

Factors influencing the success of cord blood collection: a tertiary perinatal medicine center's experience

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Background/aim: This study aimed to evaluate the effects of certain maternal, fetal, and umbilical cord blood unit factors on storage and/or discard incidence of collected cord blood units from perinatal medicine patients.

Materials and methods: A total of 273 cord blood units collected between January 2011 and December 2016 in the Division of Perinatology of Hacettepe University Hospital were evaluated retrospectively in this study.

Results: Of the collected cord blood units, 53.8% (147/273) were stored. Infant birth weight, cord blood unit volume, total nucleated cell count, and CD34+ cell count were statistically significantly different between the eligible and discarded cord blood unit groups ($P < 0.001$ for all). No cord blood units were discarded owing to contamination-related issues. The mean gestational age for pregnant women whose umbilical cord blood was stored was 36.6 ± 1.0 weeks.

Conclusion: Infant birth weight, cord blood unit volume, total nucleated cell count, and CD34+ cell count were significantly different between the eligible and discarded cord blood unit groups. The low rate of specimen storage was most likely because of the unique characteristics of perinatal medicine patients. Physicians should choose appropriate donors for cord blood collection to increase the rate of cord blood utilization.

Key words: Umbilical cord blood, cord blood bank, pregnancy

1. Introduction

Umbilical cord blood (CB) is a natural source of hematopoietic stem cells (HSCs) and is an accepted alternative to bone marrow for transplantation purposes in a variety of diseases such as leukemia, congenital immunodeficiencies, hereditary metabolic disorders, hemoglobinopathies, and bone marrow failure syndromes (1–4). Gluckman et al. reported the first successful CB transplantation for Fanconi anemia in 1989 (5). Since then, CB banks (CBBs) have been established worldwide to provide suitable CB units (CBUs) for use in allogeneic HSC transplantation (6,7).

CB has profound clinical advantages over human leukocyte antigen (HLA)-matched bone marrow, such as lower incidence of graft-versus-host disease (GVHD), immediate availability for use, low risk of transmission of infectious diseases, and better long-term immune recovery and survival rates (8).

CB can be collected and stored in two different settings: in public and private banks (7). Moreover, hybrid banks have also been recently established. Public banks collect altruistic CBUs and process, store, and release the product for clinical programs. On the other hand, private CBBs are profit-oriented institutions facilitating the collection and storage of CB from families on demand, for future autologous or family use, for a certain price (9). Both public and private CBUs can be used for directed banking, which is aimed at using stored sibling donor CB for family members when a first-degree relative is diagnosed as having a disease that can be cured using CB transplantation (10). A high percentage of CB used clinically is released through public banks, where storage of high-quality CB is ensured by both national and international standards.

CBUs altruistically collected from consenting eligible donors and stored at CBBs constitute a valuable source of a biological product with high therapeutic value that would

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otherwise be a medical waste. Nonetheless, determination of donor eligibility is crucial, especially for low-income countries with limited resources. Unit volume, total nucleated cell (TNC) count, number of CD34+ HSCs, infant birth weight, gestational age at birth, fetal distress, placental weight, previous live births, meconium-stained amniotic fluid, mode of delivery, and collection technique can affect the quality and eligibility of CBUs for storage (11–20).

The aim of this study was to evaluate the maternal, neonatal, and CBU parameters that affect the storage and discard rates of collected CBUs from perinatal medicine patients with poor obstetric histories but whose current pregnancies were being successfully managed.

2. Materials and methods

A total of 273 CBUs collected between January 2011 and December 2016 at the Division of Perinatology of Hacettepe University Hospital were evaluated retrospectively in this study. The required data were obtained from the Hacettepe University Perinatal Medicine Database.

FACT-NetCord (Foundation for the Accreditation of Cellular Therapy together with NetCord) is one of the major authorities in CB banking that establish international standards for CB banking quality control. The Ankara University Cord Blood Bank is currently the only FACT-NetCord-accredited public CBB in Turkey. This CBB is accredited for all steps of CB banking, including consent gathering and CB collection, processing, banking, and release of unrelated donations. Hacettepe University Hospital is accredited by FACT-NetCord only for CB collection and in collaboration with Ankara University Cord Blood Bank. Our study included CBUs donated voluntarily by pregnant women who delivered at our institution. CB collections for medically indicated families were excluded from this study.

