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Cord Blood Stem Cells

Use of nonvolume-reduced (unmanipulated after thawing) umbilical cord blood stem cells for allogeneic transplantation results in safe engraftment

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Summary:

Volume reduction of umbilical cord blood (UCB) units before infusion is standard in most transplant centers. We examined 26 patients who underwent transplantation from May 1997 to December 2001 with unmanipulated ($n=18$) or volume-reduced ($n=8$) UCB units for engraftment. Of 18 unmanipulated UCBT patients, 16 achieved ANC > 500/mm³, a median of 26 days (range, 16–104) post-UCBT; two died before engraftment on days +2 and +14. Of 18 unmanipulated UCBT patients, 10 achieved platelet recovery, a median of 60.5 days (range, 41–144) post-UCBT; eight patients died before platelet recovery +2 to +255 days post-UCBT. These results are similar to several reported studies and our series utilizing volume-reduced UCB units for UCBT. At a median follow-up of 29.5 months, the 100-day and 3-year overall survivals of unmanipulated UCBT were 61.1% (95% CI, 38.6–83.6) and 48.6% (95% CI, 24.8–72.4) and of volume-reduced UCBT were 60% (95% CI, 24.4–95.6) and 22.5% (95% CI, 0–58.7). There was no serious toxicity from UCB infusion using unmanipulated UCB units. We conclude that unmanipulated UCB units may be infused safely into UCBT patients with adequate engraftment and survival.
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Umbilical cord blood (UCB) has been increasingly used for transplantation to treat a variety of malignant and nonmalignant hematologic disorders in children and adults. [1–10] For patients with no suitable related donor, this alternative source of hematopoietic stem cells (HSC) offers advantages including ease of procurement, absence of donor risk, reduced likelihood of transmitting infections, rapid availability of cryopreserved samples, and decreased risk of severe graft-versus-host disease (GVHD) despite HLA disparity.[3–5,7,9,11] Survival after UCB transplantation (UCBT) in a pediatric population is comparable to that observed after bone marrow transplantation.[7–9]

While it is customary to administer cryopreserved peripheral blood or bone marrow to patients after thawing without volume reduction or washing to remove dimethylsulfoxide (DMSO), one study has shown that cell viability *in vitro* can be improved by volume-reducing UCB units in albumin/dextran solution before infusion to restore the osmolarity of the suspension and to remove DMSO.[12] Volume-reducing UCB may also decrease the incidence of adverse reactions associated with the infusion of DMSO-cryopreserved grafts.[13–14] However, removal of DMSO and/or cell lysis products by volume reduction can reduce the number of HSC infused.[14–16] Delayed engraftment because of the limited number of HSC in a single unit of UCB represents a significant problem with UCBT, especially in adults.[10] Owing to concern about HSC losses during manipulation, particularly in units with low cell doses, and the observation of prolonged engraftment in our first five volume-reduced UCBTs, we have not routinely volume-reduced UCB units prior to infusion since 1998. We report our experience of the largest series of nonvolume-reduced UCB for allogeneic transplantation.

Patients and methods

We retrospectively reviewed all UCBT recipients at Roswell Park Cancer Institute (RPCI). Between May 1997 and December 2001, 26 patients underwent unrelated UCBT for hematologic malignancies. All patients did not have a suitable HLA-compatible related or unrelated blood or bone marrow donor. No chronic myeloid leukemia (CML) patients received Gleevec® because they were all transplanted before any available clinical trials. Prior approval for UCBT was obtained from the RPCI Institutional Review Board and all patients signed informed consent. The volume-reduced UCB units were obtained from the New York ($n=4$), Duke University ($n=2$), University of Massachusetts ($n=1$), and UCLA ($n=1$) cord blood banks. The unmanipulated UCB units were obtained from the St Louis ($n=8$), New York ($n=7$), Barcelona ($n=2$), and Australia ($n=1$) cord blood banks. The methods of

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collecting, processing, and storing UCB varied among the centers.

