Cord Blood Collection for the National Cord Blood Bank in Thailand

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Abstract

Umbilical cord blood is an effective alternative source of hematopoietic stem cells transplantation in children and adolescents. However, the efficacy and safety of cord blood transplantation correlates with the quantity and quality of cord blood. To evaluate the collection systems and processing of cord blood donations, a pilot research program to optimize recruitment, collection and processing of cord blood donations was developed. The present results showed that the quality of the cord blood (volume, total white blood cells (WBC) count, CD34+ and sterility control) collected was satisfactory and discard rate of collecting units (24.2%) were comparable with data reported from other cord blood banks. To find the optimal mode of collection, comparison of 3 cord blood collection methods (Method 1 = Hanging method after delivering the placenta, Method 2 = Aspiration from *in utero* placenta, Method 3 = Aspiration from *in utero* placenta and Syringe-assisted aspiration) using the closed system showed that method 3 was the best method but it required more trained personnel and involved a complicated procedure. The National Cord Blood Bank started its activity in 2002 after several years of pre-clinical studies. To date, a number of transplants using cord blood from related and unrelated cord blood (first report in Thailand) donors have been successfully performed.

Key word : Umbilical Cord Blood, Collection Methods, Cord Blood Bank, Hematopoietic Stem Cell, Transplantation

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Cord blood contains a large number of hematopoietic stem cells and can be used as an alternative source to bone marrow transplantation[1]. 50-100 ml. of cord blood can reconstitute the hematopoietic system in small patients, usually children, although there are some reports of successful transplantation of adults[2,3]. After the first successful cord blood transplantation on human leukocyte antigen (HLA) -identical siblings (related) in 1988[4] and the first unrelated cord blood transplant[5] in 1994, an exponentially increasing numbers of such procedures is being performed. Although preliminary data shows encouraging results in cord blood stem cell transplantation, further experience is necessary to develop the optimal method for donor selection/testing, collection, separation, storage and cryopreservation of umbilical cord blood (UCB). The efficacy and safety of cord blood transplantation depends upon the quality and quantity of cord blood that correlates with the amount of total WBC cells and sterility control.

From November 2000 to March 2002, the authors conducted a pilot research project to optimize recruitment, collection and processing of cord blood donations with future incorporation of cord blood banking into the National Cord Blood Bank (NCBB). In Phase 1, the quality and quantity of cord blood collection systems and processing of cord blood donations was evaluated compared with the standard criteria for cord blood collection proposed (volume, total WBC cells count, CD34+ and sterility control). In phase 2, comparison of 3 cord blood collection methods [Method 1 = Hanging method after delivering the placenta (Placenta *ex utero*), Method 2 = Aspiration from *in utero* placenta Method 3 = Aspiration from *in utero* placenta and syringe-assisted aspiration] using the closed or semi-closed system was done to find the optimal method of collection. An important aim of the project was to evaluate the feasibility of establishing a collaborated collection network and utilized expertise to develop the NCBB. The National Blood Centre already provides processing and storage facilities for bone marrow and peripheral blood stem cell harvests. The Cord Blood Bank was established within this facility to ensure that processing was subjected to the same stringency and underlying principles required for other sources of hematopoietic stem alls.

MATERIAL AND METHOD

Recruitment

The collection of cord blood was done in the delivery and operating suite of the Department of Obstetrics and Gynecology, King Chulalongkorn Memorial Hospital by a team of specially trained physicians, nurses and technicians.

All women were interviewed concerning infectious disease history, genetic disease and medical history by a counselor and signed a detailed consent form before the onset of labor. Inclusion criteria included the following : no known genetic disease; singleton pregnancy; gestational age (37 weeks; birth weight (2,500 g and had not received antibiotics during or immediately before delivery. Exclusion criteria included positive serologic result for human immunodeficiency virus, hepatitis B virus, syphilis; maternal fever (37.8°C during labor; rupture of membranes (18 hours before delivery; obstetric complication, stillbirth or congenital anomalies. This study was approved by the ethical committee of the Faculty of Medicine, Chulalongkorn University, Bangkok. In Phase 1, 33 cord blood was collected using *in utero* placenta aspiration by the blood bag method, which is presently the standard clinical practice, from both cesarean section (n = 23) and vaginal delivery (n = 10) to compare with the standard criteria. In Phase 2, comparison of 3 cord blood collection methods (Method 1 = Hanging method after delivering the placenta (Placenta *ex utero*), Method 2 = Aspiration from *in utero* placenta Method 3 = Aspiration from *in utero* placenta and syringe-assisted aspiration) using the closed or semi-closed system was done. Collections of cord blood from 60 participants were systematically allocated into 3 groups by block randomization.

