The Impact of Obstetric Parameters on Haematopoietic Stem Cells Extracted from the Umbilical Cord Blood in the Chinese Population

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Objective: Human umbilical cord blood has been used as an alternative source of haematopoietic stem cells (HSC). However, the amount of donor blood and cell content may not be sufficient for engraftment, especially in adult recipients. We investigated the impact of obstetric parameters on cord blood stem cell content so that the quantity and quality of cord blood stem cell can be optimised.
Methods: In this prospective study, cord blood samples were obtained from Chinese women after delivery by elective Caesarean section in two regional hospitals in Hong Kong from June 2011 to June 2012. HSC were extracted from cord blood. The weight of cord blood, total nucleated cells (TNC), total HSC, TNC/gram, and HSC/gram were recorded. Statistical analyses were performed to study the correlation between quantity of HSC and obstetric parameters including maternal age, parity, sex of newborn, birth weight, and gestational age.
Results: We found a positive correlation between total HSC obtained and the birth weight of the newborn (Pearson correlation coefficient, 0.553; p=0.049). There was no significant correlation between total HSC and fetal sex, parity, maternal age, and gestational age at delivery.
Conclusion: Birth weight of the newborn is correlated with the quantity of stem cells obtained from cord blood. This finding should be taken into consideration when selecting cord blood units for potential transplantation.
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Introduction

Stem cells are multipotent and have been used to treat a multitude of diseases, conditions, and disabilities including haematological malignancies, immune deficiencies, and inherited metabolic disorders[1]. In 1990, the first haematopoietic stem cell (HSC) transplantation was performed at the Bone Marrow Transplant Centre, Queen Mary Hospital in Hong Kong. Up to the end of 2008, over 2000 transplants were performed in Hong Kong. The sources of stem cells included peripheral blood, bone marrow, and umbilical cord blood (UCB), in that order of preference[2].

UCB is recognised as an alternative source of HSC. UCB transplantation (UCBT) has been widely used in paediatric and adult patients since 1988 when it was first used to treat Fanconi's anaemia[3]. In Hong Kong, UCBT was first performed in 1994 for a 3-year-old girl with beta-thalassaemia major using stem cells from a human leukocyte antigen–matched sibling[2]. The first unrelated UCBT was performed in 1998 in a 9-year-old boy with acute lymphoblastic leukaemia[2]. Until the end of 2008, a total of 31 UCBTs were performed, mainly in paediatric and adolescent patients; only four UCBTs were performed in adults[2].

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Over 400,000 cord blood units are now stored for use in more than 100 quality-controlled public international cord blood banks[4]. National and international networks, such as the International NetCord Foundation (http://www.netcord.org) and AABB (formerly known as the American Association of Blood Banks)[5], have agreed upon standards and protocols for collection, processing, and handling of stored blood. These have also culminated in establishment of registries and accreditation of practices. Two cord blood banking options are available, private (or family) and public. Private cord blood banks obtain blood samples and store the cord blood for individual use by families. Cord blood samples stored in private banks are not searchable or available to the public[5,6]. A major criticism of private banks is that the infant for whom the blood is banked will likely never need the stored blood and the blood is not accessible to the general public for use. According to Ballen et al[7], there is an estimated 0.04-0.005% chance that an individual will develop a disease treatable with their own stored cord blood by 21 years of age using a haematopoietic transplant approach.

The major limitations of UCBT are the availability of only a single unit of UCB and the low cell dose in the UCB unit; these are also the major factors affecting the outcomes of UCBT. A low cell dose in the UCB can limit its use in UCBT, particularly in adults and adolescents who require larger volume for transplantation[8].

To improve the quantity and quality of UCB units, donor selection has been proposed[9-11]. Studies have found that obstetric factors such as birth weight, weight of placenta, sex of newborn, and mode of collection are correlated with UCB volume and cell count[12-15]. In addition, improvements in collection strategies have been proposed for collecting good-quality UCB[16,17]. The expansion of cord blood cells is also being studied[18].

