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Few and Nonsevere Adverse Infusion Events Using an Automated Method for Diluting and Washing before Unrelated Single Cord Blood Transplantation



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Graft dilution and DMSO washing before cord blood (CB) administration using an automated system may offer low incidence of adverse infusion events (AIE), ensuring reproducible cell yields. Hence, we analyzed the incidences and significance of immediate AIE, cellular yield, and engraftment after single CB infusion. One hundred and fifty-seven patients (median age, 20 years; range, 1 to 60) received a single CB unit for treatment of hematologic and nonhematologic malignancies with myeloablative conditioning after graft dilution and washing. The median total nucleated cell (TNC) doses was $3.4 \times 10^7/\text{kg}$ (range, 2 to 26) and the median post-thaw recovery was 84% (range, 45 to 178). The cumulative incidence of neutrophil engraftment at 50 days was 84% (95% confidence interval [CI], 83 to 93). A total of 118 immediate AIE were observed in fifty-two (33%) patients. All reported AIE were transient, graded from 1 to 2 by Common Terminology Adverse Events version 4. The most frequent toxicity was cardiovascular but without any life-threatening reaction. Infused TNC, recipient's weight, and rate of infusion per kilogram were risk factors associated with cardiovascular AIE in multivariate analysis (odds ratio [OR], 1.2 (95% CI, 1.1 to 1.4); $P < .001$; OR, .94 (95% CI, .9 to .97); $P < .001$; and OR, 1.5 (95% CI, 1.2 to 1.8); $P < .001$; respectively). In summary, use of an automated method for graft washing before CB administration showed low incidence of AIE without compromising cell yields and engraftment. Infused TNC dose, recipient's weight, and rate of infusion per kilogram were risk factors associated with infusion reactions.

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INTRODUCTION

Umbilical cord blood (CB) transplantation has been increasingly used over the past decades as an alternative source capable of reconstituting bone marrow after allogeneic transplantation [1].

CB-derived hematopoietic progenitor cells (HPC) is a stem cell source from residual placental blood collected for hematopoietic stem cell transplantation. It contains leukocytes (mainly granulocytes) with variable amounts of red blood cells (RBC) and limited numbers of CD34⁺. This graft requires

an optimized method for administration, including a thawing protocol that maximizes HPC yield and minimizes adverse infusion events (AIE) derived from adventitious substances used for processing, cryopreservation, and residual RBC.

Rubinstein et al. [2] originally described the preparation of CB units for infusion using albumin-dextran dilution with centrifugation for “washing” to remove the dimethyl sulfoxide (DMSO), optimizing the viability of the thawed product. However, because of the risk of cell loss, faster and easier methods, such as albumin-dextran dilution without centrifugation [3] or direct infusion, have been proposed.

The causes of AIE are still not fully determined, but the amount of infused DMSO, post-thaw cell aggregation, RBCs lysis, and presence of granulocytes or debris [4] have also been associated with AIE. In an attempt to diminish these

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risks, the CB banking field has developed a method consisting of volume reduction (with plasma and RBC depletion) before freezing, improving the clinical safety profile and facilitating the thawing procedure to prepare CB units in transplantation centers. However, severe life-threatening AIE occasionally have been reported [5].

To confront the latter concerns, we proposed the use of an automated system for CB thawing to minimize intersample variability, control timing, and ensure control of cellular losses [6,7]. Based on this strategy, we validated an automated CB processing system for volume reduction using the cell separator Sepax (Biosafe, Eysins, Switzerland) device. This automated method consists of a microprocessor-controlled cell-processing device that works as a small blood separator with functionally closed apheresis sets that has been implemented in CB transplantation.

Here, we conducted a retrospective study of 157 consecutive patients who underwent unrelated single CB transplantation using this automated method thawing strategy, and we evaluated the safety profile by assessing the occurrence and severity of AIE.