Cord blood harvesting

For all 3 methods, after the delivery of the fetus the cord was ligated at 2-3 cm from the umbilicus before clamping to prevent crushing of the tissue. The umbilical cord was then cut between the clamp and the ligation, changing the gloves and the 10-cm distal portion of the cord was disinfected with alcohol-based chlorhexidine.

Method 1 Hanging method after delivering the placenta (Placenta *ex utero*)

Wait until the placenta was delivered and then placed the placenta in a sterile supporting structure with the umbilical cord hanging through the support. The umbilical cord was then cleaned with povidone-iodine and alcohol, and the umbilical vein was canulated using a needle connected to a standard blood bag containing citrate-phosphate-dextrose anticoagulant solution. The cord blood was then collected by gravity drainage. The collection procedure conti-

nued as long as there was flow with gentle "milking", after which the blood bag was disconnected and sealed. The umbilical cord blood volume was measured by subtracting the citrate-phosphate-dextrose volume. An additional sample of undiluted umbilical cord blood was used to determine the blood count before umbilical cord blood collection.

Method 2 Aspiration from *in utero* placenta

After delivery of the child, the infant was placed on the mother's abdomen immediately after birth with no pull on the umbilical cord. The umbilical cord was clamped and transected within 30 seconds after delivery. While the placenta was still *in utero*, umbilical cord blood was collected by venipuncture of the free end of the umbilical cord into a blood-collecting bag containing citrate-phosphate-dextrose anticoagulant. Other procedures were the same as Method 1

Method 3 Aspiration from *in utero* placenta and syringe-assisted aspiration

Syringe-assisted sodium chloride solution flush and drain with a blood bag was modified from the method previously described by Elchalal[6]. Briefly, the umbilical vein was catheterized using feeding tube #8 into the placental branch while the umbilical artery was catheterized using feeding tube #5. Normal saline solution with 10 per cent anticoagulant 100-150 ml was flushed and the cord blood was collected through the umbilical vein catheter into a standard blood bag. At the time of delivery, information on several obstetric parameters, including the gender of the baby, the gestational age at delivery, and the weight of both the placenta and the infant was collected. Cord blood was sent to the bank at the National Blood Centre for processing, freezing and storage.

Cord blood processing

All cord blood units were processed within 24 hours after cord blood collection. Cryopreservative was performed within 24 hours after cord blood collection as whole blood in a final concentration of 10 per cent dimethylsulfoxide (DMSO). Before cryopreservation, the sample volumes were reduced to a final volume of about 60 ml by centrifuging the blood bag and expressing the leukocyte-rich supernatant to make a final cell concentration about 150 x 10^6 /ml The cord blood units were cryopreserved by control rate freezer and transferred to vapor phase of liquid N2 in a label cassette ready to be used and transplanted to the patient. Vials from cord blood were separately cryopreserved to be used for future diagnostic purposes. All units were stored in the vapor phase of nitrogen.

Laboratory test monitoring

1. Monitored the quantity of stem cells collected by measuring the amount of mean volume of cord blood (ml), mean total WBC cells, CD34 positive cells (by flow cytometry).

2. Monitored the quality of stem cells collected by measuring.

2.1 The aseptic technique of collection by sterility test.

2.2 The viability of stem cells.

Sterility control of cord blood samples

Since cord blood transplant recipients are at particular risk for infections, decontamination protocols were strictly implemented in the collections to reduce bacterial contamination. Extensive training was done to reduce the contamination rate. Extensive sterility tests were performed for aerobic, anaerobic and fungi cultures after collection, before and after freezing.

All units were registered in an internal database and all samples were given an internal code. The mothers' names were kept in a confidential registry in order to report all the transmissible diseases detected during the routine control.

Release and selection of transplants

The standard criteria for cord blood collection proposed were no partial clotted blood, total WBC $> 5 \times 10^8$ and negative sterility test. If cord blood unit met all the quality requirements (total volume, mononuclear cells, CD34 + cells and negative for serology and microbiology screening), a sample for quality control of cryopreservative procedure and sterility controls were taken before cryopreservation to perform full blood count, ABO and Rh typing and HLA typing (low resolution polymerase chain reaction (PCR) - sequence specific probe (SSP) of A, B and DR loci). The cord blood and vials belonging to the transplants were stored at the Nation Blood Centre.

