Cord blood volume reduction using an automated system (Sepax) versus a semi-automated system (Optipress II) versus a manual method (Hydroxyethyl Starch Sedimentation) for routine cord blood banking: *a comparative study*

Valérie Lapierre, Nadia Pellegrini, Isabelle Bardey, Caroline Malugani, Philippe Saas, Francine Garnache, Evelyne Racadot, Françoise Schillinger, Stéphane Maddens

Besançon Cord Blood Bank, 1 bd Fleming, BP1937, 25020 Besançon, France

INTRODUCTION

Umbilical cord blood (CB) contains hematopoietic stem cells (HSC) capable of long-term hematopoietic reconstitution in patients given myeloablative conditioning regimens. These findings plus the relative simplicity of CB collection led to the development of cord blood banks to increase the possibilities of unrelated HSC transplantation, particularly in children.

In 1995, the Etablissement Français du Sang Bourgogne Franche-Comté initiated a program aimed at creating the Besançon Cord Blood Bank (BCBB). Since July 2001, the BCCB has cryopreserved all its CBU using the BioArchive™ (Thermogenesis) system. CBU volume reduction is performed prior to freezing, thus providing a valuable answer to storage space problems. We initiated a study to compare the results of these 3 different volume reduction methods used consecutively in routine : Hydroxyethyl Starch Sedimentation (HES) technique, a two-step (top and bottom) (TB) extraction of the cell and plasma components and the Sepax™ (Biosafe, Eysins, Switzerland) volume reduction method.

MATERIALS AND METHODS

Quality control tests

CBU conformity is assessed first at reception, based on two criteria: the collected volume must be ≥ 80 mL without anticoagulant and the CD34+ cell content must be ≥ 2x106 /bag. CBU conformity is assessed again after volume reduction, the CD34+ cell content must by ≥1.6x106 /bag.

Data Collection

All data were prospectively retrieved from the electronic CBU file used at the BCBB. Only the CBU recorded in the France Greffe de Moelle (FGM) Registry were included in this study. Non-conforming units were excluded in cases of insufficient CD34+ cell counts after volume reduction, positive microbiology tests and/or positive mother serology, and if CBU validation was impossible at 2 months. There were no differences between the 3 techniques in terms of non-conformity for the above quality reasons (Table 1).

Statistical analysis

Comparison of initial blood sample characteristics was done by t-test for equal variances or unequal variances if necessary.

Comparison in terms of recovery between the three techniques was performed by linear regression and adjusted for the initial blood volume and the initial cell content.

For each technique, Spearman correlation coefficient was calculated between recovery and initial cell content and initial blood volume.

HES

Top and Bottom

Sepax

Hydroxyethyl Starch Sedimentation (HES)

From July 2001 to January 2002, CB bags were centrifuged after having added an adjusted volume of HES, CBU are centrifuged at 23,150g. They are then carefully removed from the centrifuge, taking care not to disturb the RBC/plasma interface. The CB bag is placed in a plasma extractor. Red blood cells are removed first and the bag is sterile-connected to the middle tube of the Pall processing set. A second centrifuge step is performed for 10 min at 400g, before the cell suspension is adjusted to precisely 21 ml using the ThermoGenesis semi-automated extractor.

Top-and-bottom method

From January 2002 to January 2003, 181 CBU were processed following the top-and-bottom method. CB bags are centrifuged at 2,230g at room temperature, and connected to the Optipress II Automated Blood Component Extractor (Baxter, Healthcare, Newbury, UK). An automated procedure allows plasma removal followed by RBC removal..

Sepax Cell Processing System

The Sepax cell separation technique (Biosafe, Eysins, Switzerland) is an fully automated method. After HES addition at a final 20% (v/v) concentration the CBU is connected to the Sepax kit (CS-530, Biosafe, Eysins, Switzerland). The closed kit includes a variable volume separation chamber, enabling the processing of a large range of blood volumes. The microprocessor-controlled protocol involves the following steps: cord blood is transferred to the separation chamber and centrifuged. The separated components are collected sequentially, with the buffy-coat directly in its freezing bag. Parameter modifications are menu driven through a simple display/keyboard. Length of procedure including kit installation is about 35 min. Moreover, we use the Coolmix system (Biosafe, Eysins, Switzerland) which introduces DMSO/Dextran automatically in a temperature and time-controlled fashion

RESULTS

CBU characteristics prior to processing are summarized in Table 1. Comparison of 3 processing are summarized in Table 2 and 3

Interestingly, with Sepax, TNC and CD34+ cell recoveries were not correlated to pre-processing values (p=0.8 and 0.4 respectively) while with HES and TB, TNC recovery was negatively correlated (p<0.0001 both) i.e., the higher the initial cell count was, the lower the recovery was. CD34+cell recovery was also negatively correlated to pre-processing values with HES method (p<0.0001) but not in the TB group (p=0.2).