METHODS

The study included 71 pediatric and 86 adult consecutive patients who received unrelated single allogeneic CB transplantation in the Hospital Vall d'Hebron (Barcelona), Hospital de Sant Pau (Barcelona), Hospital Germans Trias i Pujol (Barcelona), and Hospital Duran i Reynals (Barcelona), consisting of 4 adult and 2 pediatric transplantation programs between January 2005 and December 2013. All patients with hematological and non-hematological malignancies were eligible for enrollment if they met the following criteria: (1) allogeneic hematopoietic cell transplantation was considered the best therapeutic option, (2) a suitable related donor (HLA identical or 1 antigen mismatch) was not available, (3) there was a lack of a suitable HLA-matched unrelated donor at a reasonable time after the start of the search through international registries, and (4) there was a suitable umbilical CB unit available, as described below. Patients or their guardians gave written informed consent for their inclusion in each transplantation protocol.

Transplantation Procedure

Only patients receiving a first allograft were considered eligible for this study. The most commonly used protocol has been previously published [8]. All patients received post-transplantation granulocyte colony-stimulating factor from day +7 until neutrophil recovery.

For adult patients, precryopreservation minimum cell doses required were total nucleated cells (TNCs) $> 1.5 \times 10^7/\text{kg}$ and CD34^+ cells $> .6 \times 10^5/\text{kg}$. A degree of HLA matching between the umbilical CB unit and the recipient greater or equal to 4 or 6 (considering HLA-A at antigen level and -DRB1 at allele level) was required. For pediatric patients with malignant diseases, precryopreservation minimum cell dose required for selection was $\text{TNC} > 3 \times 10^7/\text{kg}$ and $\text{CD34}^+ > 1.5 \times 10^5/\text{kg}$ for 4 to 6/6 degree HLA mismatch. For children with nonmalignant diseases, precryopreservation minimum cell dose required was $\text{TNC} > 5 \times 10^7/\text{kg}$ and $\text{CD34}^+ > 2 \times 10^5/\text{kg}$ for 5 to 6/6 degree HLA mismatch.

Preparation of CB Units for Infusion Using an Automated Dilution and Washing Protocol

The CB bags stored in liquid nitrogen were thawed by immersion in a preheated 37°C water bath. When thawed, the CB bags were weighed, samples were taken for laboratory analysis, and the bag was then connected to a kit designed for umbilical CB cell washing and processed using the Sepax S-100 (Biosafe) in a closed system. A stock solution of 7.5% dextran-40 (molecular weight 40,000, Fresenius Kabi, Italy) and 5% human albumin (Grifols, Barcelona, Spain) was prepared and connected to the kit. The Sepax software “UCB-Washing” was used. CB units were automatically diluted 1:1 with the buffer and the product was mixed in the chamber and input bag in 5 minutes. Then, the chamber was filled with the washing solution. After a centrifugation step, the supernatant was removed and cells were diluted to the desired infusion volume. In our laboratory, the target volume was 70 mL for pediatrics and 100 mL for adults. Therefore, the aggregated dilution factor depends on the initial CB volume and ranges from 20 to 90.

Cord Blood Banks and Graft Characteristics

International and Spanish Cord Blood Banks (CBBs) provided CB units for transplantation. Prefreezing information was received from the original CBB provider. Post-thaw characteristics were determined before infusion at Banc de Sang i Teixits Cell Therapy Service, which serves as processing laboratory for all referring transplantation units.

The prethaw amounts of DMSO and dextran were estimated from the cryopreserved volume, according to the reported DMSO concentration (commonly available in the CB units' attached label). When the concentration was not reported, the banking standard of 10% DMSO and 1% dextran (v/v) was considered. The infused amount of DMSO was obtained following the formula: infused DMSO (grams) = 10% of cryopreserved volume/dilution factor. For example, for a CB unit frozen in a total of 25 mL (cryopreserved volume) and a dilution factor of 88 (25 mL of 1:1 dilution, then 170 mL to fill the chamber and an addition of 90 mL to the 10 mL pellet for a final infusion of 100 mL), the amount of DMSO infused is .03 grams.

CB Unit Assessment

Counting of TNC, RBC, and platelets was performed for each sample by using an automatic cell counter that detects and measures changes in electrical resistance (impedance) when a particle in a conductive liquid passes through the device (COULTER Ac T diff, Beckman Coulter, Inc., Miami, FL).