Eight patients received volume-reduced UCB. UCB units were thawed in the laboratory and suspended in equal volumes of 10% dextran-40 and 5% human albumin solution. The resulting 5% dextran and 2.5% human albumin solution was centrifuged at 400 g for 15 min at 10°C. The supernatant was removed and the sedimented cells were resuspended in fresh 5% dextran and 2.5% human albumin solution.[12] In total, 18 patients received unmanipulated UCB; the units were thawed at the bedside and immediately infused. We stopped volume depleting the UCB units after we observed delayed engraftment in the first five units and became concerned about loss of HSCs during the procedure; however, we reinstituted volume depletion during participation in a national multicenter study that required volume depletion by protocol.

The UCB banks provided data on the cell counts of UCB units before freezing. If the UCB units had adequate cell counts for transplantation, nucleated cell count, $CD34^+$ cell count, and test of cell viability (trypan blue dye exclusion) were performed on the samples at the time of infusion. The percent recovery of total cells was calculated from the total counts obtained before freezing and after thawing in the units that had data available from both time points.

Transplantation

Conditioning regimens were as follows: FLU/MEL/ATG: fludarabine 25 mg/m^2/day for 5 days, melphalan 90 mg/m^2/day for 2 days, antithymocyte globulin (ATG) 30 mg/kg/day for 3 days; Cy/ATG/TBI: cyclophosphamide 60 mg/kg/day for 2 days, ATG as above and total body irradiation (TBI) total dose 1200 cGy in six fractions over 3 days; BU/CY/ATG: busulfan 16 mg/kg p.o. over four days, cyclophosphamide 60 mg/kg/day for 2 days and ATG as above; MEL/ATG/TBI: melphalan 45 mg/m^2/day for 3 days, ATG as above, TBI total dose 1350 cGy in nine fractions over 3 days; BU/MEL/ATG: busulfan 12.8 mg/kg i.v. over 4 days, melphalan 135 mg/m^2 over 3 days, ATG as above (see Table 1).

GVHD prophylaxis consisted of cyclosporine and corticosteroids.[3] Patients were premedicated with diphenhydramine, lorazepam, and/or hydrocortisone prior to UCB infusion and monitored for adverse reactions. Granulocyte colony-stimulating factor (G-CSF) was administered at 10 μg/kg subcutaneously daily from day 0 until an absolute neutrophil count of $\geqslant 1000/\text{mm}^3$ for three consecutive days. Supportive therapy was given according to our standard operating procedures.

Hematopoietic recovery

Neutrophil and platelet recoveries were defined as the time from day 0 (infusion of UCB) until an absolute neutrophil count of $\geqslant 500/\text{mm}^3$ for three consecutive days and a platelet count of $\geqslant 20\,000/\text{mm}^3$ after seven consecutive days of no platelet transfusions. UCB engraftment was ascertained by chimerism assays indicating at least 90% cells of donor origin. Survival longer than 60 days after transplant without neutrophil or platelet recovery was regarded as graft failure.

Table 1 Characteristics of the recipients of unmanipulated *vs* volume-reduced umbilical cord blood transplantation

Characteristics	*Unmanipulated UCB N = 18*	*Volume-reduced UCB N = 8*
Age (y)		
Median (range)	34 (5–54)	21 (10–47)
Weight (kg)		
Median (range)	78.1 (18.5–131)	60.7 (30.2–80)
Transplant year		
1997–1998	6 (33%)	5 (63%)
1999–2001	12 (67%)	3 (38%)
Degree of HLA matching[a]		
3/6	1 (6%)	1 (13%)
4/6	8 (44%)	3 (38%)
5/6	8 (44%)	4 (50%)
6/6	1 (6%)	0 (0%)
Diagnosis		
ALL	3 (17%)	3 (38%)
AML	3 (17%)	4 (50%)
MDS	4 (22%)	1 (13%)
CML	7 (39%)	0 (0%)
MF	1 (6%)	0 (0%)
Risk[b]		
Standard	12 (67%)	1 (13%)
High	6 (33%)	7 (88%)
Conditioning regimen		
CY/ATG/TBI	12 (67%)	4 (50%)
BU/CY/ATG	3 (17%)	2 (25%)
BU/MEL/ATG	0 (0%)	1 (13%)
MEL/ATG/TBI	2 (11%)	1 (13%)
FLU/MEL/ATG	1 (6%)	0 (0%)

ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; MDS: myelodysplasic syndrome; CML: chronic myeloid leukemia; MF: myelofibrosis; CY: cyclophosphamide; ATG: antithymocyte globulin; TBI: total body irradiation; BU: busulfan; MEL: melphalan; FLU: fludarabine.
[a]A and B by serology and molecular allelic typing for DRB1.
[b]Standard risk: acute leukemia in first complete remission, chronic phase CML, untreated primary MDS; high risk: acute leukemia in second complete remission or greater, relapse, refractory, CML beyond chronic phase, secondary MDS, MDS converted to AML without induction therapy.

