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Optimizing Donor Selection for Public Cord Blood Banking: Influence of Maternal, Infant and Collection Characteristics on Cord Blood Unit Quality

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Abstract

Background—Banked unrelated donor umbilical cord blood (CB) has improved access to hematopoietic stem cell transplantation for patients without a suitably matched donor. In a resource-limited environment, ensuring that the public inventory is enriched with high-quality cord blood units (CBUs) addressing the needs of a diverse group of patients is a priority. Identification of donor characteristics correlating with higher CBU quality could guide operational strategies to increase the yield of banked high-quality CBUs.

Methods—Characteristics of 5267 CBUs donated to the Carolinas Cord Blood Bank, a public bank participating in the National Cord Blood Inventory, were retrospectively analyzed. Eligible CBUs, collected by trained personnel, were processed using standard procedures. Routine quality and potency metrics [post-processing total nucleated cell count (post-TNCC), $CD34^+$, colony-forming units (CFUs)] were correlated with maternal, infant, and collection characteristics.

Results—High-quality CBUs were defined as those with higher post-TNCC ($>1.25\times10^9$), and $CD34^+$ + CFU in the upper quartile. Factors associated with higher $CD34^+$ or CFU content included a shorter interval from collection to processing (<10 hours), younger gestational age (34–37 weeks; $CD34^+$+CFU) Caucasian race, higher birth weight (>3500grams) and larger collection volumes (>80ml).

Conclusions—We describe characteristics identifying high-quality CBUs, which can be used to inform strategies for CBU collection for public banks. Efforts should be made to prioritize collections from larger babies born before 38 weeks of gestation. CBUs should be rapidly transported to the processing laboratory. The lower quality of CBUs from non-Caucasian donors highlights the challenges of building a racially diverse public CB inventory.

Introduction

Umbilical cord blood (CB) is a rich source of hematopoietic stem and progenitor cells for hematopoietic stem cell transplantation (HSCT). Since the first unrelated donor umbilical cord blood transplant (UCBT) in 1993[1], the use of CB as a donor source for unrelated HSCT

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has become standard of care for patients without a sufficiently matched related or unrelated adult donor[2,3]. Advantages of CB donors include ease of procurement, less stringent requirements for human leukocyte antigen (HLA) matching, reduced graft-versus-host disease (GvHD) compared to other stem cell sources and improved access to transplant, especially for racial/ethnic minorities[4].

The clinical outcomes after UCBT are influenced by the number of cells available in a single cord blood unit (CBU)[5]. A typical CB bank inventory contains units with a median postprocessing total nucleated cell count (post-TNCC) of 1.04×10^9, while the median post-TNCC of units selected for transplantation from the National Marrow Donor Program (NMDP) Be the Match Registry® is 1.76×10^9 cells[6]. Effectively, only 8% of the current public inventory meets this criteria[6] suggesting that resources allocated to cord blood banking are not being used efficiently. Also, larger patients seeking a CB donor must effectively choose from a limited inventory. Previous studies have shown that potency, as represented by colony-forming units (CFU) and/or $CD34^+$ content of a CBU prior to cryopreservation or after thawing, is correlated with the engraftment potential of an individual CB unit[7–9]. Efforts to increase the CFU, $CD34^+$ and post-TNCC content of banked CBUs are necessary to increase the overall quality (i.e. racial and ethnic diversity combined with post-TNCC and potency) of CBUs. Additionally, closer HLA matching has been shown to improve outcomes of UCBT[3,10,11]. Thus, with an increasingly diverse population of patients in need of donors, strategies to bank increasing numbers of racially and ethnically diverse, high-quality CBUs that will have an increased HLA repertoire, are also necessary.

We hypothesized that clinical parameters that are readily available to the obstetrical and collection staff can be used to identify optimal CB donors. If identified, these parameters could be used to guide clinicians on how to prioritize CB collection and processing. In this report, we present the results of an analysis of over 5200 CBUs recently collected and processed by a single public cord blood bank in which we identify, update[12–18], and further define characteristics of the mother/infant donor pair and the collection that are associated with higher CBU potency and quality.

Materials and Methods

Study Overview

This is a retrospective study conducted between 2007–2009 by the Carolinas Cord Blood Bank (CCBB), a large public cord blood bank at Duke University Medical Center. CBUs donated by healthy mothers after an uncomplicated pregnancy and after written informed consent were collected at 11 sites and sent to the CCBB for processing, testing and cryopreservation. Correlations between technical parameters routinely measured on a CBU after processing and readily available clinical characteristics of the mother, infant, and collection were examined to determine characteristics that could be used to identify CBUs more likely to yield higher quality CBUs.

Cord Blood Donor Eligibility

Eligible collections included singleton gestations with an estimated gestational age of ≥34 weeks delivered by a mother who was ≥18 years of age at delivery. Collections processed by the CCBB laboratory on Mondays through Thursdays were included in this analysis (n=5267). Extensive maternal medical history and family history were obtained from a maternal donor screening questionnaire and medical records used for donor screening as per CCBB standard operating procedures (SOP). Maternal blood was obtained for donor screening, performed by the American Red Cross Testing Laboratory, in Charlotte, NC, for

infectious diseases transmittable through blood. A subset of CBUs included in this analysis (n=606, 11.5%) failed to meet banking specifications (such as maternal history exclusion, positive infectious disease screen, etc.) and were not included in the CCBB public inventory. These CBUs were included in the analysis because the exclusionary factors were found postprocessing and cryopreservation.

Characteristics of the mother, infant and CBU collection

Clinical characteristics that were examined included: maternal age, infant related characteristics such as birth weight, gestational age, gender, race/ethnicity, and collection characteristics such as delivery method, collection volume, time from collection to initiation of processing, and total cell viability after processing. All data was readily available in the CCBB database. This database was initially created by the EMMES Corporation (Rockville, MD) for the COBLT Study[4] and has been subsequently upgraded and maintained by EMMES.

The infant donor's race/ethnicity was based on the mothers' self-reporting of her own and the baby's father's race/ethnicity as part of the maternal questionnaire and determined according to definitions from the United States Office of Management and Budget as used by Health Resources and Services Administration (HRSA)[19]. Race was classified as Asian, American Indian or Alaska Native, Black or African American, Native Hawaiian or Other Pacific Islanders, and White or Caucasian. Ethnicity considered either Hispanic or Non-Hispanic origin. Assignment to a specific race indicated that both parents were of that race. A "more than one race" category included infants for whom parents were of different races. For purposes of this analysis, we treated Caucasian Hispanic infants (i.e. both parents Caucasian with at least one parent identified as Hispanic/Latino ethnicity) as a separate category. We also analyzed the American Indian or Alaska and Native Hawaiian or Other Pacific Islanders as a single group given the small numbers of patients in these groups.