This study aimed to identify the obstetric parameters which can affect HSC content in the UCB collected from patients in our local hospitals. These factors may help to identify deliveries that are likely to yield UCB of suitable volumes and quality. The cost of collecting, transferring, and storing unsuitable UCB can be avoided by appropriate selection of donors.

Methods

Recruitment

The study protocol was approved by the Hospital Authority Kowloon West Cluster Ethics Committee. Pregnant women who had registered at the Kwong Wah Hospital and Princess Margaret Hospital, Hong Kong, for elective Caesarean section were recruited from June 2011 to June 2012. They were recruited on the day of admission, which was one day before the operation. Informed consent for cord blood collection was obtained by attending obstetricians who obtained consent for Caesarean section. Women who were of non-Chinese ethnicity, with gestational age of <38 weeks, multiple pregnancies, liver disease, malaria, sexually transmitted disease, or a history of heritable immunodeficiency or coagulopathy were excluded. Cases with incomplete or abnormal placenta, or with clotted cord blood were also excluded.

Obstetric Data

Patient information and medical history were collected during recruitment of subjects by attending obstetricians. These included maternal age, parity, and presence of maternal disease. The details of delivery including weight of placenta, gender of newborn, birth weight, and gestational age were also collected after delivery. A data collection form was completed for each subject recruited.

Collection of Cord Blood

The standard labour ward protocol of the hospital was followed for the process of delivery. Before delivery of placenta, a stand with a retort ring was placed on the bench and covered with a surgical drape. A sieve was placed on top of the retort ring and also covered with surgical drape. An opening was made in the middle of the surgical drape for the umbilical cord to pass through. The placenta was taken away from the operation theatre immediately after delivery, and an umbilical cord clamp was used to prevent further loss of cord blood. The placenta was placed on the sieve and the umbilical cord passed through the opening on the surgical drape. The surface of umbilical cord was disinfected with povidone-iodine swab. A needle was inserted into the umbilical vein near the end of umbilical cord in order to collect cord blood. Then, the collection bag was lowered and the clamp on the tubing was opened so that the blood was drawn into the bag by gravity. Once the blood flow had stopped, the clamp was closed. The collection bag was gently shaken to mix the cord blood with the anticoagulant. Then, the cord blood collection bag was put into a biohazard zipper bag and transported to the laboratory within 1 hour. The cord blood was kept at 4°C during transportation.

Haematopoietic Stem Cell Isolation from Cord Blood

The cord blood collection bag was transferred into a biosafety cabinet after disinfecting the surface of the collection bag. The cord blood was transferred into a

50 mL centrifuge tube and diluted with phosphate buffered saline (PBS) in a 1:1 ratio. Then, 15 mL Ficoll-Paque Plus solution (GE Healthcare, Piscataway [NJ], US) was added into new centrifuge tubes, and slowly layered with diluted cord blood on top. Tubes were centrifuged at 400 g for 30 minutes with the brake off. After centrifugation, the topmost plasma was carefully aspirated with a pasteur pipette and discarded. The layer of lymphocytes was aspirated into new centrifuge tubes; the cell suspension was then diluted with PBS in a 1:3 ratio. The cell suspension was further centrifuged at 90 g for 10 minutes with the brake on. The cell pellet was washed once with PBS, and re-suspended in a desired volume for further analysis.

Characterisation of Haematopoietic Stem Cells

The comparative stem cell properties of cord HSCs were evaluated phenotypically by flow cytometry, and functionally in vitro by colony-forming unit (CFU) assay. The quantity of HSCs was tested by estimating the number and percentage of CD34 positive cells.

