Collection and Processing of Umbilical Cord Blood for Cryopreservation

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Abstract

Umbilical cord blood (UCB) is being increasingly used as an alternative source of hematopoietic stem cells for allogeneic bone marrow transplantation. UCB transplantation has been successfully used to treat a variety of genetic, hematological, and oncological disorders in children and adults. The objectives of this study was to establish a closed-system technique for UCB collection and buffy coat separation by Optipress I device. Thirty-four UCB were collected by triple-bag system from pregnant mothers whose fetuses were not affected by thalassemic diseases after prenatal diagnosis. The mean volumn of UCB collection were 120±5 ml (range 65-180 ml). Total WBC, CD34+ cells, the progenitor cell erythroid burst-forming unit (BFU-E) and granulocyte-macrophage colony-forming unit (CFU-GM) in the UCB units were $(9.36 \pm 0.84) \times 10^8$, $(3.61 \pm 0.52) \times 10^6$, $(9.12 \pm 1.60) \times 10^5$, and $(5.32 \pm 1.23) \times 10^5$, respectively. Good correlation between the nucleated cell and net cord blood volume could be demonstrated ($p < 0.0001$). The correlation between CD34+ cells and the following parameters: nucleated cell, BFU-E or CFU-GM were also demonstrated ($p = 0.001$, 0.0105 or 0.0001, respectively). Buffy coat was subsequently separated from 18 UCB units by Optipress I device. 70 ± 3 ml of buffy coat were collected and cryoprocessing was done by automatic controlled-rate freezer. Good recovery of total WBC, CD34+ cells, progenitor cells BFU-E and CFU-GM after buffy coat separation were observed 89 per cent, 95 per cent, 109 per cent, and 102 per cent respectively. There was no

aerobic bacterial or fungal contamination in the separated blood products. By using this technique, the UCB units were easily collected, rapidly separated within one hour, and high recovery of the hematopoietic progenitor cells could be obtained.

Key word : CD34+ Cell, Stem Cell Collection, Umbilical Cord Blood

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It is now known that umbilical cord blood (UCB) contains a number of hematopoietic progenitor cells similar to bone marrow[1] and can be used as a source of hematopoietic stem cells for human leukocyte antigen (HLA)-matched sibling donors transplantation[2-6] and more recently, unrelated transplantation[7]. UCB has many advantages over other stem cell sources, including a large, accessible donor population, ease and safety of collection, low incidence of infectious contamination and a low probability of graft-versus-host disease (GVHD)[8,9]. Several methods for collection of UCB have been described [10-13] and the recommendation is that UCB should be collected before the delivery of the placenta[14]. Many techniques for separation of UCB to reduce the final volume have been developed in order to increase storage capacity to a cost effective level. Separation of red blood cells from UCB by using simple centrifugation, lysis with ammonium chloride, differential settling in viscous media, and filtration through density gradients resulted in high progenitor cell loss[1]. Other reports found better recovery[15-17] but many steps were required, as a consequence increasing the possibility of errors and thereby exposing the blood to the risk of bacterial and fungal contamination. By using enhanced sedimentation of red cells by hydroxyethylstarch (Hespan), the progenitor cells lost during the process was 40 per cent or more[1,18,19]. Higher recovery of about 89 per cent was achieved when using pentastarch[20] but there were also many steps. By separation of the buffy coat in a semi-automate closed system, the recovery was more than 90 per cent[21,22].

The objective of this study was to develop a practical technique to collect the UCB by a closed-system and process by semi-automate separation

MATERIAL AND METHOD

Umbilical cord blood donors

Prenatal diagnosis for thalassemia was performed after informed consent in thirty-four pregnant mothers, who had previously had a thalassemic child. When the fetuses were not affected, the mothers were registered for UCB collection and processing at the Division of Hematology, Faculty of Medicine Siriraj Hospital, Mahidol University. UCB were collected at birth and HLA typing as well as testing for infectious contamination were performed.