Cord Blood Collection and Processing

CB collections were performed by trained collection staff using either *in-utero* or *ex-utero* collection procedures. At the time of collection, the umbilical cord was stabilized, sterilized with Betadine and alcohol. The umbilical vein was punctured with a 17-gauge needle attached to the collection bag containing 25 or 35 ml of CPD anticoagulant (Sterile Cord Blood Collection Unit (791-08), Pall, Corporation, Medical Subsidiary, Covina, CA). The CB drained by gravity into the collection bag while being gently rocked on a rotating scale to ensure adequate mixing.

At the completion of the collection, the total collection volume (in mls) was measured by weight excluding the anticoagulants. During the study period, a minimum CB collection volume of 40ml was required for transportation to the processing laboratory and to qualify for processing. CBUs not meeting the threshold volume were discarded at the collection site according to the CCBB SOPs. Eligible CBUs were transported to the processing laboratory in a validated shipper by a CCBB courier or via express (overnight) delivery service depending on location of collection site (ranging from 0.25 to over 600 miles from the CCBB). After receipt at the CCBB processing laboratory, trained staff verified appropriate labeling of the CBU based on accompanying documentation and visually inspected CBUs for any signs of leakage or other damage. Those CBUs passing this initial screen were then triaged by collection time and date to ensure that the oldest units were prioritized for processing first. A TNCC was enumerated prior to processing and a minimum 1.0×10^9 nucleated cells was required to continue to processing. All eligible CBUs were processed using a functionally closed automated system using the Biosafe Sepax system (starting 8/2008; CS-530.2 processing kits, Biosafe, Alexandria, VA) or by manual method using Pall

Transfer/Freezing Bag kits (791-02; Pall, Corporation, Medical Subsidiary, Covina, CA) and a refrigerated centrifuge (2007–2008). Both of these processes employ the method of RBC-depletion and plasma reduction. Hydroxyethyl starch at a 1:5 ratio (Hespan, B. Braun Medical, Inc.; Bethlehem, PA) was added prior to centrifugation to assist with the separation of the red blood cells. Following separation of the components, approximately 21 ml of buffy coat enriched product remained. A TNCC was enumerated from the product to determine final cell count recovered. DMSO/Dextran (10% dimethyl sulfoxide in 5% dextran 40, Protide, Lake Zurich, IL) was added as a cryoprotectant and the product was cryopreserved by controlled rate freezing in a 25-mL double-compartment cryopreservation bag (791-05; Pall, Corporation, Medical Subsidiary, Covina, CA). Cryopreserved CBUs were stored under liquid nitrogen.

CBU Characterization

Total Nucleated Cell Enumeration and Viability Assessment—A TNCC was performed on a sample of the collected CB to determine whether an individual CBU contained $>1\times10^9$ cells and therefore met CCBB criteria for processing. The post-TNCC was also measured after processing to determine if there was adequate recovery and banking criteria were met. The TNCC counts were measured on an automated cell counter (Sysmex K-1000 from 9/07-4/08, Sysmex XE5000 from 4/08 – 7/09; Sysmex America, Inc. Mundelein, IL). Viability was assessed by staining a post-processed CB sample with Trypan blue and scoring viable cells on a hemocytometer after incubating for 5 minutes. The CCBB required the post-processed unit to have ≥90% viability for inclusion in the banking inventory.

$CD34^+$ Enumeration—Enumeration of $CD34^+$ cells was determined after processing by flow cytometry (ProCOUNT, BD Biosciences, San Jose, CA). ProCOUNT reagent (20μL) was added to 12×75 test tubes followed by 50μL of post-processed CB sample. Samples were diluted with PBS/BSA wash (Gibco, Invitrogen, Carlsbad, CA) as needed for a concentration $<4\times10^7$ cells/ml. Tubes were mixed gently and incubated in the dark at room temperature for 15 minutes. RBCs were further lysed using 450μL of a 1:10 dilution of FACS Lysing Solution (BD Biosciences, San Jose, CA) for samples and incubated in the dark at room temperature for 15 minutes. A two laser, four-color flow cytometer was used to analyze samples. Results were analyzed by CellQuest Pro (BD Biosciences, San Jose, CA) using ProCOUNT gating strategies.

Colony Forming Unit Progenitor Cell Assay—CFU progenitor cells, including Granulocyte Macrophage (CFU-GM), Granulocyte, Erythrocyte, Macrophage and Megakaryocyte (CFU-GEMM), and Burst Forming Unit Erythroid (BFU-E), were enumerated on post-processing samples of CB. Initial samples of 2.5×10^5 CB cells were removed for testing and diluted in 0.5 ml Iscove's modified Dulbecco's Medium (IMDM) plus 2% fetal bovine serum (FBS, StemCell Technologies, Vancouver, BC). Cells were further diluted by adding 5×10^4 cells in a total of 0.5 ml IMDM plus 2% FBS to achieve a final cell count of 2.5×10^4/ml when mixed with 1.5ml of MethoCult medium 4434 (StemCell Technologies). Cells were plated in triplicate (0.5 ml/well) at 1.25×10^4 cells/well in 24-well, tissue culture plates and incubated in a humidified, 37°C, 5% CO_2 incubator for 11–14 days. Colony growth was scored in triplicate by trained personnel using an inverted, phase-contrast microscope and reported as the mean colony count per 10^5 nucleated cells. These numbers were then used to calculate the number of progenitors in the entire graft by correcting for the post-processing TNCC.

Statistical Analysis

Three multivariate logistical regression models were created using the following thresholds: CFU of >34×10^5 colonies, $CD34^+$ of >3.4×10^6 cells and post-TNCC of >1.18×10^9 cells. The thresholds were based on the median post-processing CFU, $CD34^+$ and TNCC for this cohort. As such, any reference to CFU, $CD34^+$ and TNCC content refers to measurements performed after processing unless otherwise indicated. For the purpose of this analysis, we defined pre-term, term and post-term as 34–37 weeks, 38–40 weeks and >40 weeks gestation, respectively. We estimated odds ratios (OR) and 95% confidence intervals for factors associated with higher quality CBUs. Backward selection with a p-value cut-off of <0.05 was used to create the multivariate models. First order interactions were assessed using all factors in the multivariate model. Factors associated with the time to processing from collection were analyzed in a multivariate regression model including day of delivery, time of delivery, collection site (close to CCBB vs. non-local to CCBB), race, infant gender, maternal age and year of processing (Before or After August, 2008 based on the implementation of automated processing). Generalized linear models were used to estimate adjusted means using a Bonferroni correction to describe p-values of the difference in adjusted means. Analyses were conducted using the SAS System version 9.2 (Cary, NC).

Results

We analyzed 5267 CBUs donated by healthy mothers after an uncomplicated pregnancy, labor, and delivery that were processed by the CCBB at Duke from September 2007 through July 2009. Each CBU that met initial volume and cell count criteria was mixed with Hespan, volume and RBC reduced and subsequently assessed for viability and CFUs, $CD34^+$, and post-TNCC content as part of standard testing procedures. Corresponding demographic information and clinical characteristics routinely collected by the bank for each donor CBU were included in this analysis.