The inclusion criteria for CB collection at our institution were as follows: 1) written maternal informed consent; 2) absence of familial inherited diseases; 3) negative hepatitis B, hepatitis C, HIV, and HTLV (human T-cell leukemia virus type-I/II) serology; 4) gestational age of ≥ 32 weeks at birth; 5) rupture of membranes < 12 h; 6) absence of clinical and/or laboratory findings suggesting chorioamnionitis; 7) absence of prenatally detected chromosomal anomalies and congenital abnormalities in the fetus; 8) absence of placental abnormalities; 9) absence of multiple gestations; 10) absence of maternal chronic inflammatory diseases; and 11) absence of maternal metabolic and immunological disorders. Pregnant women who did not meet these criteria were excluded from CB collection. The majority of the patients were perinatal medicine patients with poor obstetric histories but whose current pregnancies were being successfully managed.

CB was collected during the third stage of labor before delivery of the placenta by trained physicians (in utero). The umbilical cord was clamped at two different locations (5 cm and 7 cm from the newborn) about 15 s after delivery and was cleaned with 70% alcohol and iodine (in the case of vaginal deliveries). The umbilical cord was cut between the clamps, and the newborn was separated. CB was collected from the umbilical vein by using a 16-gauge needle, with the help of gravity and gentle milking until the blood flow stopped, into a sterile 350-mL collection bag containing 25 mL of CPDA (citrate, phosphate, dextrose, and adenine) anticoagulant. After the collection of CBUs, the bags were transferred to the CBB within 1–2 h for processing. Selected deliveries (mostly daytime cesarean sections [CSs], Monday to Thursday) were used for CB collection to avoid technical difficulties and problems during transportation to the CBB.

CBUs were processed and cryopreserved provided that 1) the time to transfer from collection was < 48 h, 2) the total volume was > 40 mL, 3) the TNC count was $> 10 \times 10^8$, 4) the CD34+ cell count was $> 1.5 \times 10^6$, and 5) there was no evidence of aerobic or anaerobic bacterial/fungal growth.

The CBUs were attached to a Sepax kit (Biosafe, CS-530.4) and installed onto a Sepax device (Sepax S-100) in a closed system. The final volume for HSC-rich buffy coat was set to 22 mL, and the hematocrit percentage of the CBU was included in the device settings. Red blood cell (RBC) depletion and plasma depletion were performed fully automatically, and CBUs were separated into three final fractions: HSC-rich buffy coat, CBU plasma, and RBCs. A 2-mL volume from the buffy coat was reserved for HLA typing, complete blood count analysis, and CD34 cell phenotyping. After the infusion of 5 mL of dimethyl sulfoxide into the buffy coat, units with a final volume of 25 mL were frozen to -160 °C in a controlled-rate freezer and transferred into a liquid nitrogen tank (vapor phase).

Our primary aim in this study was to evaluate the maternal and neonatal factors affecting the CBU eligibility and discard rate. All the included CBUs were grouped into two categories: eligible CBUs and ineligible discarded CBUs. Statistical analysis was conducted using SPSS 22 (IBM Corp., Armonk, NY, USA). Maternal age, gravidity, gestational week at birth, maternal hemoglobin value at birth, gestational week at birth, infant birth weight, fetal sex, CBU volume, and TNC and CD34+ cell counts of the CBUs were investigated in both the eligible and ineligible groups. Descriptive statistics were presented for each maternal, neonatal, and CBU parameter investigated. The variables were analyzed using visual histograms, probability plots, and analytical methods (Kolmogorov–Smirnov/Shapiro–Wilk test) to determine whether they were normally distributed. As the data were normally

distributed, means and standard deviations were used. The independent-samples t-test was used to compare the parametric variables between the groups. Categorical variables were compared using the chi-square test. An overall P-value of <0.05 was considered statistically significant.

Informed consent was obtained from all patients, and the study protocol was approved by the Hacettepe University Ethics Committee.

3. Results

A total of 147 deliveries were included in the eligible group (53.8%) and 126 deliveries in the discarded group (46.2%). The maternal characteristics, neonatal characteristics, and CBU parameters of the donors in terms of mean, standard deviation, minimum–maximum values, and P-values are shown in the Table for the indicated groups.

There were no statistically significant differences between groups in terms of the mean values calculated for the parameters investigated: maternal age, gravidity, parity, maternal hemoglobin value, and gestational week at birth ($P = 0.237$, $P = 0.156$, $P = 0.69$, $P = 0.51$, and $P = 0.26$, respectively).

However, the mean values of infant birth weight, CBU volume, TNC count, and CD34+ cell count were statistically different between the groups ($P < 0.001$ for all). The means and standard deviations of infant birth weight, CBU volume, TNC count, and CD34+ cell count were 2996.94 ± 349.00 g, 104.12 ± 23.68 mL, $15.27 \pm 2.20 \times 10^8$, and $3.00 \pm 0.84 \times 10^6$, respectively, for the stored (eligible) group. On the other hand, the means and standard deviations of infant birth weight, CBU volume,

TNC count, and CD34+ cell count were 2825.80 ± 364.30 g, 81.40 ± 26.32 mL, $6.39 \pm 1.24 \times 10^8$, and $0.64 \pm 1.78 \times 10^6$, respectively, for the discarded group of CBUs. There were 67 (45.6%) male and 80 (54.4%) female fetuses in the eligible group. On the other hand, there were 71 (56.3%) male and 55 (43.7%) female fetuses in the discarded group. However, there was no statistically significant difference between the groups in terms of fetal sex ($P = 0.076$).

