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Cord blood collection and processing with hydroxyethyl starch or non–hydroxyethyl starch

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Abstract

Background. Collection and processing characteristics influencing quality of cord blood (CB) units play an essential role to cord blood banks (CBBs). At many CBBs, volume reduction is performed using hydroxyethyl starch (HES) and the Sepax (Biosafe) automated cell processing system. Due to the withdrawal of HES from the European market, a validation of the nonHES protocol was performed. *Methods.* This partially retrospective study identified CB characteristics such as gestational age and CB volume/cell count correlated with higher quality. For the nonHES validation, CB was analyzed for total nucleated cell (TNC), mononuclear cell (MNC) recovery, hematocrit (HCT) and colony-forming units (CFUs). Viabilities of $CD34^+$ and $CD45^+$ cells were determined by 7-aminoactinomycin D (7-AAD) and AnnexinV (AnnV) staining and compared for 21 mL and 42 mL buffy coat (BC) samples applying the HES/nonHES protocol. *Results.* Factors affecting the potency of CB transplants were the gestational age and the volume reduction to a defined BC volume. High initial cell counts and CB volumes correlated negatively with post-processing TNC recovery for lower BC volumes. Post-processing HES and nonHES results were comparable, but nonHES revealed a significantly lower post-thaw recovery of viable $CD34^+$ cells measured by 7-AAD/AnnV (21 mL: 45.4 ± 16.4%; 42 mL: 67.3 ± 14.5%) as compared with HES (21 mL: 72.7 ± 14.4%, $P = 0.0164$; 42 mL: 83.4 ± 14.7%, $P = 0.0203$). *Discussion.* Due to the lower post-thaw $CD34^+$ cell viability ($AnnV^-/7\text{-}AAD^-$) for nonHES samples, the use of HES is recommended, ideally combined with a high BC volume. The post-processing HCT has no statistically significant impact on the post-thaw $CD34^+$ cell viability ($AnnV^-/7\text{-}AAD^-$).

Key Words: *AnnexinV, cord blood, processing*

Introduction

Umbilical cord blood (UCB) has been established as a rich source of hematopoietic stem cells (HSCs) that can effectively restore hematopoiesis [1–6]. Critical for a successful outcome is the selection of a quality cord blood unit (CBU), which will have significant engraftment potential and high potency. The potency of CBUs can be affected by events during routine CB manipulation (processing, freezing, thawing). To increase the yield of high-quality CBUs, CB characteristics predicting the potency of CBUs associated with higher quality need to be identified. Currently, the total nucleated cell (TNC) count is the only standardized and reproducible measurement of the CBU cell dose available at the time of graft selection to define CB potency. Clinical outcomes after CBU transplantation are influenced by the number of cells available in a single CBU [7–9]. Besides maternal, infantile and collection characteristics [10,11], the volume reduction to a defined buffy coat (BC) volume is one central factor affecting the cellular content of CB grafts.

Some transplantation centers also define a target dose of HSCs as a CB quality variable [3,8,9,12,13]. Therefore, the majority of cord blood banks (CBBs) report the CBU pre-freeze TNC count and the pre-freeze HSC content calculated on the basis of fresh $CD34^+$ cells and/or colony-forming units (CFUs). The potency, represented by CFUs and/or $CD34^+$ cell content, is associated with the engraftment potential [12–14] and may be superior to TNC dose measurements in predicting the quality. The clonogenic efficiency, a correlation between the amount of plated $CD34^+$ cells and counted CFUs, is a reliable indicator of potency [15]. The enumeration of HSCs by detecting the expression of the surface marker CD34 in flow cytometry following the protocol of the International Society of Hematotherapy and Graft Engineering (ISHAGE) is the most common technique to predict the potential quality of a unit [16].