If a search request was made for a specific transplant, a cryopreserved DNA vial could be used for additional testing such as confirmative of HLA typing or immunological testing.

Follow-up

Mothers and newborns were followed 3-4 days after delivery. Mothers were further followed at 6 months and 12 months, at which time, information about possible diseases developed by the newborn was also obtained.

Expected effects

The National Cord Blood Bank would be able to serve unrelated stem cell transplants for patients in Thailand. This would augment the hematopoietic stem cell pool available for transplantation for the treatment of various life threatening diseases.

Statistical methods

With twenty women in each group a test with a significant level of alpha = 0.05 would detect statistical significance. The comparison between values of the quantitative variable (volume) in the three groups was done by an analysis of variance test and included standardization in the level of significance for pair's comparisons. Results were expressed as mean (± SD). The correlation between the volume of cord blood collected and the variable factors were analyzed by means of linear regression analysis.

RESULTS

Phase 1 From November 2000 to November 2001, 33 cord blood was collected using the *in utero* placenta aspiration method from both cesarean section (n = 23) and vaginal delivery (n = 10) for use in animal research study. The standard criteria for cord blood collection proposed are no partially clotted blood, total WBC > 5 x 10^8 and negative sterility test for aerobic, anaerobic and fungi cultures. Overall, 8 (24.2%) donations allocated were discarded for reason shown in Table 1.

The mean volume, total WBC and total CD34 cells are shown in Table 2. A comparison with data obtained from cesarean delivery and vaginal delivery in the present study showed that cord blood obtained from cesarean delivery seemed to have an increase in mean volume (83 *vs* 62 ml) and WBC cells (10.16 x 10^8 *vs* 6.99 x 10^8) but similar values in mean CD 34+ cells (3.91 x10^6 *vs* 4.96 x 10^6).

Table 1. Reason for discard of cord blood donation.

Reason for unit discard	Low volume/ total WBC	Clot in sample
Cesarean delivery (n = 23)	2	2
Vaginal delivery (n = 10)	1	3

Phase 2 From October 2001 to March 2002, initially with support from the Asahi Foundation Grant, the authors began a program to collect, test, process, and cryopreserve cord blood for unrelated transplantation with future incorporation of cord blood banking into the National Cord Blood Bank. Comparison of three closed/semi-closed collection methods in umbilical cord blood collection was randomly performed.

The characteristics among the three groups were similar regarding maternal age, gestational age at delivery, birth weight, placental weight, placental diameter, and cord length (Table 3).

The total WBC cells and CD34+cells were significantly higher in method 3 "placenta *in utero* and syringe flush" compared with the other two methods. Also the discard rate for this method was the lowest (5%) of the 3 methods. No contamination of bacteria and fungi was found in all cord blood.

All validated units (HLA - typed, sterility and serology negative) were included in the Thai Bone Marrow Registry. The value of the cord blood bank was seen when we could provide a number of donor transplants using cord blood derived stem cells from prenatally diagnosed HLA-identical siblings (with beta-thalassemia/Hb E disease) and unrelated mismatched (the degree of HLA disparity was 1/6 loci) cord blood donors to a boy with severe immunodeficiency disease, Wiskott-Aldrich syndrome, aged 15 months. He had suffered from recurrent GI bleeding with pulmonary hemorrhage due to thrombocytopenia since 3 months old and required platelet transfusion once or twice every week. He also had recurrent severe pneumonia and needed ventilator support from time to time. Cord blood was collected on December 20, 2001 by the 3 rd method (placenta *in utero* and syringe flush). Total WBC cells was 5.66 x 10^7, CD34+cells = 32.10 x 10^6, Viability = 95%

HLA : Patient A*207, A*33, B*46, B*58, DRB1* 0301/19-20, DR B1 *1207
HLA : Cord blood (Asahi 26) A*203, A*33, B*46, B*58, DRB1*0301/19-20, DR B1*1207.

Table 2. Cord blood data.

Method	Cord blood volume (ml)	Cord blood total WBC cells ($x10^8$)	Cord blood CD34+cells ($x10^6$)
Cesarean delivery (n = 23)	83 (50-155)	10.16 (3.34-16.64)	3.91 (0.62-11)
Vaginal delivery (n=10)	62 (40-110)	6.99 (4.5-8.86)	4.96 (3.8-6.3)

Table 3. Characteristics of the tested groups (n = 20 women in each group).

