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Maternal and Neonatal Variables Affecting CD34+ Cell Count in the Umbilical Cord Blood

Satya Prakash[1,2], Ashish Jain[1], Deepak Pahwa[1], Jaswinder Kaur Kalra[3], Rattiram Sharma[1]

Abstract:

INTRODUCTION: Ease of collection, ready availability and lower graft-versus-host disease compared to peripheral blood stem cell favored umbilical cord blood stem cell transplantation.

AIM: To assess the maternal and neonatal predictor affecting total nucleated and CD34+ cell count in cord blood collections.

METHOD: A total of 200 Cord blood units were collected under aseptic conditions. Volume reduction was made by 6% hydroxyethyl starch followed by upright centrifugation of cord blood units to obtain a cellular pellet. Differential cell counts were done through hematology analyzer, viability testing by trypan blue exclusion test and percentage CD34, and CD45 estimation by flow cytometry. Two samples were HBsAg positive and not included in the study.

RESULT: Total CD34 positive cell counts were more in cord blood units collected from younger age mother. Higher birth weight of newborns yielded a larger volume of cord blood unit and higher absolute TNC and CD34+ cell counts. The placental weight was positively correlated with the volume of cord blood collected, birth weight of new born, and total nucleated cell count but no correlation was observed with absolute CD34+ cell counts. The gravida status, gestational age and method of delivery were not significant with total nucleated cell (TNC), mononuclear cell (MNC), and CD34+ counts.

CONCLUSION: Maternal age, birth weight, placental weight, and volume of CBUs were the most important predictor of getting increased nucleated and CD34+ cells in cord blood. Moreover, CBUs collected from pre-term deliveries were shown to have an approximately equal absolute number of CD34+ cells.

Keywords:

Maternal age, preterm birth, stem cell transplantation, umbilical cord blood

Departments of [1]Transfusion Medicine and [3]Obstetrics and Gynaecology, Postgraduate Institute of Medical Education and Research, Chandigarh, [2]Department of Transfusion Medicine, All India Institute of Medical Sciences, Bhubaneswar, Odisha, India

Address for correspondence:
Dr. Rattiram Sharma, Department of Transfusion Medicine, Postgraduate Institute of Medical Education and Research, Chandigarh, India.
E-mail: rrsdoc@hotmail.com

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Introduction

Hematopoietic stem cells (HSCs) collected from umbilical cord blood (UCB) were recognized as a valuable alternative source for transplantation purposes in various hemato-oncological disorders. The first successful cord blood transplantation in a patient of Fanconi anemia was performed in 1988 by E. Gluckman and colleagues; since then, UCB was increasingly being used for HSC transplantation.[1] Easy availability, lesser incidence of graft versus host disease, lesser human leukocyte antigen matching requirement, and no risk to donor paved the way for the use of cord blood as an alternative source of stem cell for transplantation.[2] However, a limited number of cells were obtained in single cord blood collection, which may be sufficient for transplantation in children but less effective in adults and leads to delayed engraftment as compared to peripheral blood stem cells and bone marrow.[3] Expansion of HSCs *ex vivo* with various strategies is still under development and needs significantly

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increased additional cost.[4,5] Thus, before creating a large inventory of these products, studies must focus on defining appropriate collection criteria and optimum processing techniques to obtain a product with desired cord blood unit (CBUs) cellular counts. This study aimed to assess various maternal and neonatal factors affecting CD34+ stem cell yield in cord blood collections from all live-birth deliveries.

Methods

This cross-sectional descriptive study randomly included 200 cord blood collections (*ex utero*) from all live-birth deliveries conducted in the clean labor room after obtaining informed consent. After the delivery, the cord was clamped and cleaned with 70% isopropyl alcohol and 10% povidone-iodine. CBUs were collected in a 100 ml collection bag (Mitra Industries (P) Ltd. India), having 14 ml Citrate Phosphate Dextrose Adenine-1 as an anticoagulant maintaining all aseptic precaution.

