Cord Blood Donor Criteria: A Cross-Sectional Study in Augusta

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Abstract

Background: There has been a growing interest in improving the quantity and quality of the total nucleated cell (TNC) count and the percentage of CD34+ cells obtained from umbilical cord blood (UCB). These indices influence the success of stem cell transplant outcome. Our aim was to optimize the donor selection criteria at Augusta University Medical Center cord blood bank (Augusta, Georgia, USA).

Study Design & Method: A prospective, cohort study was conducted involving women in labor presenting to Augusta University Medical Center from September 2014 to October 2015. Cord blood was processed to extract potential stem cells. The results of the TNC and CD34+ cells counts were then compared to donor characteristics extracted from electronic health records (EHR). All statistical analyses were performed using SAS 9.4. Statistical significance was set at an alpha level of 0.05. Descriptive statistics were calculated for relevant donor variables. In order to find any association between neonatal characteristics and CD34+ cells count, CD34+ cells viability percentage, and TNC we used Multivariate Linear Regression technique. Multivariate regression analysis was performed using dependent (TNC, CD34+cells count /viability percentage) and independent variables. Mean, standard deviation, range, and other statistical measures were calculated for all descriptive variables.

Results: (i) Maternal ethnicity, gravidity, and neonatal gender showed significant positive predictors for CD34+ cells count. (ii) However, for CD34+ cell viability percentages, only neonatal gender was a significant positive predictor. (iii) For TNC, gravidity and neonatal gender were both significantly positive predictors.

Conclusion: Our study suggests that maternal donor characteristics significantly influence the yield of TNC, CD34+ cells count and viability of UCB samples. These factors should be considered when attempting to improve the yield of potential stem cells in cord blood programs. A larger sample size is needed to confirm these results.

Keywords: CD34+ cells; Total nucleated cells; Umbilical; Cord blood; Stem cells; Hematopoietic

Introduction

The first clinical trial of umbilical cord blood (UCB) transplantation was performed in 1988 when stem cells extracted from UCB were used as a substitute for bone marrow in a 6-year-old boy diagnosed with Fanconi’s anemia [1]. Dr. Elian Gluckman performed this groundbreaking procedure at the Transplant Center of “St. Louis” Hospital, Paris [2]. By 2006, more than 10% of allogeneic bone marrow transplants in the USA were performed using UCB. The use of UCB for stem cell donation has revolutionized bone marrow transplantation. Indications for stem cell transplantation have broadened, and now include spinal cord injury, eye disease, and others [3]. Research now focuses on improving the process of UCB stem cell extraction. Previous research has focused on the technical aspects of cord blood cell extraction and storage [4]. Only recently research is investigating the effect of donor characteristics in the success of stem cell extraction [5-7].

The use of fetal organ tissue as a source of hematopoietic stem cells is significant ethical concerns. UCB stem cells appear to have similar bio-effects as HSC’s extracted from fetal organs.

Advantages of the Use of Umbilical Cord Blood Stem Cells

There are many potential advantages in using UCB stem cells as opposed to adult bone marrow-derived hematopoietic stem cells (HSC) [8].

UCB stem cells appear to be more potent than HSC’s extracted from adult bone marrow. It has been demonstrated that 80-120 ml of UCB contains as many hematopoietic stem cells as 1200 ml of adult bone marrow aspirate. The quality of the UCB stem cell graft is superior to that of HSC derived from adult bone marrow, although cord blood contains less mesenchymal stem cells [9]. In the Unites States, there are 4.5 million deliveries annually. In the vast majority of cases, the umbilical cord blood is discarded with the placenta. UCB stem cells are readily available and easy to process. Extraction of stem cells from UCB is a simpler process and the time from collection to transplant is shorter than HSC extraction from adult donors [10].

The graft rejection risk is significantly lower for UCB stem cells as opposed to HSC extracted from adult donors. UCB stem cells are not antigenically mature. Full HLA compatibility before transplant of UCB is not required, which allows transplantation from a wider donor pool [3]. Because UCB stem cells are immunologically immature, the fetal immune system rarely creates blocking antibodies [8]. The success rates of grafts using UCB stem cells are higher than those of peripheral blood and quite similar to those in bone marrow [10].