Quality of the unit was evaluated by cytometric assay of CD34^+ cell viability using 7-aminoactinomycin D, using a modified gating that included all dead cells [8] and colony-forming unit (CFU) assays. CFU assays were performed using a total of 10^5 cells plated in duplicate and colony growth was evaluated by light microscopy at 14 days.

As a surrogate value of CB graft potency, we used the ability of seeded CD34^+ to develop CFU (clonogenic efficiency). In our opinion, this value reflects very well the functional characteristic of the HPC contained in the bag [9].

Management of AIE

All patients received premedication with i.v. paracetamol and i.v. antihistamine (dexchlorpheniramine or diphenhydramine) before the infusion of the CBU, according to each institution's protocol. Adult patients were monitored immediately before and after progenitor infusion. Blood pressure, temperature, heart and respiratory rate, diuresis, and clinical symptoms were recorded in a registration form. Additionally, in pediatric patients hematuria or hemoglobinuria were closely monitored by Labstix strip test (Bayer, Germany) during and before infusion.

Signs and symptoms that occurred during and up to 24 hours after infusion were recorded by experienced transplantation nurses or physicians. Emergency medications, including i.v. furosemide, antihypertensive drugs, antiemetics, hydrocortisone, or oxygen, were at the patient's bedside for use, if needed.

Endpoints, Definitions, and Statistical Methods

Assessment of adverse infusion events

The primary endpoint of the study was the incidence and severity of AIE, graded by Common Terminology Criteria for Adverse Events version 4 (CTCAE v4). A severe AIE was defined as 4 and 5 (life threatening and death, respectively) or anaphylaxis, cardiac, pulmonary, or acute renal failure, seizure, transfer to intensive care unit, or death within 48 hours of CB infusion. We divided the immediate AIE in 2 main groups, depending on whether patients presented with cardiovascular or noncardiovascular events. CB units were infused in a single reduced-volume bag without requiring additional hydration before infusion.

Assessment of donor engraftment

Myeloid engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count $> .5 \times 10^9/\text{L}$ (without granulocyte colony-stimulating factor support) and transfusion-independent platelets $> 20 \times 10^9/\text{L}$ or higher for 7 consecutive days, respectively. Sustained donor engraftment was defined as sustained donor-derived count recovery with full donor chimerism ($>95\%$ donor hematopoiesis). Full donor chimerism was determined by quantitative polymerase chain reaction of informative polymorphic short tandem repeat (STR) regions of DNA from donor and recipient using AmpFISTR Identifier PlusPCR Amplification kit (Applied Biosystems, Carlsbad, CA). Patients who survived >42 days after transplantation and who failed to achieve myeloid engraftment were considered to have primary graft failure. Secondary graft failure was defined as the loss of the engraftment.

Assessment of graft-versus-host disease, nonrelapse mortality, relapse, and overall survival

Recipients were evaluated weekly for development and grading of acute graft-versus-host disease (GVHD). Acute and chronic GVHD were diagnosed and graded according to the standard criteria [10–12]. Patients dying

Table 1
Patients, Cord Blood Grafts, and Transplantation Characteristics (N = 157)

Characteristic	Value
Age, median (range), yr	20 (1–60)
Body weight, median (range), kg	52 (3–110)
Gender, male	81 of 157 (52)
Underlying disease	
AML	56 of 157 (36)
ALL	51 of 157 (32)
Immunodeficiencies	16 of 157 (10)
Metabolic diseases	4 of 157 (3)
Non-Hodgkin lymphoma	10 of 157 (6)
Hodgkin disease	10 of 157 (6)
CML	6 of 157 (4)
MDS	4 of 157 (3)
Conditioning regimen	
Thiotepa/Flu/Bu/ATG*	96 of 157 (61)
TBI/Cy/VP16/ATG	39 of 157 (25)
Bu/Cy/ATG	11 of 157 (7)
TBI/Cy/ATG	3 of 157 (2)
Other†	8 of 157 (5)
GVHD prophylaxis‡	
CsA-PDN	80 of 155 (52)
CsA-MMF	55 of 155 (36)
Other	20 of 155 (12)
HLA-A, -B antigen, -DRB1 allele match to patient	
6/6	33 of 155 (21)
5/6	63 of 155 (41)
4/6	59 of 155 (38)
ABO blood group mismatch	
Major	37 of 147 (25)
Minor	34 of 147 (23)
None	76 of 147 (52)
Donor-recipient sex match	
Male-male	37 of 141 (26)
Male-female	31 of 141 (22)
Female-male	40 of 141 (28)
Female-female	33 of 141 (24)
Donor-recipient CMV serology	
Donor and recipient negative	39 of 157 (25)