UCB collection

The UCB collection was performed in the Department of Obstetrics and Gynecology. A 450 ml Optipac triple bag system (R1789, Baxter Health Care IL, USA) containing 63 ml of citrate/phosphate/dextrose/adenine (CPD-A) was used to collect the UCB. The volume of CPD-A was reduced to 23 ml by draining 40 ml into a sterile 50 ml tube which was enough for collecting up to 170 ml UCB. After delivery of the baby, while the placenta was still *in utero*, the free end of the cord was wiped with betadine to ensure

sterility of the collection. The 16-gauge needle of the triple bag was inserted into the umbilical vein of the placenta. As much umbilical cord blood as possible was drained by gravity into the bag. After collection, 5 ml of UCB were taken for HLA-typing, 1 ml for nucleated cell count, CD34+ cell enumeration, hematopoietic progenitor cell BFU-E and CFU-GM assayed, 1 ml for bacterial and fungal culture for sterility testing. The volume of UCB unit was calculated from the weight of each collection minus the weight of the bag.

UCB processing

Sixteen UCB units with volume less than 100 ml were frozen in cryocyte freezing bags (Baxter Health Care IL, USA) as whole blood by adding equal ice-cold 20 per cent dimethylsulphoxide (DMSO) (Fluka, Switzerland) in minimal essential media (MEM) (GIBCO, New York, NY, USA) and immediately freezing in a controlled-rate freezing machine (Cryoson, Germany). The cryopreservation protocol was as follows: 1.5°C/min cooling down to -60°C, followed by a drop to -130°C, 7°C/min. The freezing product was transferred into a liquid nitrogen freezer.

In eighteen UCB units, with a volume more than 100 ml, the volume was reduced by the removal of the plasma and red blood cells using the technique as modified from previous reports[21]. The UCB unit was centrifuged at 2,620 g (Sorvall, RC-3C, USA), 22°C for 10 min. After centrifuge, the plasma and red blood cells were removed into the satellite bag by using Baxter Optipress I blood component separator (Baxter Health Care, USA). The entire process was performed in a closed system. Approximately 70 ml of buffy coat were separated and frozen as whole blood. Before being frozen, 1 ml of the buffy coat was taken for enumeration, the quantity of nucleated cells, CD34+ cells, BFU-E and CFU-GM.

Cell count and CD34+ cell analysis by flow-cytometer

Nucleated cells were counted by Sysmex K800. The CD34+ population was determined on CD45+ lymphocytes by using MoAbs againt CD45 and CD34 conjugated with fluorescein isothiocyanate and phycoerythrin (both from Becton Dickinson, San Jose, CA, USA), respectively[23]. Brifly, UCB containing 1 x 10^6 nucleated cells (volume not more than 50 µl) was incubated with the MoAb mixtures at 4°C for 30 min. After incubation, the erythrocytes were lysed by fluorescence-activated cell sorter (FACS) lysing solution (Becton Dickinson) at 4°C for 10 min. The remaining cells were washed twice with cold Phosphate-buffered saline (PBS) containing 0.1 per cent sodium azide and finally fixed with 0.5 ml of 0.5 per cent paraformaldehyde in PBS for subsequent flow cytometric analysis using CellQuest software (Becton Dickinson).

Hematopoietic progenitor cell assays

The hematopoietic progenitor cell assays were performed by the methylcellulose culture system, as previously described[24]. Briefly, mononuclear cells (MNCs) were separated from UCB after centrifugation over Ficoll-Hypaque (Nycomed, Oslo, Norway). Cells were washed twice and resuspended in Iscove's modified Dulbecco's medium (IMDM) (GIBCO, New York, NY, USA), and 5 x 10^5 MNCs were cultured in 1 ml IMDM in the presence of 30 per cent fetal bovine serum (Seromed, Berlin, Germany), 1 per cent bovine serum albumin (Terry Fox Laboratories, Vancouver, Canada), 10^{-4} nmol α-thioglycerol (Sigma, St Louis, MO, USA) and 0.8 per cent methylcellulose (Sigma). Cultures of erythroid burst-forming unit (BFU-E) and granulocyte-macrophage colony-forming unit (CFU-GM) myeloid colonies were performed by adding 1 U recombinant erythropoietin (Terry Fox Laboratories) and 10 per cent agar leukocyte-conditioned medium (Terry Fox Laboratories), respectively. Each culture was duplicated in a 35-mm petri dish (Nunc, Roskilde, Denmark) and incubated for 14 days in a fully humidified atmosphere of 95 per cent air and 5 per cent CO_2 at 37°C. Colonies were counted on day 14.