Cell replication from UCB stem cells is superior to that of HSC’s derived from adult bone marrow, presumably due to longer telomere length. UCB also contains mesenchymal stem cells, which are non-hematopoietic progenitor cells. These mesenchymal stem cells can differentiate into many types of mature cells, such as neuronal cells, osteoblasts, adipocytes, and chondroblasts [11]. As opposed to bone marrow aspiration in an adult donor, there is little no associated risks of morbidity or mortality from collecting umbilical cord blood [3]. Cytomegalovirus infection risk using UCB is lower than adult bone marrow, which reduces post-transplant complications [10].

Disadvantages of the Use of Umbilical Cord Blood Stem Cells

UCB stem cells are more primitive than peripheral blood and bone marrow stem cells; therefore, immune system recovery time is longer. This may put the patient at risk of infection for a longer period of time [12]. The volume of collected UCB stem cells is smaller than that recovered from the bone marrow aspirate of a corpulent child or an adult. This is an important limitation factor for a successful graft. Also, the viability of UCB stem cells after cryopreservation is still undetermined. A 10-year period storage of umbilical cord blood stem cells leads to a decrease in CD34+ cell number and viability. Although HLA matching is not required, bone marrow transplants still require a closer antigen match than UCB, which is not possible in the matching of grafts from related donors [10].

Conclusion

Our study suggests that maternal donor characteristics significantly influence the yield of stem cells from UCB. These factors should be considered when attempting to improve the yield of potential stem cells in cord blood programs. A larger sample size is needed to confirm these results.

Keywords: CD34+ cells; Total nucleated cells; Umbilical; Cord blood; Stem cells; Hematopoietic
cells is the current standard [8]. Longer storage times may be feasible, although this has not been studied.

In medical practice, there has been a dramatic increase in the use of umbilical cord blood (UCB) stem cells for the treatment of hematopoietic malignancies and hereditary blood diseases. Current research is ongoing to determine the feasibility of UCB stem cells for use in drug sensitivity investigations, biomedical therapies and regenerative medicine [13].

The majority of stored UCB in the United States is for the private use of families, in the event of malignancy diagnosis in the child or a relative. At present, the cost of sample preparation and storage has limited widespread acceptance of UCB as a public health resource. It was reported that more than half of the collected samples in some of UCB banks are discarded, either due to their low volume of total nucleated cells (TNC) or due to maternal factors [13].

In a previous study UCB TNC positively affects CD34+ cells count. There is a great hidden promise in this significant correlation as many efforts are focused for cell expansion and CD34+ cells proliferation that can increase the usefulness of UCB during transplantation. This correlation might help in conducting more studies on the cord blood cellular content [14].

It is essential to know the donor obstetric profile and which factors might influence the quality of the product. Higher TNC count is thought to achieve better results in hematopoietic stem cell transplantation. Many obstetrical factors can influence the choice of using a single UCB stem cell donor for transplant including blood group typing, HLA typing, birth weight, neonatal gender, parity, low venous pH, prolonged first stage of labor and Apgar scores [15]. Technical factors can also influence the choice of a single UCB stem cell donation such as TNC, CD34+ count, processing procedures and storage time [12].

In Japan (Tokyo metropolitan area), retrospective analysis of UCB stem cell transplants showed that the most important positive predictors for success were first pregnancy of the donor (primigravida) and age over 30. Other factors such as birth weight, Cord Blood Unit (CBU) collected volume, CD34+ count and gestational age showed significantly positive associations with TNC. Order of birth showed a significantly negative association [16,17].

Carolinus Cord Blood Bank defined high-quality CBU as those with higher post-TNC count (>1.25 × 10^9) and CD34+/CFU (colony forming units) in the upper quartile. They demonstrated the higher CD34+ or CFU were associated with interval from collection to processing <10 hours, gestational age (34–37 weeks), Caucasian race, birth weight >3500 grams and CBU collected volume >80ml [18].