AML indicates acute myeloid leukemia; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; MDS, myelodysplastic syndrome; Flu, fludarabine; Bu, busulfan; ATG, antithymocyte globulin; Cy, cyclophosphamide; TBI, total body irradiation; VP16, etoposide; CsA, cyclosporine; PDN, prednisone; MMF, mycophenolate mophetil; CMV, cytomegalovirus. Data presented are n (%) unless otherwise indicated.

* The most conditioning regimen used was thiotepa (10 mg/kg i.v.), Flu (150 mg/m² i.v.), Bu (9.6 mg/m² i.v.) and in vivo T cell depletion with antithymocyte globulin (6 to 10 mg/kg i.v.).

† This is a miscellaneous group including patients who received different conditioning regimen as thiotepa/Flu/Cy/ATG, Bu/Cy/melphalan (Mel)/ATG, fludarabine, AraC (cytarabine), G-CSF (FLAG)/idarubicin (Ida)/melphalan (Mel)/ATG, treosulfan/Cy, and Flu/Cy/TBI.

‡ GVHD prophylaxis included CsA (1.5 mg/kg/kg/12 hours i.v. followed by 3 to 5 mg/kg/12 hours orally when oral intake was possible and slow tapering starting between day +90 and +180 if feasible. CsA was combined with short-course of steroids (1 mg/kg/daily) from day +14 to +28 or MMF (15 mg/kg/day from –1 to +30 day).

before +100 day were not considered for chronic GVHD (cGVHD) analysis. *Nonrelapse mortality* (NRM) was defined as death from any cause without evidence of relapse. *Overall survival* (OS) was defined as survival from the time of transplantation.

Statistical methods

Baseline characteristics were described as median and range for quantitative variables and frequency and percentages for categorical variables. Comparisons of quantitative variables were done by Mann-Whitney test. Binary logistic regression was used for univariate and multivariate analyses to estimate the association of risk factors with cardiovascular AIE. Characteristics selected for inclusion in the multivariate model were those with some indication of association in univariate analysis ($P < .10$). Results were expressed as odds ratios (OR) and their corresponding 95% confidence intervals [CI]. Survival probability was calculated using Kaplan-Meier estimation [13]. Cumulative incidence curves were used in a competing risk setting to calculate the cumulative incidence of neutrophil and platelet engraftment, acute GVHD, cGVHD, relapse, and NRM [14]. Death without

Table 2
Cell Recoveries after Dilution and Wash Method

CB Unit Characteristics	All CB Units (n = 100)	FACT CBB (n = 100)	non-FACT CBB (n = 57)	P Value
TNC, median (range) $\times 10^7$ /kg				
Prethaw	3.8 (2.1–27)	4 (2–26)	3.9 (2–25)	.883
Post-thaw	3.4 (2–26)	3.9 (1.9–26)	3 (1.8–20)	.279
TNC recovery, % (range)	84 (45–178)	88 (45–178)	83 (51–157)	.075
CD34 ⁺ , median (range), $\times 10^5$ /kg				
Prethaw	1.9 (1.1–26)	2 (1.1–9)	1.9 (1–7)	.805
Post-thaw	1.3 (1–23)	1 (0.4–23)	1 (0.4–10)	.419
CD34 ⁺ recovery (%)	75 (35–163)	76 (35–163)	73 (35–146)	.339
CFU, median (range), $\times 10^4$ kg				
Prethaw	6 (10–37)	5 (10–37)	7 (5–35)	.232
Post-thaw	3 (10–68)	3 (10–64)	3 (10–65)	.155
CFU recovery (%)	61 (63–68)	67 (60–72)	44 (69–162)	.378
CD34 ⁺ cell viability, median (range), %				
Prefreezing	83 (36–100)	78 (36–100)	90 (52–100)	.069
Post-thaw	89 (50–100)	89 (50–100)	93 (51–100)	.132
Clonogenic efficiency, median (range), %				
Pre-freezing	36 (45–99)	38 (8–92)	32 (4–99)	.567
Post-thaw	24 (6–91)	27 (4–76)	21 (4–91)	.006

