Original Article

Optimizing cord blood collections: Assessing the role of maternal and neonatal factors

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Abstract:

Background: As processing and cryopreservation of cord blood is time consuming and costly, it is essential to select units with optimal CD34+ cells, total nucleated cell (TNC) number and colony forming units (CFUs). These are the most important factors affecting outcome of UCB transplantation and are influenced by various maternal and neonatal factors. **Aim and objectives:** To determine the maternal and neonatal factors affecting TNC and CD34+ cell counts in cord blood so as to aid in proper selection of cord blood units for cryopreservation. **Materials and Methods:** A total of 100 UCB units were collected from normal vaginal deliveries, processed and assessed for volume, TNC, CD34+ cell count and CFU-GM. These parameters were then analyzed to find out whether they correlated with maternal and neonatal characteristics such as mother's age, parity, gestational age, baby's birth weight, and sex. **Results:** The volume of CB collected significantly correlated with the TNC, CD34+ cell, and CFU-GM yields ($P < 0.02$). A heavier placenta ($P < 0.05$), and a heavier baby ($P < 0.002$) were associated with a significantly greater volume of CB whereas the age, parity of mother and the sex of the baby had no significant effect. **Conclusion:** The only factors found to affect the TNC and CD34+ cell counts significantly were weight of the baby and placenta and the volume of cord blood collected. Since these factors are of prognostic significance, their analysis will aid in deciding which UCB unit should be processed and cryopreserved for UCB banking and subsequent transplantation.

Key words:

CD34+ cells, hematopoietic progenitor cells, hematopoietic progenitor cells transplantation, maternal and neonatal factors, total nucleated cells, umbilical cord blood

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Introduction

Umbilical cord blood (UCB) has now been recognized as an alternative source of hematopoietic stem cells (HSC). Since the first successful cord blood transplantation (CBT) in 1988, more than 25,000 unrelated CBT have been performed globally.[1] It is increasingly being used for HSC transplantation in related and unrelated adult and pediatric patients.[2-4] The easy and widespread availability across all racial and ethnic groups and lack of ethical issues have led to the establishment of numerous cord blood banks.

As engraftment is closely correlated with the number of infused cells, attempts should be made to process and cryopreserve only those units which will have minimum engraftable total nucleated cell (TNC) and CD34+ cell numbers, as processing and storage of UCB is a time consuming and costly affair. Various maternal and neonatal parameters have been reported to affect TNC and CD34+ cells in UCB.[5-7]

Our study aimed to evaluate the effect of different maternal and neonatal factors in females belonging to low/middle socio-economic income group, on stem cell yield in the UCB, in the Indian scenario so as to optimally utilize the scarce resources.

Materials and Methods

This prospective study was carried out on 100 antenatal booked cases of the obstetric unit of a tertiary care hospital, which is affiliated to our blood bank. Both term and pre-term deliveries were included in the study. Informed consent was taken from all patients. Prior consent was taken from the Institutional Ethics Committee.

UCB collection

Standard institutional obstetric practices were followed for the delivery of the baby.

CB was collected before delivery of the placenta following the in-utero technique of collection.[8] After delivery of baby, the cord was clamped and sterilized with povidone-iodine. A 16-guage needle from the UCB bag containing 22 ml of citrate phosphate dextrose as an anticoagulant was inserted into the umbilical vein and cord blood was collected by gravity. Thereafter bags were sealed and sent to stem cell lab of the blood bank for processing.

UCB processing

All bags were processed within 3 h. The collected cord blood volume was aliquoted into 50 ml

graduated centrifuge tubes (Falcon) in which 6% hydroxy-ethyl starch was added in a ratio of 1:5.[9,10] The tubes were then kept in a rack at an angle of 45°, with loosened caps for a period of 45 min to 1 h in a CO_2 incubator at 37°C with 5% CO_2 concentration, to allow RBCs to sediment. The supernatant was taken into separate conical tubes and then centrifuged at 1500 rpm for 10 min. The resulting cell pellet was resuspended in about 5 ml of plasma. For quality control of the UCB units collected, cultures for bacterial and fungal contamination were sent to the microbiology lab.

Cell counts

TNC counts, CD34 cell count and viability was carried out using flow cytometry (BD FACS Calibur) using the BD CellQuest software (BD Biosciences). For colony forming unit-granulocyte macrophage (CFU-GM) assay, cell sample was prepared by adding 900 µL Iscove's modified Dulbecco's medium to 100 µL of processed cells and 20 µL of 2% fetal bovine serum to achieve ×10 the final concentration(s) required for plating. Commercially available methylcellulose-based stem cell culture media; MethoCult (H4034) was used as culture media. Duplicate cultures were plated in 35 mm culture dishes. These were placed in an incubator maintained at 37°C, 5% CO_2 in the air and >95% humidity for 14 days.