Statistical analysis

The results were expressed as mean ± standard error of mean (SEM). The correlation between the number of CD34+ cells and the following parameters: the number of nucleated cells, BFU-E and CFU-GM had been calculated by linear regression using Statview program. The correlation between the total number of nucleated cells and the net cord blood volume was also analysed.

RESULTS

Cord Blood collection assessment of the number of cell count

By using the triple-bag system to collect the UCB, the number of nucleated cells, CD34+ cells

and progenitor cells are shown in Table 1. The mean volume of net cord blood collected from 34 donations was 88 ± 5 ml (range 17-157). There was only one sample in which the volume was 17 ml while the others were more than 30 ml. The total volume included CPD-A in UCB units was 120 ± 5 ml (range 65-180). The total nucleated cells count of all samples was (9.36 ± 0.84) x 10^8 cells (range (1.63-20.65) x 10^8 cells) and there was a positive correlation with the volume of UCB collected (Fig. 1) ($r = 0.77$, $p < 0.0001$). The total number of CD34+ cells, BFU-E and CFU-GM were (3.61 ± 0.52) x 10^6, (9.12 ± 1.60) x 10^5, and (5.32 ± 1.23) x 10^5, respectively. There was a positive correlation between the number of CD34+ cells and the following parameters: nucleated cells, BFU-E and CFU-GM ($r = 0.62$, 0.56 and 0.70, $p = 0.001$, 0.0105, 0.0001, respectively) (Fig. 2-4). There were no aerobic bacterial and fungal contaminations in any of the 34 collections.

Buffy coat separation by Optipress I device

Volume reduction and buffy coat preparation was performed from 18 UCB units with the total volume more than 100 ml (range 105-180 ml, mean 138 ± 5 ml) by using an Optipress I machine. Table 2 shows the results on the volume, the number of cells before and after separation, and the recovery of the cells. The mean total volume of buffy coat after separation was 70 ± 3 ml (range from 55 to 110 ml). The total nucleated cells, the number of CD34+ cells, BFU-E and CFU-GM in the UCB before separation were (12.20 ± 1.08) x 10^8, (4.67 ± 0.78) x 10^6, (8.34 ±

Table 1. The number of nucleated cells and hematopoietic stem cells in the UCB collected by the triple bag system.

	Total UCB volumn (ml)	Net UCB volume (ml)	Nucleated cell (x 10^8)	CD34+ cell (x 10^6)	BFU-E (colonies x 10^5)	CFU-GM (colonies x 10^5)
N	34	34	34	32	24	24
Mean ± SEM	120 ± 5	88 ± 5	9.36 ± 0.84	3.61 ± 0.52	9.12 ± 1.60	5.32 ± 1.23
Median	120	92	8.56	2.25	7.56	3.56
Range	65-180	17-157	1.63-20.65	0.53-10.96	1.4-29.55	0.61-26.98

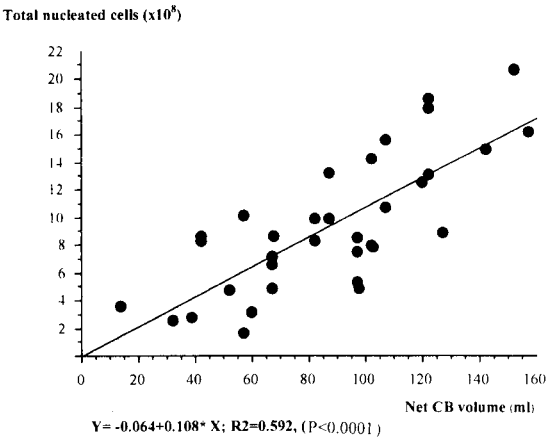

Fig. 1. Correlation between the total nucleated cells and the net cord blood volume collection. There was correlation between total nucleated cells and the net cord blood volume collection ($r = 0.77$, $p < 0.0001$)