Overall survival

Overall survival was defined as the time between day 0 (infusion of UCB unit) and death. Surviving patients were censored at the date of last follow-up. Survival was updated through 1 October 2002. For hematopoietic recovery, data were censored if the patient died before hematopoietic recovery. The probability of hematopoietic recovery and overall survival were estimated by the Kaplan–Meier product limit method.[17]

Results

Clinical characteristics

Table 1 shows the clinical characteristics of unmanipulated and volume-reduced UCBT recipients. In all, 26 patients

have undergone unrelated UCBT since 1997. Most of the patients were adults; six patients were less than 16 years old. Volume reduced UCB units were given to five patients between 1997 and 1998, and to three patients in 2001.

Characteristics of UCB grafts

Table 2 shows the characteristics of unmanipulated and volume-reduced UCB grafts. The median number of cryopreserved and infused $CD34^+$ cells/kg is provided in Table 2. Information regarding nucleated cell dose infused, $CD34^+$ cell recovery, and trypan blue viability was not available for all patients. A clinical decision was made that if the precryopreservation nucleated cell count per kilogram of body weight of the recipient was less than 2×10^7/kg, the entire UCB unit was reinfused after thawing. No additional tests for cell counts or viability were performed, and no samples were saved for future testing.

Hematopoietic recovery

Figures 1 and 2 show the Kaplan–Meier estimates for neutrophil and platelet recoveries of the 18 patients transplanted using unmanipulated UCB *vs* the eight patients transplanted using volume-reduced UCB. There was no significant difference in neutrophil or platelet recovery between the volume-reduced and unmanipulated UCBT patients. One patient in the volume-reduced UCBT group failed to engraft and is excluded from the analysis. This patient underwent a second transplant on day +72 post-UCBT from a volunteer unrelated PBSC donor and died day +5 post-PBSCT from sepsis related to infection. Two patients in the unmanipulated UCBT group died before neutrophil engraftment on days +2 and +14 and are censored at the time of death. Eight patients in the unmanipulated UCBT group died before platelet recovery on days +2, +14, +47, +47, +66, +84, +86, and +255, whereas one patient in the volume-reduced UBCT group died before platelet recovery on day +158, all of whom are censored at the time of death. The estimated probability of neutrophil recovery by day 60 after transplantation for the unmanipulated and volume-reduced UCBT, respectively, were 94% (95% confidence interval [CI], 82–100) and 100%, and the median times to neutrophil recovery were 26 (range, 16–104) and 37 days (range, 19–59). The estimated probability of platelet recovery by day 100 after transplantation in recipients of unmanipulated and volume-reduced UCBT, respectively, were 50% (95% CI, 23.3–76.7) and 80% (95% CI, 44.9–100), and the median times to platelet recovery were 60.5 (range, 41–144) and 91 days (range, 77–105). No late graft failures were observed in the engrafted recipients. Table 3 shows the comparison of hematopoietic recovery in our patient population (unmanipulated *vs* volume-reduced) to five published studies that provided details of unrelated UCB manipulation before transplant.[3–6,10] While a direct comparison cannot be made, the engraftment times are similar. As shown in Table 3, our volume-reduced UCB recipients had delayed median times to neutrophil and platelet recovery compared to our unmanipulated and other reports of volume-reduced UCBT. However, the probabilities of recovery of neutrophils by day 60 and platelets by day 100 were comparable to our unmanipulated and other reports of volume-reduced UCBT.