Colonies, which were defined as clusters containing at least 40 cells, were scored after 14 days of culture using an inverted microscope and the average value of duplicate data was used for calculation.

Maternal and neonatal data collection

The age of mother, obstetric history, blood group, medical/surgical history, infectious disease screening status and ultrasound findings of present pregnancy were recorded. Documentation of neonate included date and time of birth, sex of the neonate, birth weight (Bwt), placental weight (Pwt) and gestational age.

Statistical analysis

Data is reported as mean ± standard deviation. Descriptive statistics are presented for each maternal and neonatal factor studied. The Pearson correlation test was used for analysis of continuous variables and un-paired *t*-test was used for comparing means of categorical variables. Multiple regression analysis was carried out to estimate the independent contributions of various maternal, placental and neonatal variables. $P < 0.05$ was taken as statistically significant. SPSS version 17 (SPSS Inc., 233 South Wacker Drive, 11th Floor, Chicago, IL 60606-6412) was used for analysis.

Results

A total of 100 cord blood units were collected from random antenatal cases over a period of 6 months. All the deliveries were normal vaginal deliveries. The details of study subjects and cord blood characteristics are shown in Table 1.

Table 1: Maternal and neonatal parameters studied

Mother	Number* (%)	Mean ± SD	Range
Age (years)	100 (100)	23.63±3.42	18-35
Gestation (weeks)	100 (100)	38.22±1.46	33-41
Pre-term (<37)	9 (9)		
Term (>37)	91 (91)		
Number of previous live births			
0	60 (60)		
1	35 (35)		
≥2	5 (5)		
Gravida status			
Primigravida	44 (44)		
Multigravida			
G2	37 (37)		
G3	12 (12)		
≥G4	7 (7)		
Sex of the baby			
Male	55 (55)		
Female	45 (45)		
Bwt (kg)		2.8±0.39	
≤2.5	19	2.18±0.27	1.5-2.46
≥2.6	81	2.95±0.26	2.5-3.67
Weight of placenta (g)		450.9±30.5	260-615
≤350	9 (9)	307.7±39.29	
351-500	69 (69)	453.9±40.65	
>500	22 (69)	568.3±45.2	
Length of cord (cm)		53.76±4.04	
Volume of cord blood (mL)		83.3±21.2	30-140
Time from collection to processing (min)			
<30	45	28.33±4.52	15-30
>30	55	83.27±38.91	40-180
Positive bacterial cultures	0	—	—
Positive fungal cultures	0	—	—

*Total number of units studied 100; SD: Standard deviation; Bwt: Birth weight

We studied the correlation of various maternal and neonatal factors with the TNC, CD34+ counts and CFU-GM yields [Table 2]. Among the maternal factors, the age of the mother showed a very weak correlation with CD34+ cells ($r = 0.01$, $P = 0.92$), a weak negative correlation with TNC ($r = –0.06$, $P = 0.51$) and poor correlation with CFU-GM ($r = 0.004$, $P = 0.97$). No significant difference was seen in the cell counts between primigravida and multigravida ($P = 0.37$, 0.35 and 0.53 for TNC, CD34+ cells and CFU-GM counts respectively). Term gestations had higher median CD34+ counts when compared to pre-term deliveries, however, it was not significant ($P = 0.38$). Same was the case with CFU-GM counts ($P = 0.28$). The TNC counts between term and pre-term deliveries however showed a significant difference ($P = 0.048$). The volume of cord blood (VCB) collected significantly correlated with the Bwt of the neonate ($P < 0.002$) as well as with TNC ($P < 0.02$), CD34+ ($P < 0.002$) and CFU-GM counts ($P < 0.004$). However, it did not differ significantly with the age of the mother or the period of gestation ($P = 0.39$ and 0.19 respectively). Among the neonatal factors larger babies gave higher cord blood volume and higher TNC, CD34+ and CFU-GM yields. The Bwt of the baby significantly correlated with TNC ($P < 0.00$), CD34+ ($P < 0.00$) and CFU-GM counts ($P < 0.00$). The weight of the placenta showed a positive correlation with all the dependent variables. Pearson's correlation coefficient and *P* values for TNC, CD34+, CFU-GM and VCB collected were $r = 0.27$ and $P = 0.007$, $r = 0.25$ and $P = 0.01$, $r = 0.15$ and $P = 0.11$, $r = 0.47$ and $P = 0.00$ respectively. The sex of the baby and the birth order did not have a significant effect.