All CBUs units were processed under a biosafety cabinet (Euroclone, safe flow 1.2, Italy) within 24 h of collection. Volume reduction was made with 6% hydroxyethyl starch (HES, Fresenius India Pvt. Limited) mixed in a ratio of 1:5 (HES: UCB in ml). Upright centrifugation of the product was done at 30×g (300 rpm) in a refrigerated centrifuge (Heraeus Cryofuge, Thermo Fisher, USA) for 10 min at 4°C. The supernatant was collected into a transfer bag (J. Mitra Pvt. Ind. Ltd) with the help of a manual plasma expresser (Terumo Penpol Pvt. Limited, India). Upright centrifugation of the remaining product in the expressed bag was done at 450×g (1391 rpm) for 10 min to obtain cellular pellet and cell poor plasma. Leukocyte's poor plasma was removed in a satellite bag leaving behind a cellular button in the primary bag. The clamp was applied between the primary bag and plasma bag. The cellular button with little plasma was mixed properly. Two CBUs found positive for hepatitis B virus surface antigen were excluded from the study.

The total nucleated cell (TNC) count (/µl) and mono nuclear cell (MNC) count (/µl) were estimated through 3-part differential hematology analyzer KX21, Sysmex Corporation, Japan. The absolute CD34+ and CD45+ cellular count was estimated from percentage of CD34 and CD45 available after flow cytometry (Dual platform) based on ISHAGE protocol. The monoclonal antibodies, anti-CD34 conjugated with phycoerythrin (PE) and anti-CD45 (white blood cell common antigen) conjugated with fluorescein isothiocyanate, were used to stain the cells for flow cytometric analysis over a flow cytometer (BD FACS Aria™ II, Asia Pacific). The project was approved by the Institutional Committee on Stem Cell Research and Therapy with reference no: IC-SCRT-35/3675.

Statistical analysis

All the parameters assessed in this study were taken as mean ± standard deviation (SD). Descriptive statistics were used to measure the central tendency and dispersion. The comparisons within the same group, as well as comparison with the baseline parameters, were done by the Mann–Whitney test. For analysis of the factors affecting the stem cell yield, Pearson's product-moment correlation was used. $P < 0.05$ was taken as significant. All data were stored in Microsoft excel 2016, and analyses were performed with the statistical software XLSTAT version 2019.3.1 build 60484 (Boston, USA) for Windows 10. Boxplot was generated using statistical package R version 3.5.3.

Results

Study population

A total of 198 CBUs were analyzed from single birth live deliveries, including 147 by the normal vaginal route and 51 with a lower segment cesarean section to assess the factors affecting stem cell yield. Multigravida female contributes approximately two-third of cord blood collected, whereas only 75 were from primigravida female. Furthermore, 67% of CBUs were collected from the maternal age of more than 25 years, with a range of 19–45 years. Gestational age ranges from 28 to 41 weeks, with a mean of 36.72 ± 2.87 weeks. The majority of the newborn were term-born, and only 66 were delivered before 37 completed weeks of gestation. The proportion of Random CBUs collected was predominantly from male newborns with a male-to-female ratio of 1.5:1 [Tables 1 and 2].

Analysis of cord blood cellular parameters

The mean ± SD of the volume of CBUs collected was 72.77 ± 23.76 ml with a range of 40–150 ml (interquartile range: 50–90 ml) and the mean ± SD of absolute TNC was $6.01 \pm 3.59 \times 10^8$ with a range of 0.56×10^8 to 19.7×10^8 (interquartile range of 3.25×10^8 –8.4×10^8). Percentage of CD34+ cell ranged from 0.006% to 1.8% with a mean of 0.32% ± 0.25% and mean ± SD of absolute CD34+ cell counts was $1.91 \times 10^6 \pm 1.76 \times 10^6$ with a range of 1.53×10^4 –1.14×10^7 [Table 3].

Correlation characteristics

The evaluation and correlation of various maternal, neonatal, and collection-related variables were assessed in relation to absolute TNC, MNC, and CD34+ cell yield, as shown in Tables 1 and 2. Among maternal variables, maternal age of <25 years showed significantly higher absolute CD34+ cell counts with $P = 0.004$ [Figure 1]. However, the absolute number of TNC was not significant in these two groups, as shown in Table 1. Placental weight of more than 450 g yielded significantly increased mean volume with $P = 0.000$ and the

Table 1: Comparison of total nucleated cell count and total CD34+ cellular count with maternal variables