Method	Maternal age (y)	Gestational age at delivery (wk)	Birth weight (g)	Placental weight (g)	Placental diameter (cm)	Cord length (cm)
1	25.65 ± 6.1	38.3 ± 1.6	3,107.5 ± 234.0	560.5 ± 97.9	19.2 ± 2.8	51.8 ± 12.7
2	23.85 ± 7.8	38.6 ± 1.6	3,057.3 ± 417.3	591.5 ± 108.7	20.3 ± 2.9	53.0 ± 9.2
3	23.65 ± 4.5	38.6 ± 1.4	3,063.0 ± 300.1	575.0 ± 94.5	18.9 ± 2.5	47.7 ± 9.3

Result are presented as mean ± SD

Table 4. Reason for discard of cord blood donation.

Reason for unit discard	Placenta *ex utero* (n = 20)	Placenta *in utero* (n = 20)	Placenta *in utero* + syringe flush (n = 20)
Low volume/total WBC	4	5	-
Clots in sample	4	-	1
Maternal virology	2*	-	-
Total	10 (50%)	5 (25%)	1 (5%)

Table 5. Cord blood data.

	Placenta *ex utero* (n = 20)	Placenta *in utero* (n = 20)	Placenta *in utero* + syringe flush (n = 20)	Statistical significance*
Total WBC cells ($x\ 10^8$)	7.2 ± 4.7	8.0 ± 4.7	15.9 ± 11.0	$p < 0.001$*
CD34 + cells ($x\ 10^5$)	2.3 ± 2.1	4.1 ± 4.9	6.7 ± 8.6	$p = 0.062$
Viability (%)	96.4 ± 2.8	95.3 ± 4.6	96.0 ± 3.8	$p = 0.658$

* One way anova (the mean difference is significant at the 0.05 level)

He was transplanted on May 3, 2002. On July 12, blood cells had recovered, he has done well without GVHD, and chimerism has confirmed the donor engraftment. At present the patient is healthy. This is the first report of successful unrelated cord blood transplantation in Thailand.

The data on costs of reagents and diagnostics, disposables, maintenance, laboratory tests excluding the labor costs are shown in Table 6.

DISCUSSION

Umbilical cord blood is enriched in stem cells and has a higher proliferative capacity than bone marrow and peripheral blood[7]. The novel therapeutic modality of transplantation stem cells from umbilical cord blood was clinical introduced by the use of HLA - identical siblings cord blood[1]. But HLA match between the fetus and the sibling patient is present in only 25 per cent of the cases. The recent

Table 6. Costs of cord blood units.

	Amount (baht)
Cord blood collection fee	1,000
Cord blood cryopreservation	4,500
Infections markers and sterility test	4,350
CD34 + analysis	2,000
ABO/Rh, CBC	150
HLA typing	11,000
Total	23,000/unit/year

success in using unrelated donor cord blood stem cell transplantation has led to establishing cord blood bank where cord blood from volunteer donors can be stored[8]. However, the major problem found in cord blood transplantation is the inefficient number of stem cells and contamination of umbilical cord blood which depend on the technique of collection, processing and storage[6] several collection methods (open and closed systems) have been proposed. To optimize the collection volume and minimize microbial contamination rate, the open systems have been replaced by the closed system. In Phase 1, the quality and quantity of cord blood closed collection system (using method 2 "*in utero* placenta aspiration" which is presently the standard clinical practice) and processing of cord blood donations was compared with the standard criteria (volume, total WBC cells count, CD34+ and sterility control). The authors evaluated 33 cord blood units collected from both cesarean section (n = 23) and vaginal delivery (n = 10). The discard rate of collecting units (24.2%) was comparable with data reported from other cord blood banks[8,9]. The mean volume, total WBC and total CD34 cells are shown in Table 2. A comparison with data obtained from cesarean delivery and vaginal delivery in the present study showed that cord blood obtained from cesarean delivery seemed to be increased in mean volume and WBC cells but had similar values in mean CD 34+ cells. As a marker for hematopoietic progenitor-stem cells, CD34 is correlated with outcome after stem cell transplantation. Another study by Surbek et al previously[10] showed that the cord blood yield was similar at vaginal birth and cesarean delivery if the collection was performed with the placenta in situ.