Statistical Analysis

The data were analysed using the Statistical Package for the Social Sciences (SPSS/PC Version 19.0, SPSS Inc., Chicago [IL], US). Descriptive statistics were presented for each obstetric parameter. Mann-Whitney *U* test was used to compare the difference of medians between each group. Patients were divided into nulliparous and multiparous groups for analysis of the effect of parity. Correlation between data points was evaluated by the Pearson Chi-square test (categorical data) unless otherwise specified. Linear regression was used to investigate the relationship between two continuous variables. A p value of <0.05 was considered statistically significant.

Results

Fourteen women were recruited to participate in the study. Four of them were excluded because their cord blood had clotted. The demographic data of the 10 women included in the study are presented in Table 1. The mean ± standard deviation (SD) age of women was 31.9 ± 4.5 (range, 23-39) years. The mean gestational age was 39.0 ± 0.9 (range, 38-41) weeks. Overall, four women were nulliparous, and all women delivered by Caesarean section. The mean birth weight of the newborn was 3.4 ± 0.4 (range, 2.98-4.24) kg. Five of these 10 newborns were male. All 14 women were Chinese. There was one case of impaired glucose tolerance. The volume of cord blood collected from each case is shown in Table 2. The phenotypic characterisation of HSC was represented by the percentage of CD34 marker on the cell surface.

Effects of Maternal Parameters

In our analysis, there was no significant difference in the median (interquartile range [IQR]) values of total nucleated cells (TNC)/gram, as well as HSC/gram obtained from nulliparous and multiparous women (Table 3).

Univariate analyses found that TNC/gram and HSC/gram obtained were not independently correlated with maternal age and gestational age. Similarly, total TNC and total HSC obtained were also not correlated with these two variables (Table 4). As there was only one case of impaired glucose tolerance, this variable was not used for group comparison.

Table 1. Maternal and fetal information

Case No.	Maternal information			Fetal information		
	Age (years)	Parity	Maternal disease	Sex	Weight (kg)	Gestational age (weeks)
1	36	1	Nil	F	3.34	39 1/7
2	23	1	Nil	M	2.98	39 2/7
3	33	0	Nil	F	3.10	38 4/7
4	29	0	Nil	M	3.19	38 4/7
5	30	0	Nil	M	3.16	38 5/7
6	35	1	Nil	M	3.63	39 2/7
7	31	2	Nil	F	4.07	41 1/7
8	39	2	Nil	M	4.24	39 2/7
9	30	0	Impaired glucose tolerance	F	3.42	38
10	33	1	Nil	F	3.06	38 1/7

Abbreviations: F = female; M = male

Table 2. Volume of cord blood and characteristics of haematopoietic stem cells

Case No.	Volume of cord blood (mL)	Proportion of CD34+ cell (%)
1	31.01	0.322
2	21.50	1.067
3	49.17	0.591
4	65.47	0.358
5	7.00	0.784
6	26.86	0.596
7	18.00	0.634
8	89.48	0.580
9	23.00	0.191
10	28.00	1.734

Table 3. Comparison of haematopoietic stem cells (HSC) and total nucleated cells (TNC) by parity and gender

Group	Median (interquartile range)	p Value
TNC/gram		0.914
Primiparous	4.7 (3.2-9.4) x 10^6	
Multiparous	4.7 (2.6-7.0) x 10^6	
HSC/gram		1
Primiparous	3.1 (1.1-3.7) x 10^4	
Multiparous	3.3,(1.3-4.3) x 10^4	
TNC/gram		0.31
Male newborn	4.3 (1.8-5.5) x 10^6	
Female newborn	5.6 (3.3-11.0) x 10^6	
HSC/gram		0.095
Male newborn	1.4 (0.8-3.4) x 10^4	
Female newborn	3.5 (3.0-5.2) x 10^4	

Effects of Perinatal Parameters

The median (IQR) TNC/gram obtained from female and male babies were 4.3 (1.8-5.5) x 10^6 and 5.6 (3.3-11.0) x 10^6, respectively. The median (IQR) HSC/gram obtained from female and male babies were 1.4 (0.8-3.4) x 10^4 and 3.5 (3.0-5.2) x 10^4, respectively (Table 3). There was no significant difference in TNC/gram or HSC/gram obtained from male and female babies. The data of birth weight and total HSC are shown in Table 5. Univariate analysis showed a positive correlation between total HSC obtained and the birth weight of the newborn (Pearson correlation coefficient, 0.553, p=0.049; Table 6 and Figure). However, there was no significant correlation between birth weight and HSC/gram or the weight of cord blood (Pearson correlation coefficient, 0.536 and 0.374, respectively; p>0.05).