Maternal and Infant Donor Demographics

The majority of infants were born by vaginal delivery (59.8%; Table 1) with a median birth weight of 3580g (range, 1800–5724g) with the majority delivering between 38–40 weeks of gestation (82.1%) to mothers with a median age of 29.0 years (range, 18.0–53.0 years). Approximately half of the babies were male (49.4%) with the following infant racial/ethnic representation: Caucasian non-Hispanic (58.8%), Black or African-American (14.4%), Hispanic (12.8%), Asian (2.6%), Native American or Alaska Native (0.1%), and Hawaiian Native or Other Pacific Islander (0.1%) with 10.8% of infants identified as “more than one race”.

Cord Blood Characteristics

The median volume of CB collected (excluding anticoagulants) was 93.0ml (range, 40.0–286.0ml; Table 2). The median time from collection to the start of processing was 14.1 hours (range, 0.5–47.4 hours). After processing, the median post-TNCC and $CD34^+$ content of the CBUs was 1.18×10^9 nucleated cells (range, 0.63–5.55×10^9) and 3.21×10^9 $CD34^+$ cells (range, 0.02–9.99×10^6), respectively (Table 2). Most of the CBUs (99.9%) contained ≥90% viable cells at the conclusion of processing [median 98.5% (range, 84.0–100.0%)]. The median total CFU growth from samples obtained after processing was 34.0×10^5 colonies (range, 0.6–193.3×10^5).

Infant Gestational Age

Infants of younger gestational age were more likely to have CBUs with higher CFU and $CD34^+$ content (both p<0.0001). In multivariate analysis, preterm infants were more likely

to have higher CFU as compared to either term or post-term infants (p=0.0182 and p=0.0006, respectively; Table 3, Figure 1A). We observed similar results for $CD34^+$ content (both p<0.0001; Table 4, Figure 1B). While infants ≥38 weeks gestational age had higher post-TNCC as compared to younger infants (Table 5, Figure 1C), this did not correlate with higher potency (i.e., higher CFU and/or $CD34^+$; Figure 1A and B).

Infant Race/Ethnicity

The race/ethnicity of the infant donor was also strongly associated with having higher CBU potency. In multivariate analysis, Caucasian infants were more likely to have higher CFU content [OR 1.39 (95%CI 1.17–1.64), p=0.0001; Table 5], $CD34^+$ [OR 1.27 (95% CI 1.07–1.50), p=0.0057; Table 6) and post-TNCC content [OR 1.54 (95%CI 1.29–1.84). p<0.0001; Table 5] as compared to African American infants. To better understand if there was a biologic basis for this difference, we examined the cell density of the CBUs by race. CBUs from Caucasian infants had significantly higher adjusted mean concentrations (counts per ml of CB) of CFU, $CD34^+$, and post-TNCC (all p<0.0001, Figure 2A–C) as compared to African American infants.

Infant Birth Weight also correlated with higher quality of the CBUs. In multivariate analysis, heavier babies (>3500g) were more likely to yield CBUs with higher CFU (p=0.0003; Table 3), $CD34^+$ (p<0.0001; Table 4) and post-TNCC content (p<0.0001; Table 5). Each 500g increase in birth weight corresponded to stepwise increases in post-TNCC (p<0.0001; Figure 3C). Furthermore, Infants weighing ≥4500g were 5.37 times (95%CI 3.16–9.15) more likely to have a post-TNCC $>1\times10^9$ than infants with a birth weight of <3000g (p<0.0001) in univariate analysis. Similar findings were seen for both CFU (Figure 3A) and $CD34^+$ content (Figure 3B).

Infant Gender

When we examined the impact of gender in multivariate analyses, male infants were more likely to have higher total $CD34^+$ content than female infants (p=0.0002). This was related to the fact that male infants had higher median birth weights as compared to female infants (3671g and 3510g, respectively; p<0.0001). Slightly more male infants were born via Caesarean delivery (41.9% males vs. 38.6% for females: p=0.0155) to mothers that were slightly older (30 years old vs. 29 years old, respectively; p=0.0204). Collections from male infants had higher median collection volumes (p=0.0001), $CD34^+$ (p<0.0001) and CFU content (p=0.0013; Table 6). There were no differences between gender with respect to post-TNCC, time to processing or viability.

Maternal Age

In univariate analysis, mothers >20 years old were more likely to donate CBUs with higher CFU (p=0.0381), $CD34^+$ (p=0.0217), and post-TNCC content (p=0.0480; Tables 3–5) compared to younger mothers. This relationship was not significant in multivariate analysis.

Viability

The overall viability as measured by trypan blue exclusion was noted to decrease by small increments as the time from collection to start of processing increased. The median viability for CBUs processed <10 hours and 10–23 hours after collection was 99% and 98%, respectively, as compared to 96% for CBUs processed ≥24 hours after collection (p<0.0001). When we examined the absolute percent of viable cells as related to the post-TNCC content, higher viability (98–100%) was associated with higher post-TNCC content in univariate analysis (Table 5) but not in multivariate analysis. There was no association between cell viability and either the CFU or $CD34^+$ content.

Collection Volume and Delivery Method

CBUs with higher volumes were more likely to have higher potency and quality (all $p<0.0001$) in univariate analysis. However, when we considered the collection volume in multivariate analysis, an interaction between delivery type (i.e. Caesarean vs. Vaginal delivery) and initial collected volume was observed (Tables 3–5). Specifically, in higher volume CBUs (>80ml), collections from vaginal deliveries were more likely to result in higher CFU ($p=0.0002$) and $CD34^+$ content ($p=0.0130$) as compared to collections from babies delivered by Caesarean section. No impact of delivery type on potency was observed for smaller CBUs. Vaginal deliveries with smaller volumes (≤80 ml) were more likely to result in higher post-TNCC ($p=0.0242$) as compared to those collected after Caesarean section.

Time to Processing

We also examined the effect of time, measured from the start of collection to initiation of processing, on the potency of the processed CBU. For all three parameters, shorter time from collection to processing was associated with increased potency in the processed CBUs (Tables 3–5; Figure 4). When processing was initiated <10 hours from the time of collection, the CBUs were more likely to have higher CFU ($p=0.0004$; Figure 4A) and a trend to higher $CD34^+$ ($p=0.0687$; Figure 4B) as compared to CBUs processed 10–23 hours after collection. Furthermore, there were significant differences in overall CBU quality with increases in CFU ($p=0.0035$), $CD34^+$ ($p=0.0045$) and post-TNCC content ($p=0.0003$), in CBUs processed from 10–23 vs. ≥24 hours after collection (Figure 4). Comparing CBUs processed <10 hours to those processed ≥24 hours after collection, the CFU, $CD34^+$ and post-TNCC content (all $p<0.0001$; Figure 4A–C) were significantly higher in the CBUs that were processed closer to collection. Factors influencing time from collection to processing included distance of the collection site from the processing laboratory, prioritization protocols in the processing laboratory, local courier pickup schedules and processing staff availability (data not shown).