In Brazil, different cross sectional studies have been performed over the years to correlate maternal and fetal factors to cord blood quality as source of stem cells. An earlier study found that cord blood collection technique and newborn weight were significantly correlated with the TNC count [2]. Later, a different group demonstrated that delivery route and birth weight affect cord blood volume and TNC while gestational age affects only the cord blood volume [19].

Based on the available literature, there are significant discrepancies among recommendations for improving the quality and quantity of UCB stem cell samples. These differences may arise from the wide range of patient populations represented in the published studies. Therefore, precise, population-based information is required to determine optimum predictors for high yield TNC/CD34+ cells for UCB stem cell sampling.

The current study aimed to describe the obstetric characteristics of a defined population of cord blood donors and identify which factors were likely to provide larger UCB volumes and higher TNC and CD34+ progenitor cell yields in UCB samples. Cell dose is the main limiting factor for the widespread adoption of UCB stem cells as a source of hematopoietic progenitors. For that reason it is valuable to optimize donor selection collection method and many obstetric factors to achieve high-nucleated cell dose ([2 × 10^7/kg] for the adult transplantation and (3.7 × 10^7/kg) for children) [13].

**Materials & Methods**

**Study design**

This study was a prospective cohort study performed on the Labor and Delivery unit Department of Obstetrics and Gynecology at Augusta University Medical Center, USA from September 2014 to October 2015.

**Subjects**

A total of 100 women between 20-35 years who were scheduled for cesarean section were enrolled in the study. A written informed consent was obtained prior to the cesarean section after a thorough explanation of the procedure. Patients who were planning on private umbilical cord blood banking were excluded from the study, so as not to reduce their sample volume.

**Exclusion criteria**

Known fetal genetic defects (aneuploidy, spina bifida), premature delivery (less than 37 weeks), medical disorders complicating pregnancy (hypertension, diabetes), evidence of viral or bacterial infection, prolonged rupture of membranes (more than 12 hours), maternal fever, multiple pregnancy, history of immune deficiency, family history of autoimmune diseases, history of drug abuse, and poor obstetric history (abortion, still birth) excluded patients for enrollment in the study.

**Study variables**

The primary outcome of the study was to determine the effect of maternal donor characteristics on the quantity and quality of the hematopoietic stem cells recovered from umbilical cord blood sampling. Total nucleated cells (TNC), CD34+ cells count and viability were used as proxies for quantity and quality of the extracted samples.

Maternal demographic variables studied included age, gravidity and ethnicity (white, black or others). Newborn variables included gestational age, newborn birth weight in grams, and neonatal gender (male or female). Outcome variables included the TNC count (the leukocyte and erythroblast count percent per cubic millimeter of UCB, multiplied by the total blood volume), the CD34+ progenitor cell count and CD34+ progenitor cell viability percentage. CD34+ cells count and progenitor cell viability were calculated through cellQuest software (Becton and Dickinson, Inc., Franklin, New Jersey, USA).

**Methods**

**Umbilical cord blood (UCB) collection**

UCB collected directly from the umbilical cord (flow by gravity) after sterilization with povidone iodine as soon as possible after delivery of the baby and before the delivery of the placenta. Umbilical cord blood was collected in a UCB collection bag containing 35 ml of citrate phosphate dextrose anticoagulant (Pall Medical Corporation, Port Washington, NY, USA) until blood flow ceased. Volume collected, time of collection and time from collection to processing were measured. Units were stored at 4-7°C until taken to the laboratory and processed within 24 hours.