More than 35,000 unrelated CBUs have been distributed worldwide by public CBBs for allogeneic HSC transplantation. The usage rate of CBUs at the Ankara University CBB is 2% (30 of 1502) to date (until July 2018) (10 for related and 20 for unrelated transplantation purposes), and 34.8% of these CBUs were collected at Hacettepe University. For confidentiality reasons, the Ankara University CBB is the only authority that keeps the identities of the CBUs used for transplantation.

4. Discussion

CB can be collected using either in utero or ex utero techniques. The ex utero method relies more on the collectors' and/or clinicians' experience (21). The main limitation of CBUs is the small collection volume, which generally leads to low TNC and CD34+ cell counts, thus restricting application to children and small-sized adult patients (18). However, various strategies to increase HSC content and the homing capacity of CBUs have been developed and used successfully. Thus, CB is currently one of the most promising “off-the-shelf” products to be used both in transplantation (pediatric/adult) and regenerative medicine (22,23). In contrast to many other CBBs worldwide, where CB collection is primarily

Table. Maternal, neonatal, and CBU parameters compared between the eligible and discarded groups.

Variables	CBB status				P-value
	Eligible (147) (53.8%)		Discarded (126) (46.2%)		
	Mean \pm SD	Range (minimum–maximum)	Mean \pm SD	Range (minimum–maximum)	
Maternal age (years)	31.1 \pm 4.08	20–41	30.45 \pm 4.88	21–45	0.237
Gravidity	3.0 \pm 1.60	1–9	3.3 \pm 1.47	1–7	0.156
Parity	0.81 \pm 0.89	0–5	0.85 \pm 0.84	0–3	0.69
Maternal Hb (g/dL)	10.45 \pm 2.06	6–16	10.30 \pm 1.90	6–14	0.51
Gestational age at birth (weeks)	36.70 \pm 1.07	35–40	36.60 \pm 0.86	34–38	0.26
Infant birth weight (g)	2996.94 \pm 349.00	1470–4120	2825.80 \pm 364.30	1640–4100	<0.001
CBU volume (mL)	104.12 \pm 23.68	54–212	81.40 \pm 26.32	22–152	<0.001
TNC ($\times 10^8$) count	15.27 \pm 2.20	10–18.4	6.39 \pm 1.24	2.10–9.40	<0.001
CD34+ cell count ($\times 10^6$)	3.00 \pm 0.84	1.52–4.50	0.64 \pm 1.78	0.20–1.30	<0.001

SD: Standard deviation, CBB: cord blood bank, CBU: umbilical cord blood unit, Hb: hemoglobin, TNC: total nucleated cells.

performed by midwives, nurses, or well-trained CBB staff, obstetricians perform the collections in Turkey. Thus, the in utero technique is almost always preferred, as it is more practical, cheaper, and easier to perform (24).

CB has been considered an alternative source of HSCs needed for the treatment of certain diseases (2). Although it has advantages over bone marrow, such as lower incidence of GVHD, immediate availability for use, low risk of transmissible infectious diseases, and better long-term immune recovery and survival (8), the main drawback for the use of CBUs in the HSC transplantation setting is the low number of TNCs and CD34+ cells owing to the small collection volumes (18). Because public banks rely on financial support from the government, distinctive donor eligibility determination is of high importance, particularly for low-income countries with limited resources (25). Furthermore, the small number of collected cells restricts the application of the procedure to children and small-sized adults (18). However, progress in the enhancement of cell counts by using various stimulation methods seems to be a good innovation in this field and is expected to widen the spectrum of transplantation patients (22,23). Thus, it is important to select suitable donors for CB collection and storage to facilitate optimal use.

Faivre et al. examined several variables related to the parturient, pregnancy, labor, delivery, collection, the newborn, the umbilical cord, and the placenta in their comprehensive review that included 71 related articles (26). Maternal age at delivery was assessed in 28 of 71 articles, and it was found not to be associated with favorable CBU collection parameters in most of the studies.

Additionally, Faivre et al. indicated that iron-related maternal blood variables did not seem to affect the CBU quality according to the literature they reviewed (26). They also evaluated the effect of gestational age at birth by reviewing 24 articles. In 17 articles, no correlation was found between CBU volume and gestational age. However, a positive correlation was found in 7 articles. A positive correlation was also shown between older gestational age at birth and TNC count in 22 individual studies. In contrast, no significant correlation with CD34+ cells was reported in 18 studies, and an inverse correlation was found in 11 articles. However, 3 studies observed a correlation between CD34+ cells and older gestational age at birth. Furthermore, an inverse relation was similarly reported with colony-forming units (CFUs) in 5 articles (26).