Association between Cord Blood Potency (CFU and $CD34^+$) and post-TNCC

Many public CB banks currently use 0.9×10^9 total nucleated cells as the minimum post-TNCC for inclusion of a CBU in their inventory. This also represents the current post-TNCC threshold used to qualify CBUs for the National Cord Blood Inventory. A recent analysis of the NMDP inventory showed that 68.5% of the units listed had post-TNCCs <1.25, 15.5% between 1.25–1.49, 8.0% between 1.50–1.74 and 7.9% $\geq1.75\times10^9$, respectively. However, the median post-TNCC of units selected by transplant centers for transplantation to their patients was 1.76×10^9 cells[6]. We asked whether the practice of selecting higher post-TNCC containing units guarantees use of a unit with high potency (Figure 5). In this analysis, 11.6% of the units (n=616) had post-TNCCs $>1.75\times10^9$ (Figure 5, Regions A and C). Interestingly, defining optimal potency in CBUs as those with CFU and $CD34^+$ values in the highest quartile, ~25% of high cell count units had lower potency (Figure 5, Region C). Conversely, in the group of CBUs with intermediate post-TNCCs ($1.25–1.75\times10^9$), 39% had CFU or $CD34^+$ values in the upper quartile (Figure 5, Region B). These CBUs with intermediate post-TNCC and high CFU and/or $CD34^+$ content are likely to perform as well after transplant as the CBUs with higher post-TNCC. Using this approach, 20.7% of units are high quality with high or intermediate ($>1.25\times10^9$) post-TNCC and high potency (CFU and $CD34^+$ $>75^{th}$ percentile) (Figure 5, Region A+B).

Discussion

In this large retrospective study, we examined the clinical characteristics of maternal and infant donors and the collected CBU to identify factors that predict the quality and potency

of CBUs collected and processed by a large public CB bank. Characteristics that positively influenced the potency of the CBUs included younger gestational age, Caucasian race/ethnicity of the infant, shorter time to processing, higher birth weight and larger collection volumes. Collection staff and obstetricians could use these parameters to identify donors most likely to yield bankable, high quality CBUs.

The potency of an individual CBU correlates with its potential for engraftment after transplantation. Banks strive to store CBUs with high potency in their inventories. Practically, potency is a measure of the overall health of a CBU and reflects its ability to withstand the stresses produced during processing, cryopreservation and thaw. We previously showed that of all of the parameters measured on CBUs, CFU content, post processing and/or post thaw, best correlate with potency[9,20,21] as compared to either $CD34^+$ or post-TNCC content. In a single center retrospective study of 435 single unit UCBTs after myeloablative conditioning, higher CFU dosing was the only pre-cryopreservation graft characteristic predictive of neutrophil ($p=0.0024$) and platelet engraftment ($p=0.0063$) in multivariate analysis[9]. Likewise, post-thaw CFU content was the best predictor of neutrophil and platelet engraftment (both $p<0.0001$)[9]. Other reports have shown $CD34^+$ cell content to be a stronger predictor of engraftment as compared to post-TNCC[7,8]. Thus, for purposes of this analysis, we prioritized CFU content as the best measure of potency followed by $CD34^+$, and lastly, post-TNCC, since CBUs are conventionally selected based on the post-processed TNCC dose (adjusted for patient weight) that would be delivered to a patient. There is, however, a drawback to this approach, because potency measures are not available at the time a unit is processed and cryopreserved, post-TNCC is the only measure available in “real time” and is the parameter utilized to make the decisions “to process or not to process” or “to cryopreserve or not to cryopreserve”. Correlations of post-TNCC with $CD34^+$ and CFU will help banks set thresholds for prioritization protocols for CBU manufacturing.

Using CFU as the best biomarker of potency, shorter processing time, younger gestational age, and race/ethnicity were all highly predictive of CBUs with high potency. Similar trends were seen for $CD34^+$ content. Of note, younger gestational age was associated with lower post-TNCC, due to the smaller size of the baby and resulting smaller CBU collection, but the collections from these younger babies were enriched for both CFUs and $CD34^+$ cell content reflecting higher potency overall. Samples of CB from preterm infants have been shown *in vitro* to have higher concentration of hematopoietic progenitor cells (i.e. CFU) in addition to increased proliferative potential as compared to term infants[22]. Previous studies have also found that CBUs derived from donors of younger gestational age have higher CFU[12] and/or $CD34^+$ content[14,16,17,23,24]. These relationships are probably due to the mobilizing signals produced by placental tissue during fetal development.

Our results support previous reports demonstrating a correlation between increased birth weight and $CD34^+$ or post-TNCC[4,12,15,16,24,25] and extend this correlation to higher CFU content[12,25] content. However, at term, there is a divergence between increasing post-TNCC and baby weight and progenitor cell content (CFU and $CD34^+$); progenitor cell content decreases in post-term infants. Using a “real-world” scenario, if two infants are being delivered simultaneously and only one collection can be performed, the collector might have to decide whether to collect from a larger post-term baby at 42 weeks gestation and a smaller baby at 37 weeks. Based on the results of this analysis, we would recommend prioritizing the younger infant’s collection recognizing that, while it is likely to have a slightly lower post-TNCC, it is more likely to have higher CFUs and $CD34^+$ cells indicative of higher potency.

Whether the mother's age impacts the quality of the donated CBU is a question that remains unresolved. A recent report[26] described increased post-TNCC in units collected from 30–34 years old as compared to 20–24 year old mothers. Another report[17] described higher $CD34^+$ content in CBUs from younger mothers. However, other studies have not observed any impact of maternal age on post-TNCC content[4,12]. While we observed that CBUs donated by mothers <20 years old (comprising 7.6% of the overall cohort) were more likely to have lower post-TNCC, $CD34^+$ and CFU content in univariate analysis, this observation did not remain significant when adjusting for other characteristics. Thus, our findings do not support focusing efforts on collecting from mothers <20 years old.

This analysis also clearly demonstrated that, after adjusting for other variables, the donor's race/ethnicity is strongly associated with the quality and potency of CBUs. Most notably, Caucasian infants were more likely to have CBUs with higher potency as compared to African American infants. Furthermore, we observed that, despite similar collection volumes, CBUs from Caucasian infants had higher numbers per ml of CFU, $CD34^+$ and post-TNCC cells as compared to African American infants. These results confirm and extend other studies showing the relationship between race and post-TNCC, $CD34^+$ or CFU cell content[13,14,27,28]. Our finding of lower potency of CBUs from African American donors complicates the path to achieving the stated goals of increasing and maintaining the ethnic and racial diversity of high quality CBU in the national donor registry. Therefore, if donor/recipient matching by race is determined to be associated with superior clinical outcomes in transplantation, it will be critical to enhance recruitment of African American donors and increase resources to collect higher numbers of CBUs from this population.