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The amount of infused $CD34^+$ cells mostly correlates with the probability of engraftment in patients [12]. As reported, the potency can be lower than expected and might result in failure of engraftment [17]. The assessment of HSCs using simple flow cytometric analysis of $CD34^+$ cells leads to an overestimation of the actual potency of the transplant [18–20]. A potential loss of function and induction of apoptosis and necrosis have to be taken into consideration [21]. Duggleby et al. [22] and Radke et al. [21] demonstrated that the flow cytometric assessment of early apoptotic and necrotic cells by staining against 7-aminoactinomycin D (7-AAD) and AnnexinV (AnnV), respectively, is a feasible method for predicting the amount of CFUs. While 7-AAD [23,24] penetrates the damaged membranes of dead cells only, AnnV [25,26] stains phosphatidylserine, which is translocated to the outer membrane in apoptotic cells.

CBBs apply different methods and thus distribute products varying in characteristics such as final volume, plasma and/or red blood cell (RBC) content, the presence of manufacturing-related substances, the amount of added cryopreservative and the degree of cell viability [27–32]. As of January 2016, the José Carreras CBB of the Heinrich-Heine-University Medical Center in Duesseldorf has processed more than 25900 unrelated CB samples, 1940 of which were stored unseparated. The remaining units were volume reduced using hydroxyethyl starch (HES), which supports the RBC sedimentation during volume reduction [27]. High-molecular HES is a synthetic colloid acting as a plasma volume expander that can be infused at approximately 20 mL/kg body weight [33]. Safety issues applying HES solutions at low doses are discussed for patients with hypersensitivity to this colloid (incidence <1 in 1000) only and when applied at high concentrations (e.g., in trauma medicine). It is contraindicated in patients with bleeding disorders or renal insufficiency [34–37]. Due to side effects, the European Union (EU) has banned HES for clinical use in high doses. No side effects based on HES were observed in patients after CB transplantation because the concentration in thawed and washed CB is low. However, the production of good manufacturing practice (GMP)-graded HES only for CBBs is not profitable for suppliers. Because HES is, therefore, not available as a pharmaceutical drug in Germany/Europe anymore, a validation of the nonHES protocol (based on the current released version *UCB 321*) using the Sepax (Biosafe) automated cell processing system was performed. Results for post-processing and post-thaw TNC recovery and viability were comparable for both methods. Only the recovery of viable $CD34^+$ cells ($AnnV^-/7\text{-}AAD^-$) after thawing was significantly lower for nonHES samples than for HES samples.

Materials and methods

Study overview

This is a partial retrospective study conducted between 2006 and 2014 by the José Carreras CBB (Duesseldorf, Germany). CBUs collected from more than 80 cooperating collection centers were examined for gestational age and CB characteristics such as initial volume and cell count to select CBUs to be processed at the public CBB in Duesseldorf.

A validation of the Sepax UCB nonHES protocol versus the established HES protocol was performed in 2014/2015 to reveal potential functional and quality differences after volume reduction and thawing that might affect CBU potency.

CB donor eligibility

Donor eligibility is based on the history and risk factors of the mother according to German guidelines [38]. Eligible donations included CB collections with a gestational age of at least 36 weeks donated by a mother of legal age (≥18 years). The declaration of informed consent for the donation of UCB had to be submitted together with the anamnesis of the mother and the family, the CB and the maternal blood sample.

CB collection and processing procedures

CB collection

The collection of CB was performed using either *in utero* or *ex utero* (in case of cesarean section) collection procedures. After delivery of the baby, the umbilical cord was doubly clamped, transected, and CB was collected into the collection bag containing 29 mL of citrate-phosphate dextrose (CPD) anticoagulant (MSC 1206 DU and MSC 1209 DU, Macopharma).

Processing and cryopreservation

Processing and cryopreservation was performed in the GMP facility of the CBB within 48 h after birth. The total collection volume (in mL) including anticoagulants was measured by weight excluding the tare bag (26 g). A minimum weight of 100 g was required to qualify for processing. CBUs not meeting the threshold volume were discarded according to the CBB standard operating procedures (SOPs).

The retrospective study regarding the influence of CB characteristics on TNC count, $CD34^+$ cell count and CFU content is based on CBUs that were processed in Duesseldorf between 2006 and 2014. After the addition of HES (Hespan, Fresenius Kabi), the processing was performed by volume reduction with the Sepax to a defined BC volume of 21, 24 or 42 mL. These volumes are registered in the license of the Duesseldorf CBB due to historical reasons. Today, BC