Variable	Number (*n*=198)	UCB volume (ml) (mean±SD)	*P*	TNC count (×10⁶/unit) (mean±SD)	*P*	Absolute CD34+ cell count (×10⁴/unit) (mean±SD)	*P*
Primi	75	71.66±24.74	0.472	565.88±366.83	0.183	180.91±203.76	0.119
Multi	123	73.45±23.32		623.52±356.61		198.15±159.33	
NVD	147	76.08±24.08	0.001	635.50±353.96	0.004	198.23±176.01	0.255
LSCS	51	63.23±20.44		504.22±365.60		172.57±180.86	
Placental weight (≤450 g)	113	66.19±20.95	0.000	554.04±327.64	0.054	198.12±190.25	0.596
Placental weight (>450 g)	85	81.52±24.70		665.02±393.51		182.98±158.80	
Age (<25 years)	60	75.75±22.75	0.181	597.50±339.49	0.842	224.83±153.03	0.004
Age (≥25 years)	138	71.48±24.24		603.50±370.70		177.18±185.35	

UCB=Umbilical cord blood; SD=Standard deviation; TNC=Total nucleated cell; NVD=Normal vaginal delivery; LSCS=Lower segment cesarean section

Table 2: Comparison of total nucleated cell count and total CD34+ cellular count with neonatal variables

Variable	Number (*n*=198)	UCB volume (ml) (mean±SD)	*P*	TNC count (×10⁶/unit) (mean±SD)	*P*	Absolute CD34+ cell count (×10⁴/unit) (mean±SD)	*P*
Gender							
Male	119	71.42±23.42	0.361	606.82±354.57	0.586	190.01±166.99	0.749
Female	79	74.81±24.42		593.94±371.84		194.06±192.55	
Gestational week							
Term	132	76.97±23.73	0.000	641.10±374.93	0.026	206.57±195.40	0.226
Preterm	66	64.39±21.86		522.85±318.59		161.73±129.69	
Birth weight (kg)							
≤2.5	96	64.37±20.51	0.000	490.15±286.93	0.000	159.24±132.77	0.036
>2.5	102	80.68±24.10		706.66±391.60		222.10±206.65	
CBU volume (ml)							
≤60	79	49.68±7.89	0.000	382.20±221.79	0.000	121.75±148.17	0.000
>60	119	88.10±17.67		747.39±361.95		238.01±180.14	

UCB=Umbilical cord blood; SD=Standard deviation; TNC=Total nucleated cell; CBU=Cord blood units

Table 3: Descriptive details

Parameters (*n*=198)	Mean±SD	Range
Maternal age (years)	26.85±4.22	19-45
Placental weight (g)	450.05±56.50	300-600
Birth weight (kg)	2.49±0.65	0.81-4.21
Volume of CBU (ml)	72.77±23.76	40-150
Absolute TNC count (×1,000,000)	601.68±359.77	56-1971
Absolute CD34+ cell count (×10,000)	191.62±176.72	1.53-1144.80

SD=Standard deviation; TNC=Total nucleated cell; CBU=Cord blood units

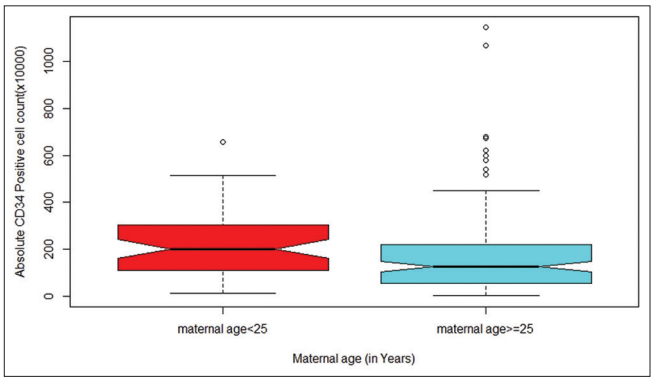

Figure 1: Correlation of maternal age with absolute CD34+ cell count

increasing trend toward absolute TNC count with $P = 0.054$ [Table 1]. CBUs collected from normal vaginal deliveries and deliveries by cesarean section did not show any significant correlation in terms of absolute CD34+ cell count. However, volume and absolute TNC count of cord blood collected *ex utero* was significantly greater in normal vaginal delivery as compared to delivery by cesarean section, as shown in Table 1. The volume of the cord blood collected, and its cellular profile did not vary significantly in relation to gravida status, as shown in Table 1.