The variables that are collection-related and that can be modified to increase CBU quality include increasing collection volumes and shortening time to processing. Larger volume CBUs were more likely to have higher potency overall compared to smaller CBUs regardless of delivery type. Thus, the collection staff should be encouraged to use careful technique and take the time to maximize the volume of CB that can be extracted. In the laboratory, strategies to prioritize the processing of recently collected CBUs should be developed. Currently, many banks receive collections from distant sites, and, therefore, delays in processing due to travel may exist. Results of the COBLT study indicated that viability, post-TNCC, and $CD34^+$ content remain stable at room temperature for >48 hours[4] leading to the rule that cryopreservation of a processed CBU must begin within 48 hours of collection. However, in this study, we found small but significant losses of CFU, $CD34^+$ cells and TNCC that occur in CB processed ≥24 hours after delivery as compared to those processed <10 hours from collection. Our findings are similar to Pope et al[29] who recently correlated decreasing viability with increased time from collection to freeze. These findings indicate that delays in processing could impact the yield of bankable CBUs. These results should inform banks designing algorithms and criteria for prioritization of CBUs in their processing laboratories. For example, in our own bank, this data prompted change in our algorithm, originally solely based on time from collection, to prioritizing processing larger (>150ml for Caucasian; >120ml for non-Caucasians) units received in the lab <24 hours from collection ahead of older units. Practically, this also indicates that banks should consider opening collection sites that are close to their processing laboratories and that courier schedules should be adjusted to transport collected CBUs back to the processing laboratory as quickly as possible. Regionalization of the public banking system should also be considered as an option in which publically donated CBUs would be referred to the geographically closest bank.

Taken at face value, our data indicates that collections from larger, Caucasian, early term babies that are processed within 10 hours of collection yield the highest quality CBUs for public banking. However, if we were to follow this formula to the letter, we would exclude

collections from racial and ethnically diverse donors, which would result in a less diverse pool of HLA phenotypes in the inventory. Whether a racially matched CBU will result in better clinical outcomes presumably because of closer HLA-matching has yet to be determined. However, results with adult donors indicate that closer HLA-matching combined with adequate cell content will result in superior clinical outcomes[11]. Larger datasets of clinical outcomes of UCBT will be necessary to definitively answer this question but if racial matching yields superior post-transplant outcomes, collection of large numbers of non-Caucasian CBUs must occur to add high-quality minority CBUs to the inventory.

Data from the NMDP registry demonstrates that CBUs with higher post-TNCC (median 1.76×10^9) are preferentially selected by transplant centers for transplantation[6]. We have shown that 75% of these units also have high potency (Figure 5, Region A). However, using the post-TNCC to select a donor unit, transplant physicians would inadvertently select a less potent CBU 25% of the time. It is possible that this discrepancy could contribute to engraftment delay in some cases. We also observed that approximately 40% of units with intermediate ($1.25–1.75\times10^9$) post-TNCC have potency in the highest quartile (Figure 5, Region B). Therefore, if transplant physicians were to consider the CFU and/or $CD34^+$ content and select more units with intermediate post-TNCC when appropriate, utilization of the existing inventory of donor CBUs would be enhanced (Figure 5, Region A+B).

We have identified several parameters related to donor characteristics, collection procedures, transportation to the processing laboratory and the actual processing procedures which could be optimized to increase the yield of high potency CBU from the pool of prospective donors. Implementing new procedures that incorporate collection priorities and processing protocols that optimize these parameters may increase this yield, but competes with the goal of rapidly expanding both the number and diversity of the national CBU inventory. Studies to objectively determine the role of race matching in cord blood transplantation should be conducted to help inform this discussion. Thus, the observations made in this report should be used to guide decisions about bank organization, location of collection sites, and prioritization of units in the processing lab to work towards creating an inventory with the highest potency for clinical applications.

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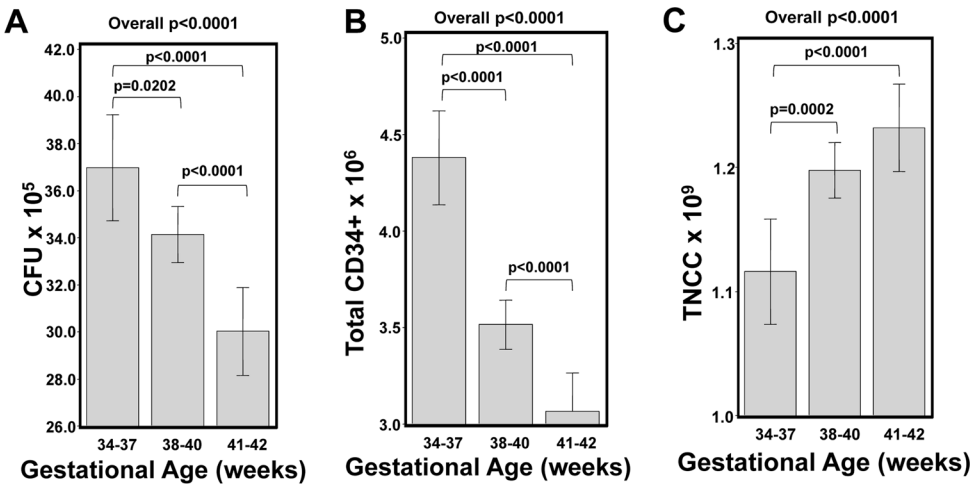

Figure 1.

Impact of infant estimated gestational age on the CFU, $CD34^+$ and post-TNCC content. In Panels A–C, the adjusted mean CFU (Panel A), $CD34^+$ (Panel B) and post-TNCC (Panel C) is shown in relationship to infant gestational age after adjusting for infant race/ethnicity, birth weight, gender, collection volume, delivery type and maternal age. Only significant p values are shown. Whisker plots represent the 95% Confidence Intervals.