relapse was the competing event for relapse. Relapse or death without developing acute or chronic GVHD was the competing event for acute GVHD and cGVHD, respectively. Death without engraftment was the competing event for neutrophil and platelet engraftment. All statistical tests were conducted using the SPSS statistical software (SPSS version 15.0, Chicago, IL). Cumulative incidence with competing risks was conducted in R software, version 2.15.2 (The CRAN project).

RESULTS

Patients and CB Units Characteristics

The main characteristics of the 157 patients included in the study are shown in Table 1. The median age at transplantation was 20 years (range, 1 to 60) and median weight 52 kg (range, 3 to 110 kg). The units were 6/6 (n = 33, 21%), 5/6 (n = 63, 41%), and 4/6 (n = 59, 38%) HLA matched to the patient. One hundred and six patients (68%) underwent transplantation for acute leukemia. Median follow-up was 36 months (range, 7 to 96).

Of all CB units used in the study, 90 (58%) were obtained from international and 67 (42%) from Spanish CBBs. Of the Spanish CBBs, 52 (33%) CB units were cryopreserved and stored in the Barcelona CBB. On the day of infusion, all CB units were thawed and washed using automated method in the Banc de Sang i Teixits cell therapy service and provided to the respective transplantation center. One hundred (64%) international CBBs were accredited by the Foundation for the Accreditation of Cellular Therapy (FACT). In 109 (69%) CB units, RBC reduction was performed. Thirty-seven (25%) patients received CB units with major ABO blood mismatch.

Characteristic of Cellular, Noncellular Components, and Cellular Recoveries

Precryopreservation, post-thaw cell doses, viability, and recoveries are summarized in Table 2. The median TNC cell doses infused was 3.4×10^7 /kg (range, 2 to 26) with a median TNC recovery of 84% (range, 45% to 178%). Median of CD34⁺ cell doses was 1.3×10^5 /kg (range, 1 to 23). For CBU obtained from FACT-accredited CBBs, median TNC, CD34⁺, and CFU were 88% (range, 45% to 178%), 76% (range, 35% to 163%), and 67% (range, 60% to 72%), respectively.

CB units had a median infusion volume of 100 mL (range, 31 to 188). After washing, median volume of residual infused RBC was .05 mL/kg (range, .001 to .108). Median content of

Table 3
Cardiovascular and Noncardiovascular AIE by Maximum CTCAE v4 Criteria

Patients with infusion reactions, n (%) [*]	52 of 157 (33%)
Total of AIE [†]	118
Cardiovascular events (n = 95)	
Grade 1 (n = 73)	
Bradycardia	23 of 73 (32%)
Tachycardia	4 of 73 (5.5%)
Hypertension	28 of 73 (38%)
Hypotension	14 of 73 (19%)
Chest pain	4 of 73 (5.5%)
Grade 2 (n = 22)	
Bradycardia	3 of 22 (13.5%)
Tachycardia	5 of 22 (23%)
Hypertension	11 of 22 (50%)
Hypotension	3 of 22 (13.5%)
Noncardiovascular events (n = 23)	
Grade 1	
Nausea and vomiting	15 of 23 (65%)
Lethargy	2 of 23 (9%)
Hemoglobinuria	6 of 23 (26%)
Grade 2	
None	-

* Percentages of AIE are referred to each grade of severity.

† In the entire cohort, there were patients who presented more than 1 AIE.