Among the neonatal factors, the birth weight of newborns was positively correlated with the volume of CBUs collected, and an absolute number of CD34+ cells, as shown in Table 2 and Figure 2. Newborn with a birth weight of ≥2.5 kg yielded larger CBU volume and had significantly increased number of TNC and absolute CD34+ cell counts than the newborn with a birth weight of <2.5Kg [Table 2]. Moreover, absolute CD34+ cell counts were found to be significantly increased with the volume of cord blood more than 60 ml [Figure 3].

CBUs collected from preterm gestation showed a comparable mean absolute CD34+ cell counts to term gestation despite the significantly lower volume and lower absolute TNC in cord blood collected from preterm deliveries [Table 2], implying thereby that preterm CBUs had a higher percentage of CD34+ cellular concentration

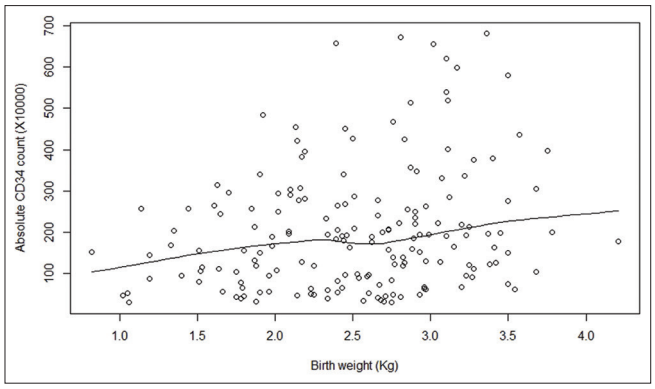

Figure 2: Correlation of birth weight of newborn with absolute CD34+ cell count

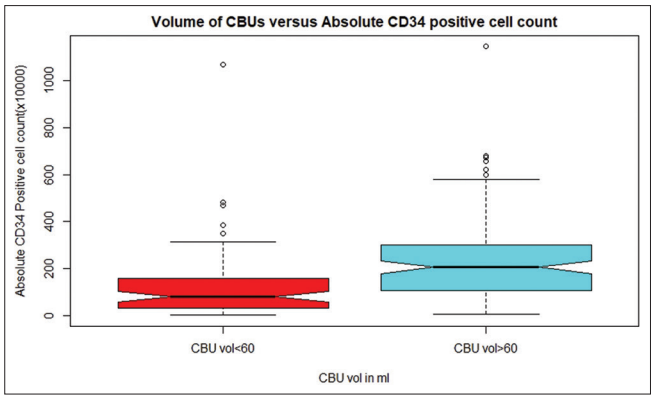

Figure 3: Correlation of volume of cord blood units with absolute CD34+ cell count

as compared to units collected from term babies. The gender of the newborn did not have any significant relation with all quality control parameters, as shown in Table 2.

Discussion

Appropriate selection and optimization of cord blood collection and processing techniques are being employed to increase the efficacy and reduce the costs of cord blood transplantation in various transplant settings in the world. We tried to assess maternal and neonatal factors affecting the CBU quality, especially in terms of cellular counts. We found that younger maternal age and normal vaginal delivery along with higher birth weight (>2.5 kg) and heavier placenta (>450 gram) yielded better cord blood volume and cellular profile. Moreover, the absolute TNC, MNC, and CD34+ cell counts among term and preterm were nearly comparable.

The correlation of stem cell yield in the form of total CD34+ cell count was found to be significantly increased with maternal age of <25 years. However, maternal age has no correlation with TNC count. Our study hereby supports the result of studies showing a statistically significant dependence between the maternal age and the concentration of CD34+ cells in the UCB. The older the women from whom UCB was collected, the lower the mean concentration of HSCs.[6-9] However, several studies on the assessment of the hematopoietic potential of CBU did not find any correlation with maternal age.[10,11] Reduction in the developmental stage to blastocyst was seen in oocyte obtained from older maternal age due to mitochondrial dysfunction and oocyte incompetence due to the shortening of telomere, cohesion dysfunction, and spindle instability. Furthermore, maternal age is a strong predictor of the success of *in vitro* fertilization.[12-14] These may be the indirect evidence to support the above finding, but the molecular and biochemical mechanism behind this remains to be elucidated.