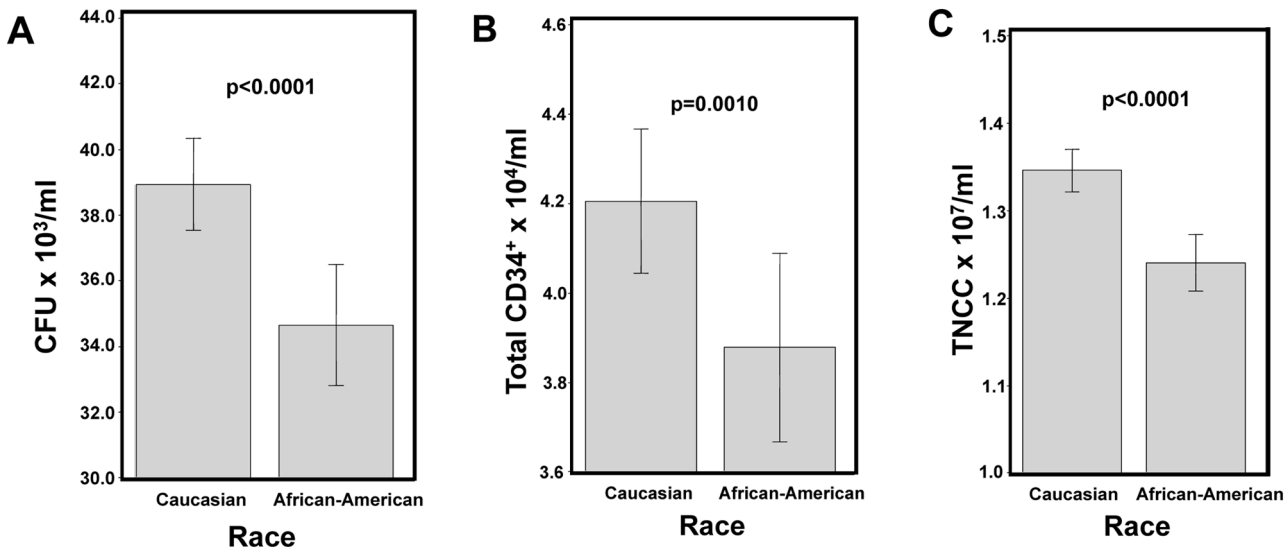

Figure 2.

Comparison of the CFU, $CD34^+$, and post-TNCC concentrations for Caucasian and African American infants. In Panels A–C, the adjusted mean CFU per ml (Panel A), $CD34^+$ per ml (Panel B) and post-TNCC per ml (Panel C) is shown in relationship to race for infants of Caucasian and African American race, respectively, after adjusting for infant gestational age, birth weight, gender, collection volume, delivery type and maternal age. Only significant p values are shown. Whisker plots represent the 95% Confidence Intervals.

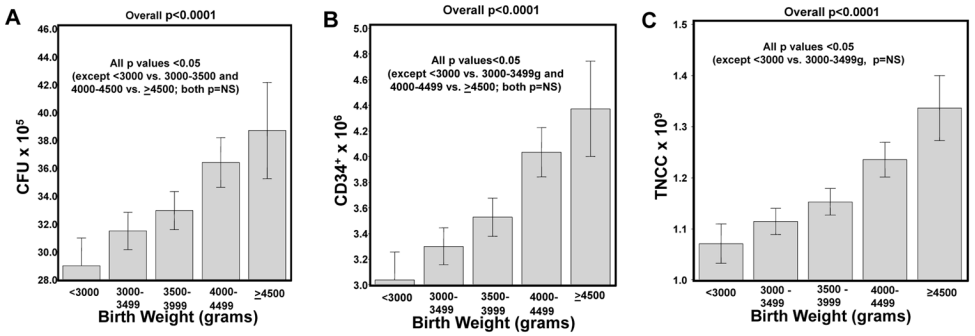

Figure 3.

Impact of infant birth weight on the CFU, $CD34^+$ and post-TNCC content. In Panels A–C, the adjusted mean CFU (Panel A), $CD34^+$ (Panel B) and post-TNCC (Panel C) with respect to birth weight is shown after adjusting for infant race/ethnicity, gestational age, gender, collection volume, delivery type and maternal age. Only significant p values are shown. Whisker plots represent the 95% Confidence Intervals.

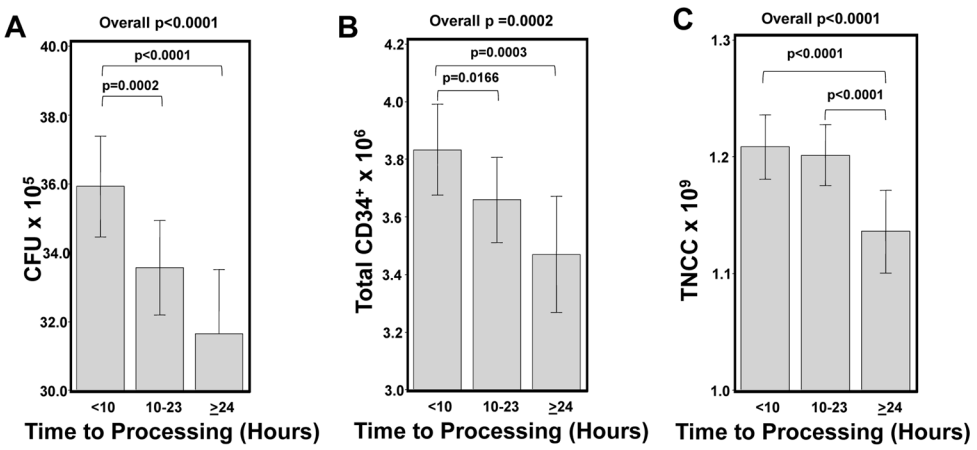

Figure 4.

Impact of time to processing on CFU, $CD34^+$ and post-TNCC content. In Panels A–C, the adjusted mean CFU (Panel A), $CD34^+$ (Panel B) and post-TNCC (Panel C) by time to processing is presented after adjusting for infant race/ethnicity, gender, gestational age, birth weight, collection volume, delivery type, and maternal age. Only significant p values are shown. Whisker plots represent the 95% Confidence Intervals.