DMSO and dextran was .01 g/kg (range, .03 to .18) and .02 g/kg (range, .01 to .14), respectively.

Infusion Procedure and Adverse Reactions

The median time to infuse CB units was 22 minutes (range, 10 to 48). The incidence and severity of AIE graded by CTCAE v4.0 criteria are summarized in Table 3. One hundred and five patients (68%) had no AIE. The remaining 52 patients (33%) presented AIE, even though all were grades mild (1) to moderate (2), according to CTCAE v4.0 criteria. Although we noticed a total of 118 infusion reactions, remarkably we did not observe any AIE with severity grade higher than 2. The most frequently AIE detected were cardiovascular events (n = 95, 81%) presented in 50 patients (32%). Six patients with grade 2 hypertension were treated with furosemide i.v or antihypertensive per os. Grade 1 nausea/vomiting was treated by antiemetics i.v. All immediate AIE occurred during the administration of CB unit and were resolved soon after receiving the appropriate above-mentioned treatment. The remaining patients who presented AIE did not require intervention.

Factors associated with cardiovascular AIE

Factors associated with cardiovascular AIE occurrence in univariate analysis were older age, lower weight, malignant disease, more than 2 previous lines of treatment, times of infusion (shorter duration, faster rate, and faster rate per kilogram), higher infused TNC cell dose, and higher dose of infused RBC and RBC-replete CB units. In multivariate analysis, higher dose of infused TNC, lower weight, and faster rate of infusion per kilogram remained as predictive for cardiovascular AIE occurrence (OR, 1.2; 95% CI, 1.1 to 1.4; $P < .001$; OR, .94; 95% CI, .9 to .97; $P < .001$; and OR, 1.5; 95% CI, 1.2 to 1.8; $P < .001$, respectively) as shown in Table 4.

General Outcomes

Engraftment

Nineteen patients (12%) died before engraftment: 8 from primary graft failure, 8 from septic shock, and 3 from multiorgan failure with unknown cause. Overall, 17 (11%) patients presented with graft failure. Among the 9 patients alive after primary graft failure, 5 received a second umbilical

Table 4
Univariate and Multivariate Analysis of Risk Factors Associated with Cardiovascular AIE according Patients and CB Grafts Characteristics

Characteristics	OR (95% CI)	P Value
Univariate analysis		
Age, yr		<.001
Adult*	24.5 (9.4-64.2)	
Gender		.338
Female*	.7 (0.4-1.4)	
Weight, kg	.9 (.89-.94)	<.001
Underlying disease		
Malignant*	2.5 (1.2-5)	.011
Previous treatments		.062
<2 lines	2.2 (.6-8)	
2 lines	.5 (1.1-2.7)	
>2 lines*		
Previous ASCT		
Yes*	1.8 (.5-6.6)	.398
Volume of infused CB products, mL	1 (.99-1.01)	.733
Duration of infusion, min	.9 (.89-.98)	.002
Rate of infusion, mL/min	1.1 (.9-1.2)	.091
Rate of infusion per kg, (mL/min)/kg	1.9 (1.8-2.2)	<.001
Number of TNC infused, $\times 10^7$ /kg	1.5 (1.3-1.7)	<.001
Dose of DMSO, g/kg	2.4 (.7-8.2)	.172
Dose of dextran, g/kg	3.2 (.6-17)	.168
RBC, mL/kg	1.9 (1.5-2.4)	<.001
RBC content		
Replete	1.7 (.9-3.3)	.083
Depleted*		
ABO incompatibility		
None	1.01 (.4-2.5)	.938
Major	1.2 (.4-3.3)	
Minor*		
Accreditation		
FACT*	.9 (.4-1.8)	.757
Multivariate analysis		
Number of TNC infused, $\times 10^7$ /kg	1.2 (1.1-1.4)	.009
Weight, kg	.93 (.9-.95)	<.001
Rate per kg, (mL/min)/kg	1.5 (1.2-1.8)	<.001

ASCT indicates autologous stem cell transplantation.

* Reference category.