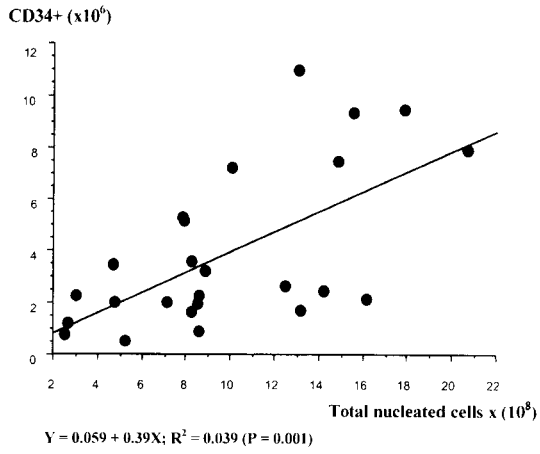

Fig. 2. The relationship between cord blood CD34+ cells and the total number of nucleated cells. The total CD34+ cells was correlated with total numbers of nucleated cells. A positive correlation of $r = 0.62$ was found ($p = 0.001$).

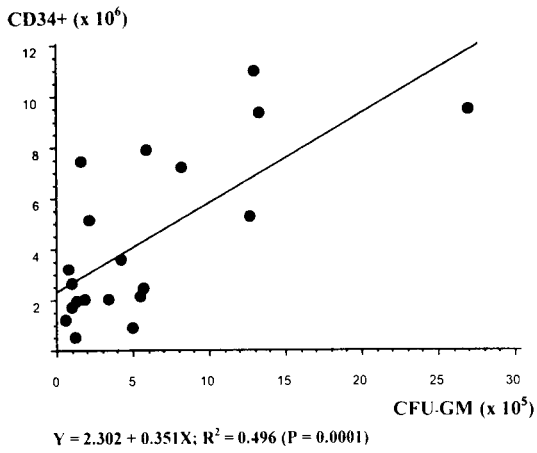

Fig. 3. **The relationship between the quantity of cord blood CD34+ cells and progenitor cell CFU-GM. The total CD34+ cells was correlated with total numbers of progenitor cell CFU-GM. A positive correlation of r = 0.70 was found (p = 0.0001).**

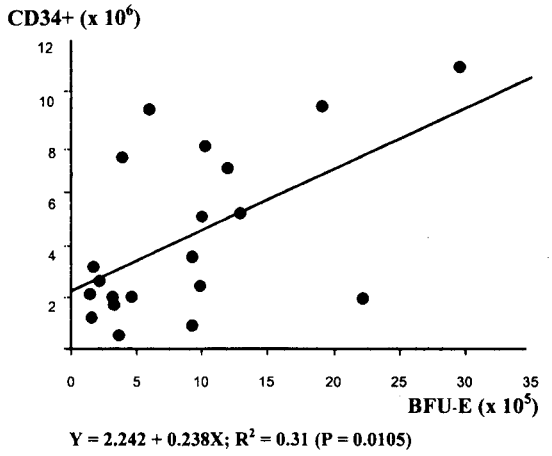

Fig. 4. **The relationship between the quantity of cord blood CD34+ cells and progenitor cell BFU-E. The total CD34+ cells was correlated with total numbers of progenitor cell BFU-E. A positive correlation of r = 0.56 was found (p = 0.0105).**

Table 2. **Comparison of the number of nucleated cells and hematopoietic stem cells in the UCB before and after separation by Optipress I device.**

	Total UCB volumn (ml)	Total nucleated cell (x 10^8)	Total CD34+ cell (x 10^6)	Total BFU-E (colonies x 10^5)	Total CFU-GM (colonies x 10^5)
N	18	18	18	14	14
Before separation					
Mean ± SEM	138 ± 5	12.20 ± 1.08	4.67 ± 0.78	8.34 ± 2.13	6.66 ± 2.01
Median	135	12.76	2.90	9.88	5.87
Range	105-180	4.80-20.65	0.53-10.96	1.40-29.55	0.81-26.98
After separation					
Mean ± SEM	70 ± 3	10.56 ± 0.86	4.03 ± 0.60	7.74 ± 1.60	5.88 ± 1.56
Median	67.5	10.38	3.03	7.12	3.53
Range	55-110	4.06-18.83	0.41-8.57	0.91-23.10	0.78-19.67
% Recovery					
Mean ± SEM		88.93 ± 3.52	94.67 ± 7.70	108.82 ± 13.40	102.55 ± 10.54
Median		90.60	90.60	91.80	91.80
Range		59.80-110.83	47.66-179.44	65.16-224.00	65.16-204.96