The TNC and CD34+ cells showed a moderate correlation which was statistically significant ($r = 0.578$, $P = 0.000$). The volume of blood collected showed a moderate correlation with CD34+ cells ($r = 0.527$, $P = 0.000$) [Figure 1] and a weak correlation with TNC ($r = 0.395$, $P = 0.000$). CFU-GM counts showed a very good correlation with CD34+ cells ($r = 0.920$, $P = 0.000$) [Figure 2] and a moderate correlation with TNC ($r = 0.544$, $P = 0.000$). The

Table 2: Correlation of various maternal and neonatal factors with the TNC, CD34+ counts and CFU-GM yields

Characteristics	Total UCB volume (mL)			CD 34+ cell concentration (×106)			Total nucleated cells (×108)			CFU-GM (×106)		
	Mean ± SD	Range	*P*	Mean ± SD	Range	*P*	Mean ± SD	Range	*P*	Mean ± SD	Range	*P*
Bwt (kg)												
<2500	61.58±17.73	30-105	0.002	1.5±0.77	0.54-3.27	0.0002	3.8±2.55	0.86-11.52	0.0003	0.31±0.2	0.07-0.8	0.007
≥2500	88.4±8.63	50-140		2.86±1.51	0.58-7.5		7.0±3.51	1.15-16.32		0.62±0.48	0.01-2.8	
Total UCB Vol (mL)												
30-100				2.37±1.32	0.54-7.5	0.002	5.99±3.58	0.86-16.32	0.029	0.5±0.41	0.01-2.8	0.004
101-140				3.47±1.79	1.27-7.5		7.89±3.17	1.5-14.35		0.81±0.54	0.29-2.2	
Pwt (g)												
≤450	65±19.2	30-105	0.002	2.4±0.9	0.54-2.90	0.002	3.5±1.9	1.5-7.8	0.02	0.33±0.2	0.06-0.4	0.03
>450	92±20.4	70-140		3.5±1.1	2.52-7.5		7.6±2.8	3.7-15.5		0.61±0.3	0.09-0.8	
Gravida												
Primi	86.41±21.94	30-140	0.19	2.76±1.59	0.54-7.5	0.37	6.77±3.57	0.86-16.32	0.35	0.59±0.51	0.05-2.8	0.53
Multi	80.86±20.4	45-135		2.48±1.41	0.66-6.5		6.09±3.57	1.15-14.64		0.54±0.41	0.01-2.1	
Period of gestation (weeks)												
<37 (pre-term)	74.9±22.3	45-105	0.19	2.21±1.58	0.84-6.1	0.38	4.29±2.33	1.51-8.34	0.048	0.39±0.2	0.08-0.5	0.28
≥37 (term)	82.2±20.9	30-140		2.65±1.48	0.54-7.5		6.23±3.6	0.86-16.32		0.54±0.3	0.1-2.1	
Sex												
Male	82.42±21.45	30-135	0.65	2.64±1.52	0.6-7.5	0.77	6.49±3.26	1.5-14.6	0.8	0.59±0.5	0.05-2.8	0.49
Female	84.3±21.0	45-140		2.55±1.47	0.54-7.5		6.31±3.82	0.86-6.32		0.53±0.39	0.01-2.2	

CFU: Colony forming unit; GM: Granulocytes-macrophage; TNC: Total nucleated cell; VCB: Volume of cord blood; UCB: Umbilical cord blood; SD: Standard deviation; PWt: Placental weight; Bwt: Birth weight