CB transplantation, 2 received haploidentical transplantation as salvage treatment, and the remaining 2 patients had autologous recovery.

The remaining 121 (77%) patients reached neutrophil engraftment with a median of 26 days (95% CI, 24 to 28). The cumulative incidences of neutrophil engraftment at 30 and 50 days were 62% (95% CI, 54 to 70) and 84% (95% CI, 83 to 93), respectively (Figure 1A). One hundred nineteen patients who engrafted showed full donor chimerism at the time of recovery. Five patients who presented engraftment by day +42 experienced secondary graft failure. One hundred twenty-one patients had platelet engraftment by day +42. The cumulative incidences of platelet engraftment at 30 and 50 days were 20% (95% CI, 14 to 26) and 69% (95% CI, 74 to 86), respectively (Figure 1B).

GVHD

Grade II to IV acute GVHD occurred in 43 patients (grade II in 24 patients, grade III in 10 patients, and grade IV in 9 patients) at a median time of 24 days (range, 14 to 97). The cumulative incidence of grade II to IV acute GVHD at day 100 after CB transplantation was 29% (95% CI, 22 to 36). Twenty (17%) of 115 evaluable patients developed cGVHD. cGVHD was limited in 9 patients and extensive in 11 patients. The median time to the development of cGVHD was 124 days (range, 117 to 2295). The 1- and 2-year cumulative incidences of cGVHD were 11% (95% CI, 5 to 17) and 17% (95% CI, 10 to 24), respectively.

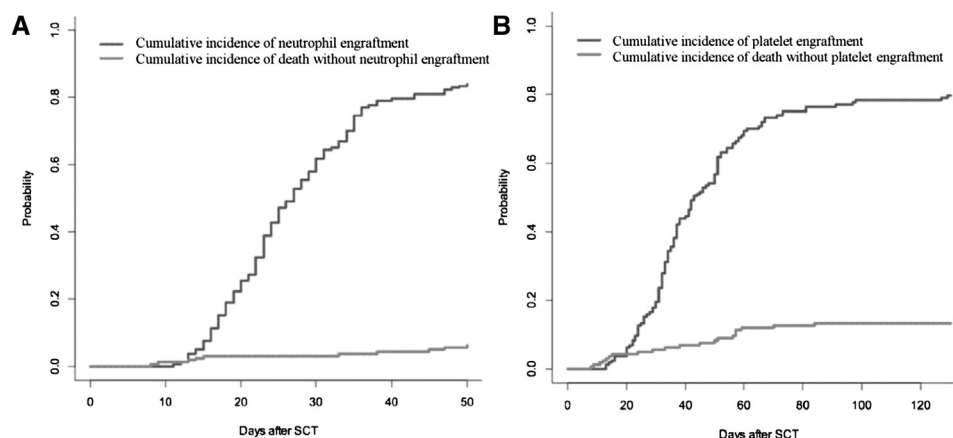


Figure 1. (A) Shows neutrophil recovery for the whole cohort. (B) Shows platelet recovery for the whole cohort.

NRM

A total of 81 (52%) patients died of transplantation-related causes. Infectious complication was the main cause of death ($n = 46$, 29%) followed by GVHD in 16 (10%) patients (14 for acute and 2 for chronic), graft failure in 8 (5%), multiorgan failure in 7 (4.5%) patients, and other causes in 4 (2.5%) patients. The cumulative incidence of NRM at 1, 2, and 3 years was 36% (95% CI, 28 to 44), 39% (95% CI, 31 to 47), and 42% (95% CI, 34 to 50), respectively.

Relapse and OS

Twenty-two (14%) patients relapsed at median time of 6 months (range, 1 to 75) and 9 (6%) died from this cause. The 1-, 2-, and 3-year cumulative incidences of relapse were 9% (95% CI, 4 to 14), 12% (95% CI, 7 to 17), and 14% (95% CI, 8 to 20), respectively. The probabilities of OS at 1, 2 and 3 years were 55% (95% CI, 44 to 60), 53% (95% CI, 45 to 61), 45% (95% CI, 37 to 53).

