Factors predicting the hematopoietic stem cells content of the umbilical cord blood

Suleimman A. Al-Sweedan [a,*], Lama Musalam [b], Basil Obeidat [b]

[a] Department of Pediatrics, Jordan University of Science & Technology, P.O. Box 4614, Irbid 21110, Jordan
[b] Jordan University of Science & Technology, Irbid, Jordan

ARTICLE INFO

ABSTRACT

Umbilical cord blood (UCB) has been demonstrated to be alternative source of hematopoietic stem cells (HSCs). Unfortunately, the wide use of UCB Transplantation is limited due to the low number of HSCs. The aim of this study was to determine factors that affect the number of HSCs collected from UCB. 200 eligible donors were included for HSCs testing, including total nucleated cells (TNCs) and CD34+ cell number, by using univariate and multivariate analysis. In univariate analysis, factors positively associated with higher number of TNCs were maternal weight ($P = 0.002$), preeclampsia ($P = 0.03$), neonatal weight ($P < 0.001$), neonatal platelet count ($P = 0.02$), neonatal Rh ($P = 0.03$), gestational age ($P = 0.04$) and delivery type ($P < 0.001$). Factors positively associated with higher number of CD34+ cells were maternal weight ($P < 0.007$), preeclampsia ($P = 0.02$), maternal hypertension ($P = 0.02$) neonatal weight ($P < 0.001$), neonatal Rh type ($P = 0.02$) and delivery type ($P = 0.04$). In multivariate analysis, factors significantly influence TNCs were neonatal weight ($P < 0.001$), preeclampsia ($P = 0.008$), neonatal Rh type ($P = 0.02$) and delivery type ($P < 0.001$). While factors significantly influence number of CD34+ cells were maternal weight ($P = 0.025$), neonatal weight ($P = 0.005$), neonatal Rh ($P = 0.006$), nuchal cord ($P = 0.026$) and delivery type ($P = 0.009$). Conclusions factors significantly influence TNCs content of UCB were neonatal weight, preeclampsia, neonatal Rh and delivery type. While factors significantly influence number of CD34+ cells were maternal weight, neonatal weight, neonatal Rh, nuchal cord and delivery type.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Hematopoietic stem cells (HSCs) are primitive cells that maintain the structural and functional integrity of the hematopoietic system [1,2]. Bone marrow (BM), peripheral blood and umbilical cord blood (UCB) are sources that provide HSCs [3,4]. HSCs transplantation is an important key for treating certain hematologic, genetic and malignant disorders [5,6]. Access to BM transplantation is limited by difficulties in finding suitable HLA-matched donors for patients without matched siblings [7,8]. Even with fully matched allogeneic BM grafts, its success is limited by a

high frequency of severe graft versus host disease (GVHD) in the recipient [7]. Last decade has witnessed an increase in the use of UCB as an alternative source of HSCs for allogenic transplantation due to several advantages of UCB [9–15]. Practical advantages include ease of collection of material that is discarded routinely, exists in almost limitless supply [16], without any discomfort or risk to the donor, its prompt availability as a frozen graft and unlikely to transmit infectious agents to the recipient [17,18]. Moreover, UCB Transplantation permits more liberal HLA-matching [19]. So, it can be given to fully or partially HLA-matched related or unrelated recipients [11,14]. UCB shows decreased immune responses to alloantigen, with a reported low incidence of severe acute and chronic GVHD [20–22]. Efficacy of UCB Transplantation correlates mainly

---

* Corresponding author. Tel.: +962 799051255.
E-mail address: sweedan@just.edu.jo (S.A. Al-Sweedan).

with HSCs dose and success being associated with the total nucleated cells (TNCs) and CD34+ cells content infused [23–26]. TNCs infused has been considered a reliable and reproducible indicator of transplantation outcomes [27,28]. Number of CD34+ cells is used as an additional parameter to assess hematopoietic potential of UCB unit [29,30]. Unfortunately, one restriction that limits the use of UCB Transplantation, especially in large children and adult patients, is the relatively low number of HSCs harvested from single UCB unit [15,31]. This results in slower time to engraftment and higher transplant related mortality [16,32].