Discussion

UCB is an effective alternative source of HSC for stem cell transplantation. The main drawbacks of cord blood are the small volume and the low number of cells in the unit, making it unsuitable for transplantation. Donor selection has been studied to improve the collection of UCB units. The cost of collecting, transferring, and storing unsuitable UCB can be avoided by appropriate selection of donors. However, local data in our population are lacking. We thus analysed the TNC content of the UCB collected from patients in our local hospitals.

Some previous studies[13,19-22] have reported that maternal age affected neither TNC nor CD34+ cell counts. Our data are consistent with these findings; we did not find any correlation between maternal age and TNC. Moreover, there was no correlation between gestational age and TNC. However, some authors did find a correlation between TNC and gestational age. Ballen et al[13] studied cord blood obtained from babies delivered at 37 to 42 weeks' gestation and found that gestational age was positively correlated

Table 4. Correlation between haematopoietic stem cells (HSC) and total nucleated cells (TNC) obtained and maternal and gestational age

Variable	Dependent variable	Pearson correlation coefficient	Dependent variable	Pearson correlation coefficient	p Value
Maternal age	TNC/gram	0.365	Total TNC	0.444	NS
	HSC/gram	0.265	Total HSC	0.496	NS
Gestational age (days)	TNC/gram	0.187	Total TNC	0.011	NS
	HSC/gram	0.380	Total HSC	0.078	NS

Abbreviation: NS = non-significant

with TNC. Also, Solves et al[23] studied cord blood obtained from babies delivered at 25 to 42 weeks' gestation and found that gestational age correlated positively with TNC.

Table 5. Birth weight of newborns and haematopoietic stem cells (HSC) obtained

Birth weight (kg)	Total HSC obtained in umbilical cord blood unit
3.34	441,280
2.98	764,292
3.1	1,772,409
3.19	2,534,640
3.16	195,216
3.63	917,840
4.07	602,300
4.24	6,148,000
3.42	140,268
3.06	282,545

Table 6. Correlation between birth weight of newborn and maternal / cord blood parameters

Variable	Pearson correlation coefficient	p Value
Weight of cord blood	0.374	0.129
Total HSC	0.553	0.049
HSC/gram	0.536	0.055

Abbreviation: HSC = haematopoietic stem cells

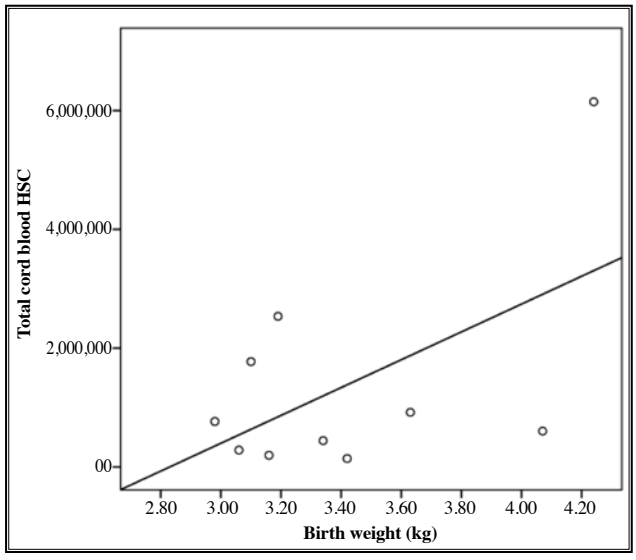

Figure. Correlation between birth weight and total haematopoietic stem cells (HSC) obtained

