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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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A B S T R A C T

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 3.4×10^7/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.

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) $> 1.5 \times 10^7$/kg and $CD34^+$ cells $> .6 \times 10^5$/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 TNC $> 3 \times 10^7$/kg and $CD34^+ > 1.5 \times 10^5$/kg for 4 to 6/6 degree HLA mismatch. For children with nonmalignant diseases, precryopreservation minimum cell dose required was TNC $> 5 \times 10^7$/kg and $CD34^+ > 2 \times 10^5$/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 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 (dexclorpheniramine 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 $> .5 \times 10^9$/L (without granulocyte colony–stimulating factor support) and transfusion-independent platelets $> 20 \times 10^9$/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 AmpFlSTR Identifiler 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