The parity status of the mother in our study had not shown any significance in terms of various cord blood parameters [Table 1]. The study of Ballen *et al*. showed that their primigravida female and women with fewer previous live birth produced CBUs with higher cellular yield with a decline of 3% in volume, 12% decrease in total nucleated count, and17% decrease in CD34+ cell counts. However, a subsequent study by Nakagawa *et al*. did not find any such association.[9,10] The possible explanation for this could be babies born in subsequent birth order are bigger, which may nullify the effect of birth order as observed by Ballen *et al*.[10]

The placental weight had been found to be a major contributing factor in volume and other laboratory parameters of CBUs collected. Although the mean placental weight in our study is lower in comparison to other studies quoted below, placental weight of more than 450 g had a significant positive correlation with the volume of CBU collected and had a positive trend toward absolute TNC count but not with absolute CD34+ cell counts, as shown in Table 2. Askari *et al*. reported similar observations with the placental weight of >500 g with higher cord blood volume, TNC counts, and CD34+ cell counts.[11] Another study by Donaldson *et al*. also revealed that placental weight with a mean of 602.5 g significantly affected cord blood volume and cellular profile of the product.[15] Thus, placental weight is one of the most important variables affecting the volume and cellular profile of CBUs, and placental weight is also positively correlated with the birth weight of a newborn. A heavier baby was associated with a heavier placenta, which, in turn, resulted in a large volume of cord blood collected and subsequently higher cell counts.[10] Thus, the birth weight of a newborn should always be taken into consideration for the selection of cord blood collection.

The volume of cord blood collected was significantly positively correlated with birth weight, placental weight, and gestation period in our study, as shown in

Tables 1 and 2. Most of the study has shown a strong correlation of cord blood volume with a birth weight of newborn and placental weight.[9-11,15] The total gestational period also had a similar effect on UCB volume, and there is no doubt regarding increased birthweight and placental weight with progressive gestational age and thus subsequently increased volume of cord blood collected.[16] Mode of delivery had an impact on UCB volume, and many studies quote increased volume with cesarean delivery.[11,17,18] However, UCB volume in our study was significantly high in normal vaginal delivery as compared to cesarean delivery, and a similar finding was also observed in a study by Al-Sweedan *et al*.[19] This difference may be attributed due to the difference in the method of collection and delay in clamping the cord as *in utero* collection during cesarean delivery has shown the increased volume of cord blood collected.[17] Significantly increased UCB volume collected had been observed in normal vaginal delivery, but the mode of delivery had not affected the absolute CD34+ cell count, and a similar result was reported earlier.[20]

Several studies had shown a positive correlation between females born with higher TNC counts.[6-9,11] However, another study had also quoted higher CD34+ cell counts in UCB collected from male born. A recently published review article concluded that gender alone could not be a guide toward selection for the collection of CBUs. Our study also supports this view as there was no correlation of sex of the baby with cord blood volume, MNC, and absolute CD34+ cell counts and is similar to the observation by Ballen *et al*. and Donaldson *et al*.[10,15,16]

Absolute TNC, MNC, and CD34+ cell counts were comparable in both term and preterm born babies. However, the volume of CBUs collected was significantly less in preterm and more in the full-term born baby [Figure 1]. From this point of view, although the volume of cord blood collected in the preterm born baby was less, absolute cell counts were found to be comparable. The higher percentage of absolute CD34+ cell count in cord blood collected from preterm born was also supported by Podesta M. and a few more authors.[21-27] This indicates that hematopoietic progenitors from preterm cord blood may be suitable for transplantation. Further animal studies have done previously shown impaired homing of HSCs from preterm born in spite of higher proliferative potential, but this problem may be sorted out by direct intraosseous delivery of these potential HSCs as evidenced by a study of Bonifazi *et al*. showing early immune reconstitution.[28,29]

Predicting maternal and neonatal variables in cord blood collection is important and this study better depicts the correlation of these variables with absolute TNC, MNC, and CD34+ cellular counts. However, this study was carried out on a small number of CBUs collected *ex utero* after delivery of the placenta. Further study with a greater number of units collected *in utero*/*ex utero* is required to assess collection-related variables to define appropriate collection criteria.

Conclusion

This study concludes that younger maternal age and normal vaginal delivery along with higher birth weight (>2.5 kg) and heavier placenta (>450 gram) yielded better cord blood volume and cellular profile. The absolute TNC, MNC, and CD34+ cell counts among term and preterm were nearly comparable, and thus, cord blood collected from preterm delivery may be considered for clinical use. Consideration of these maternal and neonatal factors definitely aids in selecting CBUs for potential use in transplantation.

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Conflicts of interest
There are no conflicts of interest.

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