The cell counts from UCB collected at gestational age between 34 and 37 weeks were higher than those collected at gestational age of >37 weeks[23]. This was attributed to the fetal stress at premature gestation. The reason for the difference in our results may be because only women with gestational age of ≥38 weeks were included in our study. Nevertheless, in a study by Ballen et al[13], the CFU-granulocyte/macrophage (CFU-GM) and CD34 cell counts dropped when gestational age was >37 weeks, suggesting a loss of haematopoietic potential with rising gestational age. This hypothesis deserves to be addressed in future studies.

Ballen et al[13] found that the first baby had higher volume and total cell counts compared with those in each subsequent live birth. Omori et al[24] also found that primigravida was associated with higher cell count in a cord blood unit. In our study, we did not find any significant association between parity and cell counts. The hypothesis of a weakening of the placental vasculature with rising parity has been proposed to explain the relationship between parity and cord blood cell yield. However, there are no convincing data to support if there is any stem cell exhaustion or an ageing effect.

Shlebak et al[19] found that birth weight of ≥2.5 kg correlated positively with UCB volume. Ballen et al[13] also found that bigger babies were more likely to produce cord blood units with larger volumes and higher cell counts. The birth weight of the babies analysed in this study[13] ranged from 2.155 to 5.925 kg. In our study, we studied newborns with a mean (± SD) birth weight of 3.4 ± 0.4 (range, 2.98-4.24) kg. We also found that there was a positive correlation between total HSC obtained and the birth weight of the newborn (Pearson correlation coefficient, 0.553; p=0.049), though there was no correlation with the weight of cord blood. This supports the hypothesis that the bigger the baby, the higher the total HSC extracted from cord blood.

It has been proposed that there is an association between the gender of the baby and the yield of cord blood. Solves et al[14] found that female babies had significantly higher TNC but not CD34+ count or CFU. In contrast, Ballen et al[13] found that female babies had significantly lower CFU-GM count, but the effect of the gender of the baby was less evident in the volume of cord blood collected, CD34+ cell counts, or TNC. Our data found no significant difference in the TNC obtained from newborn male and female babies. This suggests that good-quality UCB can be obtained from newborns of both genders.

The mode of UCB collection in this study was based

on the Standards for Cellular Therapy Product Services published by the AABB, which is an international, non-profit association representing individuals and institutions involved in the field of transfusion medicine and cellular therapies. There are two methods for UCB collection, in utero (before placental delivery) and ex utero (following placental delivery). The UCB collection in this research was all ex utero, after the delivery of baby and placenta, by venipuncture of the umbilical vein.

The limitations of our study were that we only recruited women with term gestation and the newborns in our study were mostly healthy. Some studies[25,26] have suggested that prematurity and fetal compromise may increase the quantity and quality of cord blood stem cell because of the stress in the fetus. Further study is needed to explore this hypothesis. In addition, there was only one case of maternal disease, so it was not used for between-group comparisons. The influence of maternal disease on the quantity and quality of cord blood stem cell should be addressed in further studies.

Recent studies have revealed a new way for harvesting stem cells from a woman's placenta. Human placenta can now serve as a novel source of human mesenchymal progenitor cells for in-vitro expansion. One study showed that mesenchymal progenitor cells from human placenta could expand the CD34 cells from UCB in vitro[27]. Some data further suggested that the placenta provides a niche for haematopoiesis resulting in HSC, which is more primitive than those in the cord blood[28-31]. Further research in this field is needed for the importance of placenta and UCB in clinical practice.

In conclusion, we found that the birth weight of the newborn has an impact on the quantity of UCB obtained. To overcome the limitation of UCB units used for transplantation, our finding should be taken into consideration during selection of potential donors.

Declaration

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