2.13) x 10^5, and (6.66 ± 2.01) x 10^5, respectively. The number of these cells in the buffy coat after separation were (10.56 ± 0.86) x 10^8, (4.03 ± 0.60) x 10^6, (7.74 ± 1.60) x 10^5, and (5.88 ± 1.56) x 10^5, respectively. The recovery of the nucleated cell ranged from 59.8 to 110.83 per cent (mean 88.93 ± 3.52%). The recovery of the quantity of CD34+ cells, BFU-E and CFU-GM were 94.67 ± 7.70 per cent, 108.82 ± 13.40 per cent, and 102.55 ± 10.54 per cent , respectively.

DISCUSSION

UCB is enriched with enough hematopoietic stem cells and progenitor cells to be used for stem cell transplantation. Collection, separation and process-

ing of UCB are important so that maximal number of stem cells can be achieved and stored. A previous study indicated that the UCB should be collected before placental delivery with better yields of cells (14). In the present study, the UCB was collected after delivery of the new born, while the placenta was still *in utero*. The triple bag system was used to collect the UCB. The total volume of UCB was 120 ± 5 ml and ranged from 65-180 ml. The net cord blood volume was 88 ± 5 ml and ranged from 17 ml to 157 ml. The results are similar to other studies(12,21,22). The number of total nucleated cells, CD34+ cells, the hematopoietic progenitor cell BFU-E and CFU-GM were (9.36 ± 0.84) x 10^8, (3.61 ± 0.52) x 10^6, (9.12 ± 1.60) x 10^5, and (5.23 ± 1.23) x 10^5, respectively. These findings were similar to other studies that used the same technique to collect the UCB(12,21,22). There was correlation between the number of CD34+ cells and the following parameters: the nucleated cells, BFU-E and CFU-GM, respectively in the UCB collection. Correlation between the number of nucleated cells and the net cord blood volume was also observed.

Several methods were performed in order to reduce the UCB volume and ultimately to reduce the space for storage. Almici et al(25) compared three different densities of percoll (1.069 g/ml, 1.077 g/ml, 1.084 g/ml), sedimentation over poligeline (Emagel) and sedimentation over poligeline followed by separation over Ficoll/Hypaque (F/H). They found that separation by sedimentation over poligeline followed by Ficoll/Hypaque yielded the highest depletion of RBC while maintaining high recovery of the cells. However, these techniques required many steps and were performed in an open system with increasing the possibility of identification errors and thereby exposing the blood to the risk of bacterial and fungal contamination. Rubinstein et al(11) used hydroxyethyl starch (HES) to sediment the red blood cells in a closed system. By using a modification of a triple bag system and adding a solution of HES, the UCB was separated by two centrifugation steps into three components: buffy coat, red cells and plasma fraction. The overall recovery of the cells was good(12). High recovery was found by using pentastarch(20) but this technique required many steps of centrifugation, increasing the risk of damage to the bags and the processing time. Good recovery of more than 90 per cent was observed when separating the buffy coat by the semi-automatic closed system(21,22). The UCB in the triple bag system was centrifuged for one step and buffy coat was separated by Optipress II automatically. The modification from those techniques was used in the present study by using Optipress I machine to separate the buffy coat from the UCB unit. The buffy coat was separated from 18 UCB units by using Optipress I blood cell separator after centrifugation. The recovery of the nucleated cells, the number of CD34+ cells, BFU-E and CFU-GM were 89 per cent, 95 per cent, 109 per cent and 102 per cent, respectively which is similar to other investigators(21,22) but the volume of the buffy coat after separation was 70 ± 3 which was more than previously reported(21,22). It may be due to many factors such as the centrifugation rate, the difference of the protocol of the Optipress I or II device, or the size of the separation bag, each of which needs further study.