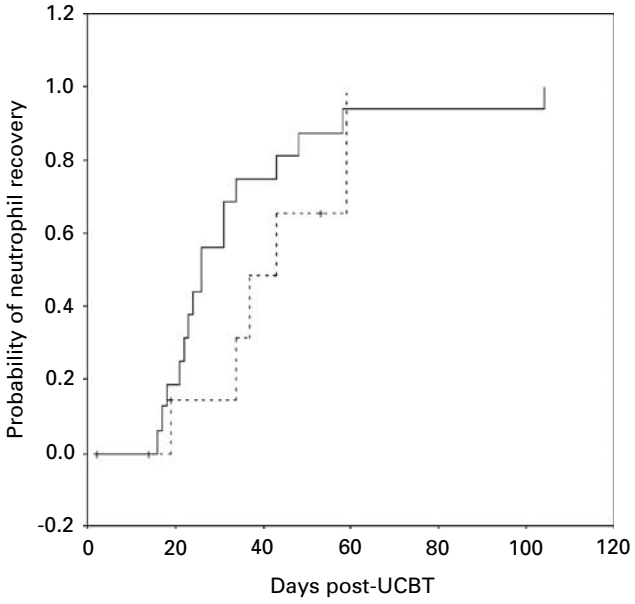

Figure 1 Kaplan–Meier estimate of neutrophil engraftment following unmanipulated *vs* volume-reduced umbilical cord blood transplantation. UCBT: umbilical cord blood transplant; tick marks indicate censored patients, solid line is the unmanipulated UCBT group; dashed line is the volume-reduced UCBT group; *P*-value >0.1.

Survival

The median follow-up was 29.5 months (range, 2.4–47.7). Figure 3 displays the Kaplan–Meier overall survival curves

Table 2 Characteristics of unmanipulated and volume-reduced umbilical-cord blood (UCB) grafts

Characteristics	*Unmanipulated UCB N = 18*	*Volume-reduced UCB N = 8*
Volume (ml)	36 (25–287) ($n=18$)[a]	146 (25–380) ($n=8$)
Nucleated cells cryopreserved/kg ($\times 10^7$)	2.4 (1.1–4.1) ($n=18$)	2.1 (1.4–3.6) ($n=8$)
Nucleated cells infused/kg ($\times 10^7$)	3.3 (2.0–3.5) ($n=3$)	1.4 (0.7–2.0) ($n=8$)
Nucleated cell recovery (%)	100 (92–100) ($n=3$)	47 (39–99) ($n=5$)
CD34+ cells cryopreserved/kg ($\times 10^5$)	1.1 (0.2–12.8) ($n=11$)	1.1 (0.1–1.3) ($n=3$)
CD34+ cells infused/kg ($\times 10^5$)	0.7 (0.2–3.7) ($n=7$)	0.8 (0.2–2.0) ($n=4$)
Cell viability (%)	98 (70–100) ($n=5$)	98 (92–100) ($n=6$)

[a]Median (range) (number of patients with available data). The cell viability estimated the trypan blue exclusion in mononuclear cells.

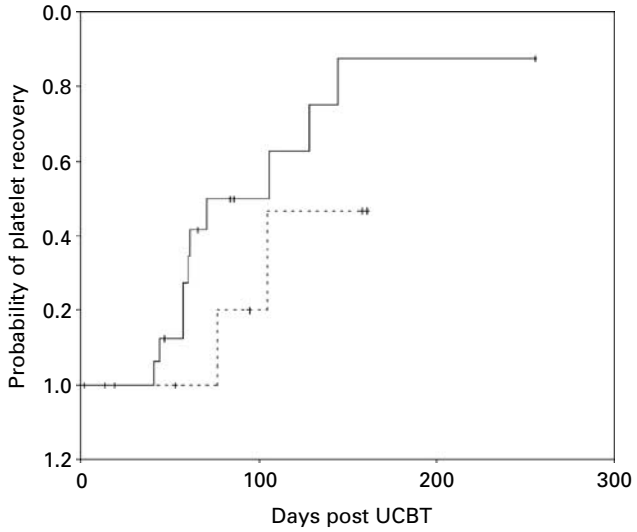

Figure 2 Kaplan–Meier estimate of platelet engraftment following unmanipulated *vs* volume-reduced umbilical cord blood transplantation. UCBT: umbilical cord blood transplant; tick marks indicate censored patients, solid line is the unmanipulated UCBT group; dashed line is the volume-reduced UCBT group; *P*-value >0.1.