In light of the literature, Faivre et al. recommended to limit CBU collections after a defined gestational age for cost-effectiveness issues. The effect of parity was also another parameter evaluated in this review (26). In 25 articles, the researchers evaluated the impact of previous births on CBU volumes, TNC count, CD34+ cell count, and/or CFUs. Overall, no correlation was found between

previous births and CBU quality, irrespective of the variables tested. Although some studies showed a slight increase of TNC and CD34+ cell counts in primiparous women, the authors attributed these findings to the higher rates of fetal stress due to longer delivery times. Thirty-seven studies assessed the effect of delivery type on CBU parameters included in this review (26). CBU volume was shown to be significantly higher with CS delivery, and TNC counts were observed to be significantly higher with vaginal delivery. Faivre et al. also reviewed 45 studies that evaluated the effect of birth weight on CBU quality (26). Although heavier birth weight was associated with favorable CBU parameters in a vast majority of the studies, no significant impact of fetal sex was shown.

Infant birth weight, CBU volume, TNC count, and CD34+ cell count were statistically significantly different between the groups in our study. The CS rate was very high in our study population (269 of 273, 98.2%) because physicians preferred CS cases for CB collection to avoid technical difficulties and transfer problems. There were 3 vaginal deliveries in the eligible CBU group, whereas only 1 vaginal birth was present in the ineligible discarded group. These findings revealed that heavier infants, higher volumes, and higher TNC and CD34+ cell counts were associated with higher CB storage rates, compatible with the current literature (11–15,18,19). However, there were no statistically significant differences in the mean values of the parameters investigated, including maternal age, gravidity, parity, maternal hemoglobin value, and gestational age at birth, between the groups ($P = 0.237$, $P = 0.156$, $P = 0.69$, $P = 0.51$, and $P = 0.26$, respectively).

We did not consider the influence of mode of delivery, fetal distress, meconium-stained amniotic fluid, placental weight, and collection techniques on CBB outcomes because of lack of information. Selected deliveries (mostly daytime CSs, Monday to Thursday) were used for CB collection to avoid technical difficulties and problems in transportation to the CBB. In our series, all CBUs were collected in utero and only 53.8% of them were stored. This low rate of specimen storage is most probably because of the unique characteristics of the patients admitted to the Division of Perinatal Medicine. The majority of the patients were perinatal medicine patients with poor obstetric histories but whose current pregnancies were being successfully managed.

The mean gestational age for the pregnant women whose CBs were stored was 36.6 ± 1.0 weeks. The study population consisted of women with early term or late preterm deliveries with poor obstetric history, as mentioned earlier. We did not observe any microbiological contamination, and the perinatal medicine staff seemed to be more devoted to the collection process and collaborated more easily than the obstetrics ward staff. Uncomplicated

term deliveries are considered ideal for CB collection. However, “successfully managed risky pregnancies” (like in our cohort) should also be kept in mind, especially in places with low CB collection costs. However, encouraging more pregnant women to donate CB, increasing the number of public CBBs, training medical staff for better collection techniques, choosing term and heavier fetuses for CB collection, facilitating CB collection even in emergency deliveries, and increasing CB collection rates in vaginal deliveries may increase the utilization rate of CBUs for treatment. CB banking and CB collection are relatively new issues in the daily practice of obstetricians in Turkey. Additionally, more comprehensive studies are needed about the quality assessment of CB collection procedures that are carried out at various institutions in Turkey. Thus, our study is a critical step for the evaluation of various factors that affect the storage rate of CBUs, as our institution is one of the leading national facilities for CB collection.

To ensure successful treatment and the true realization of the potential of CBUs, it is crucial that each and every collection maintains the high quality defined in international standards. Storing altruistic CBUs that were selected according to the highest eligibility criteria

makes the products more cost-effective and beneficial for the patients, preventing unnecessary costs from the need to acquire the product from other international CBBs. A well-organized national public bank such as the Ankara University CBB is a perfect opportunity not only for Turkish patients but also for international patients worldwide.

The main strengths of our study were the relatively high number of patients and the application of a standardized procedure for all patients (same medical staff and same conditions). However, the retrospective design, single-center experience, and homogeneity of the patients (perinatal medicine patients with poor obstetric histories) were the main limitations of our study.

In conclusion, infant birth weight, CBU volume, TNC count, and CD34+ cell counts were statistically significantly different between the eligible and discarded CBU groups. Physicians should choose appropriate donors for CB collection to increase the rate of CB utilization.

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