The current study utilized an “intrauterine technique” of UCB collection. The “intrauterine technique” requires that the cord blood sample be collected prior to the removal of the placenta at the time of cesarean section. One prior study comparing the “intrauterine technique” to the “extra-uterine technique (collection of the UCB sample after removal of the placenta)” showed that the intrauterine collection technique provides larger sample volumes [20], Surbek et al. [21].
found a significant difference among different collection techniques in a randomized trial, with a 30–50% gain in total blood volume and TNC count when cord blood collection was intrauterine, with no harm to the newborn. Disadvantages of intrauterine collections include: the need for a skilled and well-trained professional, as collection requires concentration and dexterity under time-restricted conditions, and total UCB volume may be reduced in fetuses with short umbilical cords or small and preterm placentas [22]. Extra-uterine collections also require dexterity in handling the placenta after birth, in order to avoid damage to the cord during placental removal. The “extra-uterine technique” technique showed the highest percentage of discarded units (93%). The mixed technique showed larger UCB total blood volumes and TNC counts compared with intra- and extra-uterine collections alone [22]. However, this type of collection is not frequently used in obstetrical practice.

### Pre-processing of samples

1 ml of umbilical cord blood was taken from the sample chamber for TNC. Cell counts from this sample of UCB were analyzed using automated total blood analyzer.

### Umbilical cord blood cell separation

Mononuclear cells (MNC) were isolated from UCB by centrifugation on HES (Hetastarch 6% in 0.9 sodium chloride, 500mL Bag- Catalogue No. 89-192JT, GRU Clinical Pharmacy). CD34+ cell quantification and viability percentage for MNC samples were performed using a Flow Cytometer and a stem cell enumeration (SCE) kit (BD Biosciences laboratories, San Jose, CA, USA catalog No. 344563). Hetastarch was added to the anticoagulated UCB in a flow rate of 0.5 ml/second through the filter. The added amount of Hetastarch to the UCB was calculated based on the following formula: amount of Hetastarch = volume of UCB (UCB weight/1.05) × 0.2ml. The thermal clamp closed the filter upstream and the filter was removed for disposal. A two-step centrifugation manual method for separation used resulting in buffy coat [23]. After processing, 0.5 ml of separated buffy coat was kept in Trucount tube (BD Biosciences laboratories, San Jose, CA, USA) for CD45+ cells, CD34+ cells, and viability determination.

### Analysis of CD34+, CD45+ cells, and viability

Umbilical cord blood samples were submitted to Augusta University’s Flow Cytometer Core Facility for analysis. The Flow Cytometer procedure was adapted for the analysis of fixed human UCB stem cells. The 7AAD dye in SCE kit (Becton Dickinson, Franklin Lakes, New Jersey, USA) was replaced with FVS620 dye (BD catalog #564996). A 10X volume of ammonium chloride was added to the blood sample volume after the hetastarch processing, incubated 8-10min, spun down and washed with 2ml stain buffer containing fetal bovine serum (FBS) to dilute the ammonium chloride. A second and third wash with PBS was performed before staining with the FVS 620 dye for analysis.

20 ul of stem cell reagent was added to Trucount tube for each 100 ul of dye-treated sample volume. The tube was vortexed for 5 seconds and then incubated in the dark for 20 minutes. 500ul of fixation buffer (BD cytotox CAT number 554722) was added and the mixture was incubated in the dark for 5 minutes. Samples were run immediately using fluorescence-activated cell sorting (BD FACS Calibur; Becton Dickinson Company, Franklin Lakes, New Jersey, USA). Control samples provided by the manufacturer (Becton Dickinson) were run against patient samples for quality control. CD34+ stem cells were identified using sequential gating technique. The numbers of absolute CD34+ cells, viability, and CD45+ cells were calculated by using specific equations implemented in cellQuest software, which were the end points for this study.

### Results

#### Descriptive analysis of the donor population predictors

The characteristics of the donors and neonates, including maternal age/weight/ethnicity/gravidity, gestational age, birth weight, and neonatal gender were extracted from patients’ electronic health record (EHR). Patient ages ranged from 20-35. Donor body weight ranged from 64.5-180 kg. 80% of recruited donors were gravida 3 or less. The most common ethnic categories were African-American and Caucasian (44% each). The mean newborn birth weight was 3.6 kg.56% of newborns were female. Donor and newborn characteristics are displayed in Figures 1-4 and Tables 1 and Table 2.