In conclusion, UCB collection using the triple bag system is a simple and useful technique to collect the blood while the placenta is still *in utero*. The buffy coat separation using Optipress I machine reduced the volume of the UCB to 70 ml. Our methodology is rapid; buffy coat separation is performed by one step of centrifugation, partial removal of red blood cell and plasma by Optipress I automatically, and is processed within one hour before cryopreserved processing. Good recovery of the hemopoietic cells was observed. The volume reduction for cord blood banking set up will be modified, including the use of the smaller volume of the collecting bag, the centrifugation rate and the protocol of the optipress I device, which need further study.

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REFERENCES

1. Broxmeyer HE, Douglas GW, Hangoc G, et al. Human umbilical cord blood as a potential source of transplantable hematopoietic stem/progenitor cells. Proc Natl Acad Sci USA 1989; 86: 3828-32.
2. Vilmer E, Sterkers G, Rahimy C, et al. HLA-mismatched cord blood transplantation in a patient with advanced leukemia. Transplantation 1992; 53: 1155-7.
3. Gluckman E, Broxmeyer HE, Auerbach AD, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical cord blood from an HLA-identical sibling. N Engl Med 1989; 321: 1174-8.
4. Issaragrisil S, Visuthisakchai S, Suvate V, et al. Transplantation of cord blood stem cells into a patient with severe thalassemia. N Engl J Med 1995; 332: 367-9.
5. Issaragrisil S, Suvate V, Visuthisakchai S, et al. Bone marrow and cord blood stem cell transplantation for thalassemia in Thailand. Bone Marrow Transplantation 1997; 19 (Suppl 2): 54-6.
6. Wagner JE, Kernan NA, Steinbuch M, et al. Allogeneic sibling umbilical-cord-blood transplantation in children with malignant and non-malignant disease. Lancet 1995; 346: 214-9.
7. Kurtzberg J, Laughlin M, Graham ML, et al. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated patients. New Engl J Med 1996; 335: 157-66.
8. Broxmeyer HE, Hangoc G, Cooper S. Clinical and biological aspects of human umbilical cord blood as a source of transplantable hematopoietic stem and progenitor cells. Bone Marrow Transplant 1992; 9 (Suppl 1): 7-10.
9. Wagner JE, Rosenthal J, Sweetman R, et al. Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: Analysis of engraftment and acute graft-*versus*-host-disease. Blood 1996; 88: 795-802.
10. Issaragrisil S, Visuthisakchai S, Tangnaitrisorana Y, et al. Collection of cord blood stem cells for transplantation in thalassemic patients. Stem cells 1995; 13 (Suppl 3): 71-5.
11. Rubinstein P, Dobrila L, Rosenfield R, et al. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc Natl Acad Sci USA 1995; 92: 10119-22.
12. Reboredo NM, Diag A, Castro A, Villaescusa RG. Collection, processing and cryopreservation of umbilical cord blood for unrelated transplantation. Bone Marrow Transplant 2000; 26: 1263-70.
13. Armitage S, Warwick R, Fehily D, Navarrete C, Contreras M. Cord blood banking in London: The first 1,000 collections. Bone Marrow Transplant 1999; 24: 139-45.
14. Wong A, Yuen PMP, Li K, Yu A LM, Tsoi WC. Cord blood collection before and after placental delivery: Levels of nucleated cells, hematopoietic progenitor cells, leukocyte subpopulations and macroscopic clots. Bone Marrow Transplant 2001; 27: 133-8.
15. Harris DT, Schumacher MJ, Rychlik S, et al. Collection, separation and cryopreservation of umbilical cord blood for use in transplantation. Bone Marrow Transplant 1994; 13: 135-43.
16. Bertolini F, Corsini C, Lauri E, Gorin F, Sirchia G. Umbilical cord blood banking: Evaluation of the collection procedure and of CFU-GM content. J Hematother 1993; 2: 233-4.
17. Pahwa RN, Fleischer A, Than S, Good RA. Successful hematopoietic reconstitution with transplantation of erythrocyte-depleted allogeneic human umbilical cord blood cells in a child with leukemia. Proc Natl Acad Sci USA 1994; 91: 4485-8.
18. Fraser J, Cairo MS, Wagner E, et al. Cord blood transplantation study (COBLT): Cord blood bank standard operating procedures. J Hematother 1998; 7: 521-61.
19. Sousa T, De Sousa ME, Godinho MI, et al. Umbilical cord blood processing: volume reduction and recovery of CD34+ cells. Bone Marrow Transplant 1997; 19: 311-3.
20. Yang H, Acker JP, Abley D, McGann LE, Akabutu J. High-efficiency volume reduction of cord blood using pentastarch. Bone Marrow Transplant 2001; 27: 457-61.
21. Armitage S, Fehily D, Dickinson A, Chapman C, Navarrete C, Contreras M. Cord blood banking: Volume reduction of cord blood units using a semi-automated closed system. Bone Marrow Transplant 1999; 23: 505-9.
22. Ademokun JA, Chapman C, Dunn J, et al. Umbilical cord blood collection and separation for hematopoietic progenitor cell banking. Bone Marrow Transplant 1997; 19: 1023-8.
23. Pakdeesuwan K, Muangsup W, U-pratya Y, Issaragrisil S, Wanachiwanawin W. Clinical paroxysmal nocturnal hemoglobinuria is the result of expansion of glycosyl-phosphatidyl-inositol-anchored protein-deficient clone in the condition of deficient hematopoiesis. Inter J Hematol 2001;73: 64-70.
24. Issaragrisil S. Correlation between hematopoietic progenitors and erythroblasts in cord blood. Am J Clin Pathol 1983; 80: 865-7.
25. Almici C, Carlo-Stella M, Mangoni L, et al. Density separation of umbilical cord blood and recovery of hematopoietic progenitor cells: implications for cord blood banking. Stem Cells 1995; 13: 533-40.