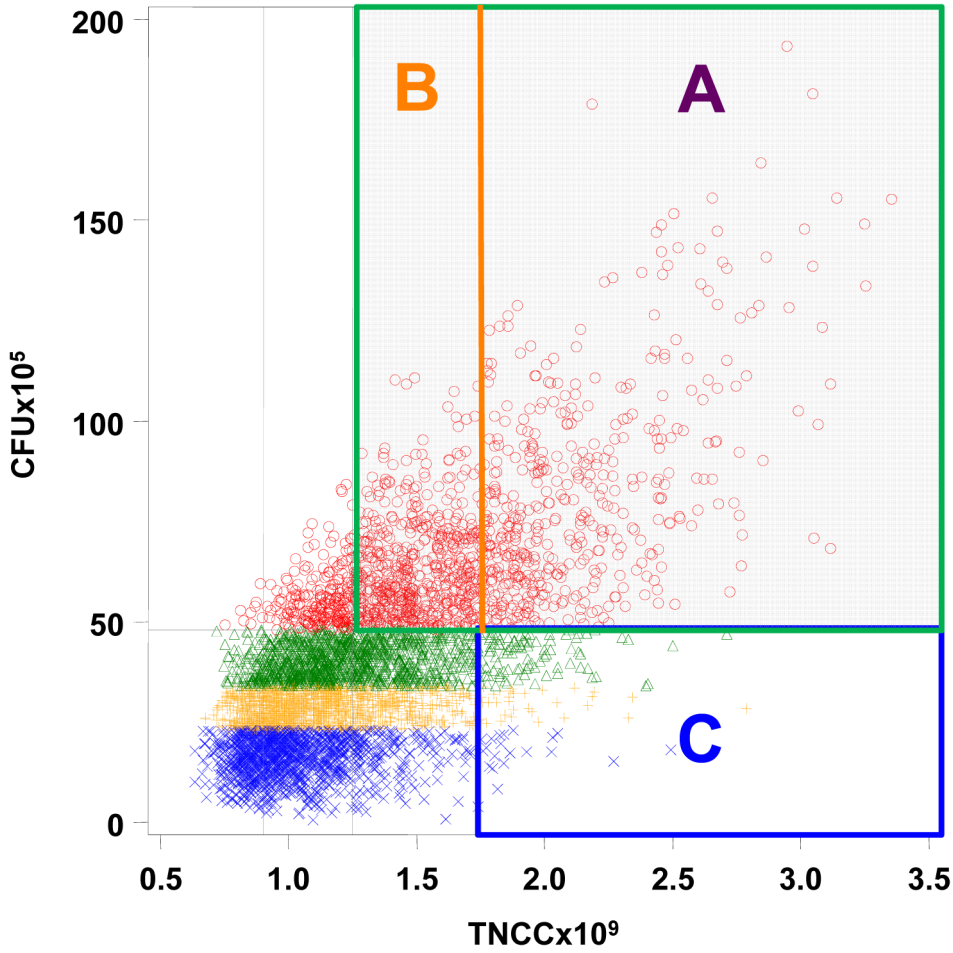

Figure 5.

Considering the potency (CFU content) along with post-TNCC in donor selection. The relationship between post-TNCC and the CFU content (i.e. potency) is shown for the study cohort (n= 5267 CBUs). The vertical lines represent (from left to right): the minimum post-TNCC required for banking (0.9×10^9), an intermediate post-TNCC (1.25×10^9), and the median post-TNCC of CBUs selected for transplantation (1.75×10^9)[6]. Quartiles for the CFU content are also represented [upper quartile: red circle, second quartile: green triangle, third quartile +: gold, and lower quartile: blue x]. Region A refers to CBUs with post-TNCC>1.75x 10^9 and CFU in the highest quartile. Region B refers to post-TNCC 1.25–1.75×10^9 with CFU in the highest quartile. Region C refers to CBUs with post-TNCC >1.75×10^9 and CFU in the lower three quartiles.

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Table 1

Clinical characteristics of maternal and infant donors (n=5267).

	N (%)
Infant gender	
Male	2602 (49.4)
Female	2665 (50.6)
Infant race/ethnicity	
White/Caucasian (Non-Hispanic)	3099 (58.8)
Black/African-American	758 (14.4)
Hispanic	675 (12.8)
More than one race	568 (10.8)
Asian	136 (2.6)
Other including Native Hawaiian, Pacific Islander or Native American	31 (0.6)
Infant gestational age (weeks)*	
34–37	361 (6.9)
38–40	4305 (81.7)
41–42	573 (10.9)
Delivery method	
Vaginal	3149 (59.8)
Caesarean	2188 (40.2)

	Mean (SD)	Median (range)
Birth weight (grams)	3596 (442)	3580 (1800–5724)
Maternal age (years)	29.3 (5.6)	29.0 (18.0–53.0)

* Infant gestational age is unknown for 28 units. The mean and median gestational ages were 39 weeks (SD 1.1; range, 34–42 weeks).

Table 2

Characteristics of the processed cord blood units (n=5267) including post-processing cellular parameters.

	Mean (SD)	Median (range)
Collection volume (ml)	97.5 (28.6)	93.0 (40.0–286.0)
Time to processing (hours)	14.5 (8.9)	14.1 (0.5–47.4)
Total CFU ($\times 10^5$)	38.3 (22.3)	34.0 (0.6–193.3)
Total CFU per ml collected ($\times 10^3$/ml)	39.99 (20.94)	36.60 (0.38–173.13)
$CD34^+$ ($\times 10^6$)	3.66 (2.05)	3.21 (0.02–9.99)
$CD34^+$ per ml collected ($\times 10^4$/ml)	3.91 (2.21)	3.44 (0.01–15.32)
Post-TNCC ($\times 10^9$)	1.27 (0.40)	1.18 (0.63–5.55)
Post-TNCC per ml collected ($\times 10^7$/ml)	13.46 (3.65)	12.99 (4.20–43.76)
Viability (%)	97.8 (2.40)	98.5 (84.0–100.0)

Table 3

Clinical characteristics predictive of CFU >34×10^5 (median) in univariate and multivariate analysis.

Characteristics	Univariate	Multivariate
	Odds Ratio (95% CI*), p-value	
Infant gestational age (weeks)		
Overall Effect	p=0.0264	p=0.0027
34–37 vs. 38–40	1.23 (0.99–1.63), p=0.0567	1.32 (1.05–1.65), p=0.0182
34–37 vs. 41–42	1.44 (1.10–1.87), p=0.0071	1.63 (1.23–2.16), p=0.0006
38–40 vs. 41–42	1.17 (0.98–1.39), p=0.0859	1.24 (1.03–1.49), p=0.0213
Infant Race/Ethnicity+		
Overall Effect	p=0.0020	p=0.0076
Caucasian compared to African American	1.37 (1.17–1.61), p=0.0001	1.39 (1.17–1.64), p=0.0001
Hispanic compared to African American	1.19 (0.97–1.47), p=0.0946	1.29 (1.03–1.60), p=0.0233
Infant birth weight (g)		
>3500	1.43 (1.28–1.59), p<0.0001	1.25 (1.10–1.41), p=0.0003
≤3500	1.00	1.00
Infant gender		
Male	1.18 (1.06–1.32), p=0.0023	NS
Female	1.00	
Maternal age (years)		
>20	1.24 (1.02–1.53), p=0.0381	NS
≤20	1.00	
Collection volume (ml) and delivery type		
≤80		
Vaginal	NS	NS
Caesarean		
>80		
Vaginal	1.18 (1.03–1.34), p=0.0142	1.30 (1.13–1.49), p=0.0002
Caesarean	1.00	1.00
Processing time (hours)		
Overall Effect	p<0.0001	p<0.0001

Characteristics	Univariate	Multivariate
	Odds Ratio (95% CI*), p-value	
<10 hours vs. 10–23 hours	1.29 (1.15–1.46), p<0.0001	1.25 (1.11–1.42), p=0.0004
<10 hours vs. ≥24 hours	1.63 (1.36–1.95), p<0.0001	1.64 (1.36–1.98), p<0.0001
10–23 hours vs. ≥24 hours	1.26 (1.06–1.50), p=0.0098	1.31 (1.09–1.57), p=0.0035

CI: Confidence intervals; GA: gestational age; BW: birth weight;

*Characteristics that were predictive of higher CFU content in either univariate or multivariate analysis were included. Viability of the cord blood cells was not associated with CFU content and was therefore not included in this table.

