BMT (N = 21)	CBT (N = 22)	*P*-value
Neutrophil engraftment (median day)	17 (12–27)	20 (13–41)	25 (13–56)	0.013[b]
Platelet engraftment (median day)	29 (18–90)	32 (18–947)	48 (18–125)	0.009[b]
Rejection	0%	2 (9.5%)	0%	0.18
Acute GVHD ≥ Grade II	0%	5 (26.3%)[a]	1 (5%)[a]	0.055[c]
Chronic GVHD	0%	4 (21%)[a]	0%	0.045[c]
TRM	0%	0%	3 (13.6%)	0.11
OS	100%	100%	86.4%	0.56
Probability of 5 year EFS ± SEM	100%	90% ± 0.067	86.4% ± 0.07	0.42

[a]Patients with primary graft failure or early TRM not analyzed for GVHD (competing risk). [b]CBT > BMT ∼ co-transplant. [c]BMT > CBT ∼ co-transplant.

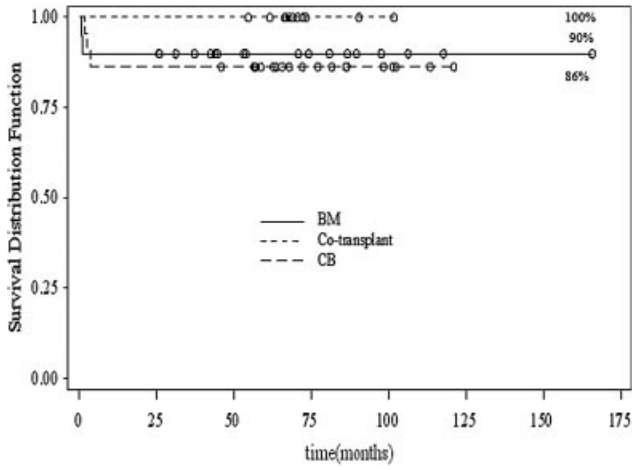

Fig. 1. Probability of event free survival

durable engraftment in patients with non-malignant disorders [19]. Even though stable engraftment is possible with the administration of single CB units, the neutrophil and platelet recovery is delayed compared to BMT and this contributes to a higher TRM caused by infection and hemorrhage during the prolonged period of pancytopenia that follows CBT [9,20,21]. Hence, strategies to enhance engraftment kinetics are required to improve outcomes after CBT for non-malignant disorders.

One strategy to increase the cell dose of CB unit and thereby enhance engraftment is to supplement the CB with a BM harvest from the same donor and co-infuse both the grafts. This is particularly helpful if the volume and cell content of the CB unit is small, a common feature of family cord blood collections that lack the rigor and banking requirements of dedicated public banking operations. This study is the first demonstration that co-transplantation from the same sibling donor is feasible and successful. This retrospective study includes the largest cohort of patients with hemoglobinopathies who underwent HSCT with a co-infusion of CB and BM from the same HLA-identical sibling. We also compared the outcomes in consecutive patients treated either by CBT or BMT alone for hemoglobinopathies at the same institutions, who were prepared with myeloablative preparative regimens during the same time period as co-transplants were performed to minimize selection, period and center bias. Patients treated with CBT or BMT were closely matched to the co-transplant group in terms of age and duration of follow-up. The engraftment rates, kinetics, incidence of GVHD and outcomes of the comparison groups (CBT or BMT) were similar to the outcomes reported in literature for related CBT and BMT performed for hemoglobinopathies, suggesting that comparison groups are representative of transplant outcomes for these disorders [5,9,22].

The neutrophil and platelet engraftment was rapid in patients who received co-transplantation and was significantly faster than after CBT. This might have contributed to the absence of TRM in the co-transplant group, in contrast to CBT. A TRM of 5–10% was observed in prior studies of pediatric non-malignant disorders after HLA-identical BMT or CBT [4,9,22]. While this rate of TRM is acceptable after related donor HSCT, our study suggests that combining the two graft sources may further reduce the TRM risk by enhancing the kinetics of CB engraftment. In addition, cyclosporine and methotrexate for GVHD prophylaxis after co-transplantation had no obvious detrimental effect on engraftment kinetics, unlike the disadvantage of methotrexate noted after CBT [6].

Traditionally, the incidence of acute GVHD $\geq$grade II after HLA-identical sibling BMT is approximately 15–30% in pediatric non-malignant disorders [5,6,9,23]. A lower incidence of acute and chronic GVHD has been reported in the related donor CBT setting [6,9]. Therefore, an uncertainty of the approach combining BM and CB was whether the benefit of a lower risk of GVHD associated with CB graft might be transferred to the co-transplantation procedure or rather would the addition of BM increase the GVHD incidence. Our data strongly suggest that the lower risk of GVHD is retained with co-transplantation, although a larger cohort of patients will be needed to confirm this initial observation. The reason for this is uncertain, but it is possible that features such as an expanded T-regulatory population in the CB might maintain its activity to attenuate the occurrence of GVHD after co-transplantation. While we believe that our observation is best explained by unique biology of the CB graft as the GVHD prophylaxis was same in both the co-transplantation and BMT groups, the effect of other factors such as the cumulative dose of ATG in the conditioning regimen, a lower BM cell dose in the co-transplant group compared to BMT alone cannot be excluded in this retrospective analysis.

CBT from an HLA-identical donor has distinct advantages (ease of collection, ready availability, and lower GVHD risk) and it is safe and successful in most patients with hemoglobinopathies [8]. Here, we suggest that an important disadvantage of CBT, that is, delayed engraftment and risk of graft rejection, might be mitigated by augmentation of CB unit with BM from same donor, while the risk of non-beneficial GVHD associated with BMT may be attenuated by co-infusion of CB. Our results show that this is a feasible and effective strategy that warrants further investigation. Moreover, it also illustrates the importance of directed donor family CB banking activities.

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