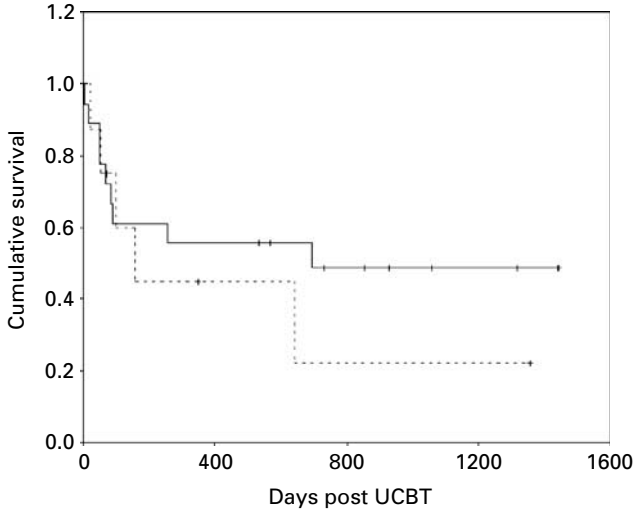

Figure 3 Kaplan–Meier estimate of overall survival of the recipients of unmanipulated *vs* volume-reduced umbilical cord blood transplantation. UCBT: umbilical cord blood transplant; tick marks indicate censored patients; solid line is the unmanipulated UCBT group; dashed line is the volume-reduced UCBT group; *P*-value >0.1.

of patients given unmanipulated *vs* volume-reduced UCB units. The probability of survival at 100 days and 3 years after transplantation in recipients of unmanipulated UCB was 61.1% (95% CI, 38.6–83.6) and 48.6% (95% CI, 24.8–72.4), and of volume-reduced UCB was 60% (95% CI, 24.4–95.6) and 22.5% (95% CI, 0–58.7). At the time of last follow-up, nine (50%) of the 18 unmanipulated UCBT patients had died of infection ($n=3$), relapsed or refractory leukemia ($n=3$), organ failure ($n=2$) or GVHD ($n=1$), and five (62.5%) of the volume-reduced UCBT patients had died of infection ($n=3$), relapsed leukemia ($n=1$), or organ failure complicated by infection ($n=1$).

UCB infusion-related adverse reactions

Four of 18 (22%) recipients of unmanipulated UCB had adverse reactions related to infusion. The reported reactions were chest tightness, increased blood pressure, decreased heart rate, nausea, and/or tingling sensation in the fingers or abdomen. These reactions were mild and

Table 3 Comparison of neutrophil and platelet recovery in this and other reported series of umbilical cord blood transplants

Reference	*Number of patients*	*Median (range) time in days to ANC $>500/mm^3$*	*Estimated probability of ANC $>500/mm^3$ by day 60*	*Median (range) time in days to platelet count $>20\,000/mm^3$*	*Estimated probability of platelet $>20\,000/mm^3$ by day 100*
Hahn *et al*, 2003					
Unmanipulated UCB units	**18**	**26 (16, 104)**	**94%**	**60.5 (41, 144)**	**50%**
Volume-reduced UCB units	8	37 (19, 59)	100%	91 (77, 105)	80%
Rubinstein *et al*[5]					
Volume-reduced UCB units	562	28 (10, 120)	91%	90 (16, 250)[a]	58%[a]
Laughlin *et al*[10]					
Unmanipulated and volume-reduced UCB units[b]	68	27 (13, 59)	90%[c]	58 (35, 142)	NS
Locatelli *et al*[6]					
Volume-reduced UCB units	60[d]	33 (12, 56)	79%	85 (16, 159)	78%[e]
Kurtzberg *et al*[3]					
Volume-reduced UCB units	25[f]	22 (14, 37)	NS	56 (35, 389)	NS
Wagner *et al*[4]					
Volume-reduced UCB units	18	24 (16, 53)	100%	54 (39, 130)	NS