+All races/ethnicities were compared against each other in both univariate and multivariate analysis. Only the relationships that are statistically significant are shown in the table.

Table 4

Clinical characteristics predictive of $CD34^+$ 3.21> $\times 10^6$ (median) in univariate and multivariate analysis

Characteristics*	Univariate	Multivariate
	Odds Ratio (95% CI), p-value	
Infant gestational age (weeks)		
Overall Effect	p<0.0001	p<0.0001
34–37 vs. 38–40	1.89 (1.51–3,20), p<0.0001	2.20 (1.74–2.79), p<0.0001
34–37 vs. 41–42	2.44 (1.86–3.20), p<0.0001	3.14 (2.35–4.18), p<0.0001
38–40 vs. 41–42	1.29 (1.08–1.53), p=0.0049	1.42 (1.18–1.71), p=0.0002
Infant Race/Ethnicity*		
Overall Effect	p=0.0510	p=0.1172
Caucasian compared to African American	1.29 (1.10–1.51), p=0.0018	1.27 (1.07–1.50), p=0.0057
Hispanic compared to African American	1.21 (0.98–1.49), p=0.0726	1.28 (1.03–1.60), p=0.0259
Infant birth weight (g)		
>3500	1.64 (1.46–1.83), p<0.0001	1.53 (1.36–1.73), p<0.0001
≤3500	1.00	1.00
Infant gender		
Male	1.36 (1.22–1.51), p<0.0001	1.24 (1.11–1.40), p=0.0002
Female	1.00	1.00
Maternal age (years)		
>20	1.27 (1.04–1.56), p=0.0217	NS
≤20	1.00	
Collection volume (ml) and delivery type		
≤80		
Vaginal	NS	NS
Caesarian		
>80		
Vaginal	NS	1.19 (1.04–1.37), p=0.0130
Caesarian		1.00
Processing time (hours)		
Overall Effect	p<0.0001	p=0.0004

Characteristics*	Univariate	Multivariate
	Odds Ratio (95% CI), p-value	
<10 hours vs. 10–23 hours	1.16 (1.03–1.30), p=0.0139	1.12 (0.99–1.28), p=0.0687
<10 hours vs. ≥24 hours	1.47 (1.23–1.76), p<0.0001	1.46 (1.21–1.76), p<0.0001
10–23 hours vs. ≥24 hours	1.27 (1.07–1.51), p=0.0068	1.30 (1.08–1.56), p=0.0045
Viability (%)		
98–100	NS	NS
90–97		

CI; Confidence Interval; NS: not significant;

*Characteristics that were predictive of higher $CD34^+$ content in either univariate or multivariate analysis were included. Viability of the cord blood cells was not associated with $CD34^+$ content and was therefore not included in this table. All races/ethnicities were compared against each other in both univariate and multivariate analysis. Only the relationships that are statistically significant are shown in the table.

Table 5

Clinical characteristics predictive of Post-TNCC >1.18×10^9 in univariate and multivariate analysis.

Characteristics*	Univariate	Multivariate
	Odds Ratio (95% CI), p-value	
Infant gestational age (weeks)		
Overall Effect	p<0.0001	p<0.0001
34–37 vs. 38–40	0.63 (0.51–0.79), p<0.0001	0.62 (0.49–0.79), p=0.0001
34–37 vs. 41–42	0.49 (0.38–0.64), p<0.0001	0.51 (0.38–0.68), p<0.0001
38–40 vs. 41–42	0.78 (0.66–0.93), p=0.0060	0.82 (0.67–0.99), p=0.0409
Infant race/ethnicity*		
Overall Effect	p<0.0001	p<0.0001
Caucasian compared to African American	1.44 (1.25–1.66), p<0.0001	1.54 (1.29–1.84), p<0.0001
Caucasian compared to More than One Race	1.24 (0.87–1.77), p=0.2288	1.27 (1.04–1.55), p=0.0193
Caucasian compared to Hispanic	1.22 (1.03–1.43), p=0.0213	1.14 (0.94–1.37), p=0.1752
Asian compared to African American	1.19 (0.92–1.56) p=0.1914	1.59 (1.05–2.40), p=0.0275
Infant birth weight (g)		
>3500	1.85 (1.65–2.07), p<0.0001	1.37 (1.21–1.56), p<0.0001
≤3500	1.00	1.00
Maternal age (years)		
>20	1.31 (1.00–1.71), p=0.0480	NS
≤20	1.00	
Collection volume (ml)+		
≤80		
Vaginal	1.41 (1.05–1.90), p=0.0228	1.41 (1.05–1.91), p=0.0242
Caesarian	1.00	1.00
>80		
Vaginal	1.18 (1.03–1.35), p=0.0170	1.25 (1.08–1.44), p=0.0023
Caesarian	1.00	1.00
Vaginal		
≤80	0.15 (0.13–0.18), p<0.0001	0.15 (0.12–0.17), p<0.0001
>80	1.00	1.00

Characteristics*	Univariate	Multivariate
	Odds Ratio (95% CI), p-value	
Caesarian		
≤80	0.12 (0.09–0.16), p<0.0001	0.13 (0.10–0.17), p<0.0001
>80	1.00	1.00
Processing time (hours)		
Overall Effect	p<0.0001	p=0.0001
<10 hours vs. 10–23 hours	1.18 (1.05–1.32), p=0.0061	1.08 (0.94–1.23), p=0.2674
<10 hours vs. ≥24 hours	1.60 (1.34–1.91), p<0.0001	1.54 (1.26–1.88), p<0.0001
10–23 hours vs. ≥24 hours	1.36 (1.14–1.61), p=0.0006	1.42 (1.18–1.73), p=0.0003
Viability (%)		
98–100	1.09 (0.97–1.22), p=0.1649	NS
90–97	1.00	

CI: Confidence Interval; NS: Not significant;

* Infant gender was not significantly associated with TNCC content and therefore was not included in this table.

* All races/ethnicities were compared against each other in both univariate and multivariate analysis. Only the relationships that are statistically significant are shown in the table.

Table 6

Comparison of significant differences between male and female infants in overall cohort.*

	Male	Female	P value
Delivery type (Vaginal)	N=1512 (58.1%)	N=1634 (61.4%)	0.0155
	Median (range)		
Birth weight (grams)	3671 (2227–5473)	3516 (1800–5724)	<0.0001
Maternal Age (years)	30 (18–45)	29 (18–53)	0.0269
Collection Volume (ml)	95 (40–286)	92 (40–284)	<0.0001
Total CFU ($\times 10^5$)	34.9 (0.6–181.5)	33.1 (2.3–193.3)	<0.0001
$CD34^+$ ($\times 10^6$)	3.4121 (0.02–9.99)	3.0455 (0.252–9.92)	<0.0001

* There were no differences between male and female infants when comparing gestational age, race/ethnicity or the median TNCC.