2. Materials and methods

2.1. Study design

A cross-sectional study design was used to examine the association of certain study factors on the TNCs and CD34+ cells content of the UCB units.

2.2. Study population

2.2.1. Sampling population

Hospitals based study was carried out on 200 women admitted to Princess Badea'a Hospital/Irbid/Jordan over 7 months period between Aug/2008 and Feb/2009. All study subjects provided written informed consent in accordance with the Helsinki declaration which was approved by the Institutional Review Board at Jordan University of Science and Technology.

2.2.2. Criteria for donor eligibility

Medical record of each mother participated in UCB donation was reviewed. Mothers having any inherited, infectious, including HIV I, HIV II, HBV and HCV, or any other diseases or taking medications during pregnancy were excluded. Other exclusion neonatal criteria were the presence of congenital abnormality in the newborn, twins, stillbirth or neonatal death. All infants 5-min Apgar score was (9–10) and the results of the cardiotocogram (CTG) were normal.

2.3. Data collection

2.3.1. UCB collection

UCB units were harvested ex-utero using open system from both spontaneous vaginal and cesarean sections deliveries with the help of the obstetricians and midwives.

Umbilical cord was clamped immediately after the delivery process as close as possible to the infant within 30 s of birth. Then, a second clamp was applied around 5 cm from the first and the cord was cut between the two clamps.

Two blood samples were drawn from the umbilical vein at the most distal possible vein puncture site (closest to the second clamp to minimize the loss of UCB) using needles attached to EDTA anticoagulant blood tubes. After that, the umbilical vein was un cannulated and blood was drained by gravity from the transected end of the cord into

graduated sterile container until blood flow stopped. Total volume of UCB was recorded, before these containers had been discarded. Samples were kept in the refrigerator until sent to the laboratory at KAUH/Irbid/Jordan within 24–48 h of the collection for testing.

2.3.2. UCB testing

Two anticoagulant UCB specimens were drawn from each subject for TNC and CD34+ cells measurement. Nucleated cells content per micro liter of UCB (WBC counts) were enumerated by an automated autoanalyzer (using Pentra 80 autoanalyzer) at the Hematology Lab. This was used to calculate the TNC content per UCB sample by multiplying WBC count with the UCB volume which is illustrated by the following equation: TNC count (per unit) = WBC count $\times$ total UCB volume. The percentages of CD34+ cells (the fraction of CD34+ cells within the leukocyte population) were quantified by standard flow cytometry using (FACScalibur cytometer with CellQuest Pro software) which is based on using phycoerythrin (PE) labeled monoclonal anti-CD34+ antibody after RBCs lysis using lysing solution. Then, the absolute number of CD34+ cells per UCB sample was measured by multiplying the relative CD34+ cell count (percentage) with the of total nucleated cells as detailed in the following equation: Absolute number of CD34+ cells (per unit) = % CD34+ cells TNC count.

2.3.3. Data collection of maternal, neonatal and obstetric factors

Data collection sheets that included maternal, neonatal and obstetric factors were filled from each participant using medical record review or direct observation.

Maternal factors obtained include (Age, weight, smoking status, ABO-Rh blood group, hypertension and preeclampsia. While neonatal factors obtained include: (gender, neonatal weight, birth order, ABO-Rh blood group, hemoglobin concentration, platelet count and gestational age). Finally, the examined obstetric factors were: delivery type of either spontaneous vaginal or cesarean sections and presence of nuchal cord (which is wrapping of umbilical cord around the neck of the baby).

2.3.4. Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSSs, version 15.0) software. Descriptive statistics were provided to display the characteristics of UCB HSC variables and maternal, neonatal and obstetric factors. Data were analyzed for univariate analysis using Spearman's correlation coefficient for continuous variables and Student's t-test for categorical variables. Multiple linear backward regression was also performed to examine the effect of the various maternal, neonatal and obstetric factors on the UCB HSC variables. Statistical significance for all tests was considered at P value < 0.05.

3. Results

UCB was collected from 200 women; their mean age was 28.29 years with a range of 18–44 years. 92% of the