[a]platelet count $>50\,000/mm^3$.
[b]The manuscript states 'some' units were volume reduced, but it does not state how many were in each group and reports the aggregate. Also, 11 unmanipulated and three volume-reduced UCBT patients included in Hahn *et al*, 2003, were included in Laughlin *et al*, 2001.
[c]measured by day 42 post UCBT.
[d]The total reported cases were 102 of which 60 received unrelated UCBT.
[e]The estimated probability of platelet recovery was calculated by day 180.
[f]Includes three unmanipulated and 22 volume-reduced UCB units.
ANC: absolute neutrophil count; UCB: umbilical cord blood; NS: not stated in manuscript.

transient with no serious toxicity observed. These reactions cannot be compared to other published studies as adverse reactions during UCB infusions have not been reported. However, the incidence and severity were low when compared with our experience using larger volumes of cryopreserved bone marrow and blood (unpublished observation). Patients with adverse reactions had a significantly larger volume reinfused than those who did not have an infusion reaction (median 106.5 *vs* 35 ml, $P = 0.021$). However, after adjusting for recipient weight, there was no relation between the volume per kilogram of body weight infused and development of an adverse reaction to the infusion (median 1.06 *vs* 0.57 ml/kg, $P > 0.1$).

In summary, unmanipulated UCBT showed no adverse effects on neutrophil and platelet engraftment or survival. Indeed, the outcomes of UCBT using unmanipulated units were comparable to the published literature reporting the use of volume-reduced units.

Discussion

It is postulated that volume reduction of UCB units in albumin/dextran solution before infusion into patients results in improved cell viability. This is based on an *in vitro* study by Rubinstein *et al*,[12] which showed that cell viability could be improved by volume reducing the UCB units before infusion to restore the osmolarity of the suspension and to remove the DMSO-containing supernatant. It was suggested that this process could protect the cells from the severe osmotic stress associated with infusion of cells suspended in medium with high concentrations of DMSO.[12] However, this study was conducted *in vitro* with no *in vivo* engraftment correlates. In addition, the products were only diluted 1 : 20 with media in this study, which does not reflect the physiology of DMSO dilution and catabolism in a human recipient. Neutrophils were the major cell population affected by the *in vitro* incubation whereas mononuclear cells that include the pluripotent stem cells were relatively resistant to the *in vitro* toxic effects of DMSO.

By reducing the volumes of both DMSO and cell lysis products, washing may also decrease the adverse reactions associated with the infusion of cryopreserved units.[13,14] However, volume-reducing UCB grafts after thawing can reduce the number of HSC infused into the patients because of cell loss during manipulation.[14–16] Many studies have shown that infusing a high nucleated cell dose is a good prognostic factor for both engraftment and survival in UCBT.[1,5,10] It is known that the number of cells infused during UCBT is one log less than in a standard allogeneic bone marrow transplant.[7,8] In addition, the UCB manipulation may cause qualitative changes in the product that may effect engraftment. The slow engraftment because of the limited number of HSC available in a single unit of UCB may contribute to high peritransplant mortality and limit the success of UCBT especially in adult patients.[6,8,10,12] Therefore, any process that may result in HSC loss or adversely affect HSC viability, that is, manipulation, should be avoided especially in UCB units with low numbers of HSC.

An earlier study observed delayed neutrophil recovery in three patients receiving unmanipulated UCB.[3] However, these patients received methotrexate as part of GVHD prophylaxis that impacts on hematopoietic recovery. This contrasts with the findings in our study of 18 nonvolume-reduced UCB recipients who did not receive methotrexate as part of GVHD prophylaxis. We found that the hematopoietic recovery and survival of the recipients of unmanipulated UCB were comparable to those of volume-reduced UCB in the literature.[3–6,10] The incidence of infusional adverse reactions was low, mild, and reversible. The administration of a relatively small quantity of DMSO to patients receiving unmanipulated UCB units resulted in acceptable toxicity with no effect on engraftment.

In conclusion, it is reasonable to infuse UCB into patients immediately after thawing and without manipulation to simplify the procedure and reduce cell loss, especially in cases of borderline cell doses when there is concern that further cell loss and UCB manipulation during volume reduction might adversely affect the outcome of UCBT.

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