DISCUSSION

Our study shows that dilution and wash using the automated method to process CB units before infusion provides high percentages of cell recoveries and engraftment with a very low incidence of AIE and no serious toxicities.

The aim of the washing approach is to decrease AIE after CB transplantation. Although the true incidence of AIE remains unknown, prior studies report an incidence of AIE between 60% and 80%, which is higher than the incidence we found in our population (33% of patients). Moreover, we did not have any grade 3 to 5 reactions and all AIE after CB infusion were described as mild and transient. Additionally, the most frequent AIE reported previously were cardiovascular (hypertension) [15,16], as well as in our study.

AIE after CBT have been related to cryoprotectant agents, dead cell debris, lysis of RBC, or ABO incompatibility between donor and recipient. Serious AIE (especially cardiovascular) have been attributed to components of the cryopreservation solution, such as DMSO and/or dextran [17,18], as these could promote an acute extreme volume expansion.

The occurrence of AIE has been directly attributed to DMSO administration [17] in a dose-dependent manner and, in an attempt to minimize AIE, many institutions have chosen to limit the total amount of DMSO infused, whereas other groups have adopted washing protocols [18,19]. In our study, the median dose of DMSO after washing was very low, minimizing the incidence of AIE.

Recently, some reports have suggested that dextran is a potential causative agent contributing to severe AIE [4,20–22], such as myocardial ischemia or anaphylactic reactions. Dextran could promote acute volume expansion and idiosyncratic reactions, even at low concentrations. In our study, the median infused dextran dose was lower than the previously described dose capable of triggering AIEs [23,24], which could potentially explain why we did not detect any severe or lethal reactions immediately or over the first 24 hours after infusion.

Renal impairment after CB infusion has been previously associated with free hemoglobin and cellular debris [25]. Chow et al. [26] evaluated AIE after RBC-replete infusions and showed more hemoglobinuria in nonwashed infusions than in washed infusions. Barker et al. [27] developed a “nonwash” method to prepare units before double CB transplantations, adopting the policy of infusing exclusively RBC-depleted CB units, in an attempt to minimize AIE related to RBC debris leading to acute renal failure [28,29]. Based on our experience, we do not consider units containing RBC as a limitation to selecting CB units for infusion, as we apply the dilution and “wash” method, decreasing hemolyzed RBC, free hemoglobin, and membrane fragments. Of note, we did not find renal impairment in our patients, but we did observe the use of RBC-replete CB units as risk factor for cardiovascular AIE, with a trend to statistical significance. Highlighting the occurrence of serious or life-threatening reactions associated with CB infusions, the National Marrow Donor Program and the Food and Drug Administration recently provided recommendations for thawing and washing RBC-replete CB units [5]. On the other hand, premedication administered before infusion may help to reduce AIE.

As risk factors associated with AIE occurrence, we confirmed higher TNC cell dose infused, lower recipient's weight, and rate of infusion per kilogram, suggesting special consideration in small pediatric patients. Nonetheless, our study presents limitations, such as the retrospective nature of the analysis, the wide diversity of population regarding the age (and inherently the weight of patients), and underlying diseases affecting the recipients.

Preparation of CB units by albumin-dextran dilution and centrifugation (wash) before infusion has been challenged because of its risk of cell loss. This study demonstrates that the dilution and wash method allows minimization of the amount of DMSO that could affect the function of HPC, influencing short- and long-term engraftment. We

confirmed a high rate of sustained donor neutrophil and platelet engraftment, which supports that this automated method maintains cell viability and functionality, reducing the risk of cell loss, and our results are in line with those of other groups using dilution without centrifugation. Additionally, in accordance with other authors, we observed higher rate of recoveries in CB units provided by FACT-accredited banks, showing differences in CB banking practices [30].

In conclusion, our study demonstrates that dilution and wash with this automated method is a feasible option in CB transplantation, with a safe profile regarding AIE, that yields good recovery of cells and provides a high rate of engraftment in patients who receive unrelated single CB units for transplantation. The availability of this automated method for graft dilution and wash would facilitate the widespread use of this approach for CB administration, improving safety and, probably, outcomes.

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