In all cases TNC and CD34+ cell counts after volume reduction were significantly lower than with the whole blood (Table 4).

Table 1: Reasons for CBU non-corformity after volume

	HES	TB	Sepax	p
Conforming at reception	583	251	286	
Non -conforming after volume reduction				
Technical Problem	14 (2.4)	6 (2.4)	12 (4.2)	p=0.30
Quarantine impossible	56 (9.6)	25 (10.0)	20 (7.0)	p=0.44
Positive Microbiology	66 (11.3)	39 (15.5)	41 (14.3)	p=0.21
Recorded in the FGM Registry	447 (76.7)	181 (72.1)	213 (74.5)	p=0.87

Number (%)
CBU: Cord Blood Unit HES: Hydroxyethyl starch sedimentation
TP: Top and bottom method Sepax: Sepax® cell processing system
FGM: *France Greffe de Moelle* (French Unrelated Hematopoietic Stem Cell Registry)

Table 2: Initial CBU characteristics

Technique	No.	Volume (mL)	Total nucleated cell counts (10^9)	% CD34+ cells	Total CD34+ cell counts (10^6)
HES	447	111 (± 23)	1.60 (± 0.50)	0.35 (± 0.20)	5.56 (± 3.35)
TB	181	106 (± 21)	1.57 (± 0.49)	0.35 (± 0.16)	5.60 (± 3.24)
Sepax	213	106 (± 20)	1.57 (± 0.50)	0.33 (± 0.15)	5.12 (± 3.00)

Mean (± standard deviation)
CBU: Cord Blood Unit
HES: Hydroxyethyl starch sedimentation
TP: Top-and-bottom method
Sepax: Sepax® cell processing system

Statistical analysis
Volume: HES vs TB p= 0.01; HES vs Sepax p=0.003 ; TB vs Sepax p = 0.89
TNC counts: HES vs TB p= 0.55; HES vs Sepax p=0.52 ; TB vs Sepax p = 0.99
CD34 + cell counts: HES vs TB p= 0.88; HES vs Sepax p=0.10 ; TB vs Sepax p = 0.13
% CD34 + cells: HES vs TB p= 0.95; HES vs Sepax p=0.06 ; TB vs Sepax p = 0.08

Table 3: Total nucleated cell and total CD34+ cell recoveries

Technique	No.	TNC	CD34+ cells
HES	447	76.82 (± 9.10)	81.46 (± 12.50)
TB	181	60.72 (± 13.55)	81.99 (± 17.68)
Sepax	213	80.26 (± 7.73)	86.08 (± 11.63)

HES: Hydroxyethyl s tarch sedimentation
TP: Top -and -bottom method
Sepax: Sepax® cell processing system

Statistical analysis
CD34+ cell recovery: Sepax vs HES p< 0.0001; Sepax versus TB p=0.008
HES versus TB p=0.72
Total nucleated cell recovery: Sepax vs HES p< 0.0001; Sepax vs TB p<0.0001
HES vs TB p<0.0001

Table 4: Comparison of total nucleated cell and total CD34+ cell counts in the CBU recorded in the *France Greffe de Moelle Registry (French unrelated hematopoietic stem cell registry)*

Technique	No.	TNC counts	CD34+ cell counts
Whole blood	1214	1.53 (± 0.53)	5.39 (± 3.61)
HES	447	1.22 (± 0.38)*	4.44 (± 2.52)*
TB	181	0.93 (± 0.29)*	4.54 (± 2.70)*
Sepax	213	1.26 (± 0.40)*	4.40 (± 2.64)*

TNC: Total nucleated cells
HES: Hydroxyethyl starch sedimentation
Sepax: Sepax® cell processing system
TP: Top-and-bottom method

Statistical analysis
Comparison with whole blood p<0.0001

In conclusion, the closed Sepax system offers the advantage of a time-saving CBU volume reduction method that allows good cell recoveries as compared to either the HES or TB method. In addition all the process of volume reduction is performed as a closed system preventing contamination and therefore limits infrastructure constraints. Moreover, cell recovery rates are independent of the initial TNC and CD34+ cell counts