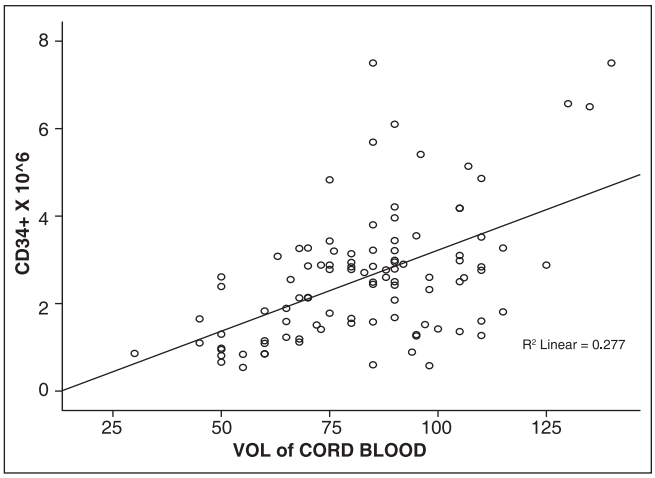

Figure 1: Correlation of CD34+ cells with volume of cord blood collected

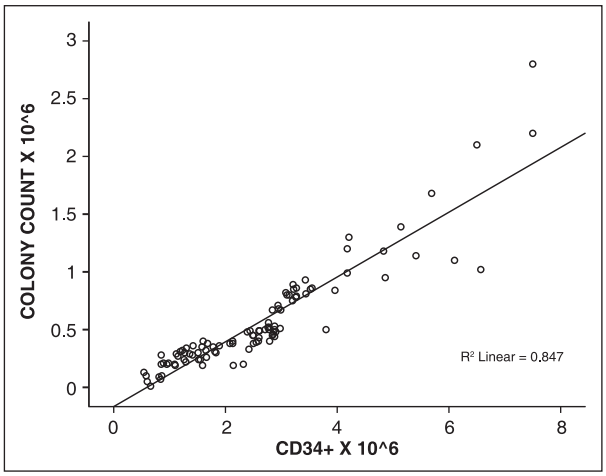

Figure 2: Correlation of colony forming unit-granulocytes-macrophage with CD34+ cells

multivariate linear regression analysis [Table 3] showed that the numbers of TNC and CD34+ cells were influenced by the Pwt and UCB volume whereas the CFU-GM counts were affected by only UCB volume.

All the units collected were sero-negative for the infectious diseases screened. The bacterial and fungal cultures were also negative.

Discussion

Today UCB is an accepted alternative to stem cell transplantation using bone marrow or peripheral blood stem cell, although limitations of TNC and CD34+ cell counts suitable for transplant remain. As the number of cord blood units required to be banked is increasing, it is necessary to improve the quality of the units for cost efficient management of the banks. We studied the maternal and neonatal factors affecting these counts in a study group which included an urban population of Western India.

The maternal age did not affect the TNC, CD34+ cell or CFU-GM counts ($P = 0.92$). This observation was similar to that reported by Ballen *et al.*[11] Our study did not find any significant correlation between the gravida or para status of the female and TNC, CD34+ cell or CFU-GM counts. Though the counts for all the three dependent variables were slightly higher in primigravidas, it was not statistically significant ($P > 0.05$ for all three). A study by Ballen *et al.*[11] showed that primigravida females and those with fewer previous live births produced cord blood units with higher TNC levels. However, Nakagawa *et al.*[12] reported no such association. The possible reason is that babies born in subsequent birth orders are bigger, which may nullify the effect of birth order. In our study, a higher period of gestation was associated with a significantly more TNC count ($P = 0.048$), however the

Table 3: Multivariate regression analysis of TNC count, CD34+ cell counts and CFU-GM counts in selected donor and collection related variables

Variables	TNC count	CD34+ count	CFU-GM count
Age (years)	Negative correlation with increasing maternal age *P*=0.25	No correlation *P*=0.46	No correlation *P*=0.486
Parity	Negative correlation with increasing parity *P*=0.41	Negative correlation with increasing parity *P*=0.44	No correlation *P*=0.464
Sex of neonate	Negative correlation with male sex *P*=0.40	No correlation *P*=0.38	No correlation *P*=0.246
Bwt	Positive correlation with increasing Bwt	Positive correlation with increasing Bwt	No correlation *P*=0.055
Pwt	Positive correlation with increasing Pwt *P*=0.003	Positive correlation with increasing placental weight *P*=0.005	No correlation *P*=0.057
VCB	Positive correlation with greater UCB volume *P*=0.000	Positive correlation with greater UCB volume *P*=0.000	Positive correlation with greater UCB volume *P*=0.000

CFU: Colony forming unit; GM: Granulocytes-macrophage; TNC: Total nucleated cell; VCB: Volume of cord blood; UCB: Umbilical cord blood; PWt: Placental weight; Bwt: Neonatal birth weight

association with VCB collected, CD34+ cells and CFU-GM counts was not significant ($P > 0.05$ for all). Nakagawa *et al.*[12] and Ballen *et al.*[11] stated that the babies of longer gestational age had higher cell counts, but lower CD34+ cell counts and CFU-GM. On the corollary, infants with shorter gestational age had higher CD34+ cells.