Recently Elchalal et al[6] reported that syringe-assisted saline flush and drain with the practically closed system resulted in higher total WBC count with a 16 per cent contamination rate. In Phase 2, the authors compared the three methods of closed or semi-closed cord blood collection system after vaginal deliveries in a randomized trial. The present study showed that "aspiration from *in utero* placenta and syringe-assisted aspiration method" which was adapted from Elchalal et al resulted in higher mean volume; cell counts (total WBC and CD34+) and without the problem of contamination. Also the "*in utero* placenta" (methods 2 and 3) appeared to perform better than the "placenta *ex utero*" (method 1). This correlated with the study from Surbek et al[10], which showed that cord blood sampling was more efficacious if performed before delivery of the placenta. Also, the discard rate of cord blood units in method 3 was the lowest (5%) compared with method 2 and 1 (25% and 50%, respectively). Even though, this collection method seemed to be beneficial and safe, it involved more complicated procedure and required more personnel. Therefore, it might be preferable for targeted related cord blood stem cell transplantation than in general unrelated cord blood bank. The mean total WBC cells, CD34+cells and viability in phase 1 and phase 2 (Table 2 and 5) were considered highly satisfactory compared with other cord blood banks data[8,9]. Out of 60 cord blood units, 45 units passed the standard criteria and a sample for sterility control, full blood count and Hb typing ABO = Rh typing, and HLA typing were taken before cryopreservation. This cord blood bank was later incorporated into the National Cord Blood Bank, which was established in 2002. Up to January 2003, 150 cord blood units have been registered into the Unrelated Stem Cell Donor Registry Program. One of these cord blood units has been used for transplantation (Wiskott-Aldrich Case). Its quantities and quality of collected cells were suitable for clinical transplantation (more than 2 X 10^7/kg total WBC cells)[8]. This lead to the first successful unrelated mismatched allogeneic cord blood transplantation in Thailand.

Cord blood is an expensive resource but these costs compare favorably with those of other hematopoietic stem cell sources. Centralized banking with high quality standards has been shown to cost less[11] So far, however, funding has been limited and inconsistent. With money coming from small research grants and the Thai Red Cross Society, a limited number of cord blood units could be provided. To provide a greater number of units for stem cell transplantations, a lot more funding support is needed.

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การเก็บเลือดสายสะดือทารกสำหรับ ธนาคารเลือดสายสะดือทารกแห่งชาติในประเทศไทย

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เลือดสายสะดือทารกเป็นแหล่งเซลล์ต้นกำเนิดเม็ดเลือดที่มีประสิทธิภาพอีกแหล่งหนึ่งในการปลูกถ่ายไขกระดูกให้ผู้ป่วยวัยเด็กหรือวัยรุ่น อย่างไรก็ดีประสิทธิภาพและความปลอดภัยของการปลูกถ่ายเลือดสายสะดือทารกขึ้นกับปริมาณและคุณภาพของเลือดที่เก็บได้ ได้ทำการศึกษาวิจัยถึงประสิทธิภาพของวิธีการเก็บและขบวนการดำเนินการก่อนแช่แข็ง โดยพิจารณาจากปริมาณเลือดที่เก็บได้ จำนวน white blood cells, CD34+cells และการไม่พบเชื้อจากการเพาะเชื้อแบคทีเรีย (aerobe และ anaerobe) และ fungus เทียบกับเกณฑ์มาตรฐาน (Phase 1) พบว่าจำนวนร้อยละของเลือดสายสะดือทารกที่เข้าเกณฑ์มาตรฐานมีร้อยละ 75.8 นอกจากนี้ได้ศึกษาเปรียบเทียบวิธีการเก็บเลือดสายสะดือทารกในระบบปิด 3 วิธี คือ วิธีที่ 1 การเก็บโดยการแขวนรกหลังคลอด (Hanging method) วิธีที่ 2 การดูดเลือดสายสะดือทารกที่ยังไม่คลอดและวิธีที่ 3 การดูดเลือดสายสะดือทารกที่ยังไม่คลอด ร่วมกับการดูดจาก Syringe พบว่าวิธีที่ 3 มีประสิทธิภาพดีที่สุด แต่มีวิธีการเก็บที่ซับซ้อนและอาศัยบุคลากรจำนวนมากกว่า (Phase 2) ได้นำประสบการณ์การเก็บเลือดสายสะดือทารก มาพัฒนาจัดตั้งธนาคารเลือดสายสะดือทารกแห่งชาติ และเริ่มดำเนินการเมื่อปี พ.ศ. 2545 ปัจจุบันได้มีการปลูกถ่ายเลือดสายสะดือทารกที่ได้จากพี่น้อง (related cord blood transplantation) และจากบุคคลอื่น [Unrelated cord blood transplantation (รายงานครั้งแรกในประเทศไทย)] เป็นผลสำเร็จ

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