#### Multivariate Linear Regression to test association among predictors’ characteristics and response variables

Factors associated with CD34+ cells count and viability percentage: Primigravida donors and donors with less than 2 prior pregnancies had higher CD34+ cells counts (P=0.0094*). Maternal age and weight showed no significant influence to CD34+ cells count. Maternal ethnicity data analysis showed significant differences in CD34+ cells count (P= 0.0328*). The results also showed that female newborn gender had a higher CD34+ cells count and viability percentage as compared to male newborns (P=0.0024*, P=0.0117*). No significant influence was observed among other donor characteristics studied including gestational age and neonatal birth weight (Table 4 and Table5).

**Factors associated with the TNC:** The TNC count was higher in the group of donors with a gravidity of 3 or less (P=0.0124*). Moreover, it was higher in the units collected from female newborns as compared with male newborns (P=0.0007*). Neonatal weight, order

![Figure 1: Participants distribution according to the resulting neonatal gender.](image)

![Figure 2: Maternal ethnicity among the participants.](image)

of birth, gestational age, maternal weight/age and ethnicity showed no statistically significant difference in TNC count.

Discussion

Cellular therapy is a branch of medicine, which deals with treatment of disease using stem cells. There has been a dramatic increase in the use of human stem cells for the treatment of disease over the past two decades. Hematopoietic stem cells obtained from adult bone marrow aspirate are still a vital source for both clinical therapy and experimental research in the stem cell biology field. However, bone marrow HSC numbers and differentiation potential decrease with age. Hence, it is important to continue to discover alternative sources for HSC from alternative sources.

Multiple studies have shown that UCB contains both hematopoietic and mesenchymal stem cells which can be used as an alternative source of HSC. In a large study, Page et al. [18] stated that high-quality umbilical cord blood samples should be defined as those with a larger collection volume, higher TNCs and high CD34+ cell count [18].

In our study, we found that (i) maternal ethnicity, gravidity, and neonatal gender have a significant effect on CD34+ count. In addition, (ii) neonatal gender was a significant factor for CD34+ viability percentage, while (iii) gravidity and neonatal gender both were significant factors for TNC (Tables 2-5).

The average volume of cord blood collected in our study was approximately 75.5 ml. The average TNC per unit averaged of $5 \times 10^8$ cells. The average CD34+ cells count was $2.2 \times 10^5$ cells per unit, with a viability of 95%.

The results obtained from our study are similar to prior published

Table 2: Descriptive statistics for discrete predictors.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal ethnicity</td>
<td>4</td>
<td>4.00</td>
</tr>
<tr>
<td>Black</td>
<td>44</td>
<td>44.00</td>
</tr>
<tr>
<td>Hispanic</td>
<td>8</td>
<td>8.00</td>
</tr>
<tr>
<td>White</td>
<td>44</td>
<td>44.00</td>
</tr>
<tr>
<td>Neonatal gender</td>
<td>44</td>
<td>44.00</td>
</tr>
<tr>
<td>Male</td>
<td>56</td>
<td>56.00</td>
</tr>
<tr>
<td>Female</td>
<td>44</td>
<td>44.00</td>
</tr>
</tbody>
</table>

Table 3: Response variable CD34+ cells count.

<table>
<thead>
<tr>
<th>Source</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal ethnicity</td>
<td>0.0328*</td>
</tr>
<tr>
<td>Gravidity</td>
<td>0.0094*</td>
</tr>
<tr>
<td>Parity</td>
<td>0.2246</td>
</tr>
<tr>
<td>Neonatal gender</td>
<td>0.0024*</td>
</tr>
<tr>
<td>Maternal age</td>
<td>0.2118</td>
</tr>
<tr>
<td>Maternal weight</td>
<td>0.8460</td>
</tr>
<tr>
<td>Fetal gestational age</td>
<td>0.5796</td>
</tr>
<tr>
<td>Neonatal birth weight</td>
<td>0.1795</td>
</tr>
</tbody>
</table>

Note: A *P- value indicates that there is an association between a predictor characteristic and the response variable, C34 count, at 5% significance level.

Table 4: Response variable CD34+ cells viability percentage.