The mean VCB collected in our study was 83.3 ml (range: 30-140) which included 22 ml of anticoagulant in the umbilical cord bag and the mean TNC was 6.3×10^8. It is similar to collections reported by Nakagawa *et al.*[12] and Sparrow *et al.*[13] (60.8 ml and 63 ml excluding the anticoagulant, TNC 5.86×10^8 and 12.1×10^8 respectively). However, in studies done on western population Donaldson *et al.*[14] collected a mean volume of 93.5 ml with a mean TNC of 13.1×10^8. Cairo and Wagner[15] reported a mean collection of 103 ± 49 ml with an additional 8 ± 85 ml collected by needle aspiration of the umbilical vessels. The median TNC was 1.4×10^9. These values are higher as compared to our study thus suggesting that a lower cut-off should be set for the qualifying UCB volume and TNC counts for units to be cryopreserved in our country. Our study showed a significant correlation of UCB volume with the Bwt of the baby. Other parameters including the gravida status of the female and the sex of the baby did not significantly affect the UCB volume collected. Positive correlation between neonatal Bwt and the UCB volume was shown by Jan *et al.*[5] Chandra *et al.*[16] and Solves *et al.*[17] which corroborated with our study. Our study showed a significant correlation between the UCB volume and TNC, CD34+ cells and CFU-GM counts ($P < 0.05$ for all). This was similar to that reported by many other researchers.[12,16]

Among the neonatal parameters analyzed, our study found that a higher Bwt was associated with a significantly higher CD34+ cell count ($P = 0.00025$) as well as higher TNC counts ($P = 0.00031$) and CFU-GM ($P = 0.007$). A heavier baby was associated with a larger and heavier placenta ($P = 0.03$), which in turn was associated with a higher VCB collected and greater cell counts. Jan *et al.*[5] and Wen *et al.*[6] reported a significant positive correlation between Bwt of the neonate and TNC and CD34+ cell yields.

Other investigators have reported a positive correlation of Bwt with each of the three dependent variables; TNC, CD34+ cells and CFU-GM units.[11,12,17] Choong *et al.*[18] reported an association of Bwt with TNC only. Neonatal sex has also been reported to have an effect on the above mentioned parameters. Our study found a slightly higher CD34+ count in male babies; however, it was not statistically significant. Jan *et al.*[5] in their study have reported that cord blood collected from male babies had higher CD34+ cells and blood volume but low TNC counts. A study by Aroviita *et al.*[19] found significantly more CD34+ cells and CFU-mixed cell counts in male babies. However, the CFU-GM concentrations between male and female neonates did not show a significant difference.[19] In our study, the median TNC count was slightly greater in female babies; however, it was not statistically significant. Ballen *et al.*[11] and Nakagawa *et al.*[12] reported that female sex was associated with a higher TNC count; however no such association was seen with CFU-GM counts. The higher nucleated cell counts in female babies could be due to a higher neutrophil concentration found in them. Our results corroborated with the findings of M-Reboredo *et al.*[20] who found no association between infant sex and cell counts.

In summary, many factors have been proposed for donor selection to ensure collection of cord blood units with optimal cell counts. Different studies have shown that both TNC and CD34+ cells correlate with transplant outcome and hence are the ultimate measure of product quality. CD34+ cell count has been shown to predict the hematopoietic potential of a cord blood unit better than TNC count due to its better correlation with CFU content.[21] However, Kurtzberg *et al.*[2] stated that the number of nucleated cells transfused per kilogram of body weight correlated with the rate of myeloid engraftment and that cell dose may be a more important indicator of engraftment than CD34+ cell or CFU-GM values. Given the variability in CD34+ cell count quantification between institutions and the inconsistency in CFU content measurement, TNC count becomes an important outcome variable when a standard is required to be set; that might be utilized by many banks. The volume of collected UCB units may also be considered as an indicator of product quality, as in our study it correlated with all three outcome variables; TNC, CD34+ cells and CFU-GM units, even though it has not been shown to correlate directly with transplant outcome. Proper donor selection may predict better product volume and hence make the system more cost-effective by increasing the probability of processing mostly those units that are more likely to be banked. Cord blood banks with limited resources can focus on variables that produce products with better volume and TNC counts, which in this study were higher Bwt of the baby and a heavier placenta. These can be easily measured without the need of sophisticated equipment's like flow cytometer and be utilized in public cord blood banks which do not have a private funding. Hence, we can cost-effectively increase our cord blood donor database and subsequently the unrelated cord blood transplants performed in the country.

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