<table>
<thead>
<tr>
<th>Source</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal ethnicity</td>
<td>0.8798</td>
</tr>
<tr>
<td>Gravidity</td>
<td>0.1555</td>
</tr>
<tr>
<td>Parity</td>
<td>0.8406</td>
</tr>
<tr>
<td>Neonatal gender</td>
<td>0.0117*</td>
</tr>
<tr>
<td>Maternal age</td>
<td>0.1551</td>
</tr>
<tr>
<td>Maternal weight</td>
<td>0.9939</td>
</tr>
<tr>
<td>Fetal gestational age</td>
<td>0.3780</td>
</tr>
<tr>
<td>Neonatal birth weight</td>
<td>0.5456</td>
</tr>
</tbody>
</table>

Note: A *P- value indicates that there is an association between a predictor characteristic and the response variable, CD34 viability percentage, at 5% significance level.

Table 5: Response variable TNC (Total nucleated cell count).

<table>
<thead>
<tr>
<th>Source</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal ethnicity</td>
<td>0.0866</td>
</tr>
<tr>
<td>Gravidity</td>
<td>0.0124*</td>
</tr>
<tr>
<td>Parity</td>
<td>0.0937</td>
</tr>
<tr>
<td>Neonatal gender</td>
<td>0.0007*</td>
</tr>
<tr>
<td>Maternal age</td>
<td>0.9481</td>
</tr>
<tr>
<td>Maternal weight</td>
<td>0.4558</td>
</tr>
<tr>
<td>Fetal gestational age</td>
<td>0.8876</td>
</tr>
<tr>
<td>Neonatal Birth weight</td>
<td>0.9924</td>
</tr>
</tbody>
</table>

Note: A *P-value indicates that there is an association between a predictor characteristic and the response variable, TNC, at 5% significance level.

studies that confirmed a higher yield of TNC and CD34+ cells counts obtained from patients with lower gravidity and female gender newborn. These factors should theoretically increase the possibility of success in transplantation trials [15,24].

Our study showed no significant influence of maternal age on outcome variables such as CD34+ cells count, viability or TNC count. Our findings differ from previous published studies that suggested an influence of maternal age on UCB stem cells samples [13]. This difference in findings may be attributed to the fact that maternal age increases the risk of donor medical complications, which were excluded in the current study.

Studies have suggested that UCB obtained from singleton births and Cesarean deliveries have a higher TNC counts than multiple births and vaginal deliveries [25]. The lower volume of collected cord blood with vaginal deliveries, which might be attributed to the use of ecobolics resulting in coordinated uterine muscle contraction leads to reduced utero-placental circulation [2].

Different researchers found that the higher the newborn weight, the larger the total volume of UCB collected [24,26-28]. George et al. [27] reported that the ideal fetal weight for UCB donation should be more than 3000 g. The current study confirms these findings and showed that newborn weight is associated with trend of increase of UCB volume; TNC and CD34+ cells count (Tables 2-5).

Our study has two main limitations that need to be addressed in future studies and directions: firstly the small samples size from Augusta population, Secondly the tight timeframe for the study, which limit the analysis. As an eye on the future, our goal to start further elaborate extended studies recruiting more sample size to find more associations optimizing the collections and units’ quality for transplantation purposes. Moreover, we are aiming to go beyond the observational studies and start the translational medicine implementation by using the stem cells we will isolate in both diagnostic and therapeutic experimental animal models.

**Conclusion**

In conclusion, maternal age should not be a factor in selecting umbilical cord blood donors. Several maternal donor and newborn factors positively influence the quality and quantity of UCB stem cell samples including maternal gravidity less than 4, maternal race and newborn female gender. Based on the differing results of multiple prior studies, each specific patient population should be studied to determine the optimal donor characteristics for UCB stem cell samples. Standardization in collection, processing and storage procedures for UCB stem cells should also improve the success rates of HSC stem cell transplants from UCB donors.

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**Conflict of Interest**

Mohamed SA, Mashaly AF, Sayed MT, Elchennawi FA, Darwish A, Shalaby S, Browne P and Al-Hendy A reported no conflict of interest.

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