การเก็บและเตรียมเลือดจากสายสะดือทารกแรกคลอดเพื่อเก็บไว้ในไนโตรเจนเหลว

เยาวลักษณ์ อู่ปรัชญา, วทม, สิริขวัญ บุญเหมาะ, วทบ*, อรทัย พรหมสุวิชา, วทบ*, จรรยา ธีระพิทยานนท์, วทม*, ลักษมี กะลันชัย, วทบ**, วิรัตน์ จันทร์เจอบุญ, วทม**, กรกช ศิริมัย, พบ***, สนั่น วิสุทธิศักดิ์ชัย, พบ*, ศศิธร เพ็ชรจันทร์, พบ**, สุรพล อิสรไกรศีล, พบ**

เลือดสายสะดือทารก (cord blood) มีเซลล์ต้นกำเนิดเม็ดเลือดจำนวนมากซึ่งเพียงพอแก่การปลูกถ่ายไขกระดูกชนิด allogeneic transplantation ในเด็ก ซึ่งนิยมทำการรักษาด้วยวิธีนี้มากยิ่งขึ้น ดังนั้นการเก็บเลือดและขบวนการเตรียมเลือดสายสะดือทารกจึงเป็นสิ่งสำคัญ การศึกษานี้ได้ทำการเก็บเลือดสายสะดือทารกด้วยถุงเก็บเลือด Top-Buttom Bag จากหญิงตั้งครรภ์ที่ได้รับการตรวจวินิจฉัยแล้วว่าทารกในครรภ์ไม่เป็นโรคธาลัสซีเมียจำนวน 34 ราย พบว่าสามารถเก็บเลือดสายสะดือทารกได้เฉลี่ย 120 $\pm$ 5 มิลลิลิตร (ปริมาตรตั้งแต่ 65-180 มิลลิลิตร) จำนวนเม็ดเลือดขาว (WBC) เฉลี่ย (9.36 $\pm$ 0.84) x 10^8 เซลล์ CD34+ เฉลี่ย (3.51 $\pm$ 0.52) x 10^6 เซลล์ต้นกำเนิดเม็ดเลือดแดง (BFU-E) และเซลล์ต้นกำเนิดเม็ดเลือดขาว (CFU-GM) เฉลี่ย (9.12 $\pm$ 1.60) x 10^5 และ (5.32 $\pm$ 1.23) x 10^5 ตามลำดับ และพบว่าจำนวนเซลล์ CD34+ มีความสัมพันธ์กับจำนวน WBC, BFU-E และ CFU-GM อย่างมีนัยสำคัญ (p เท่ากับ 0.001, 0.0105 และ 0.0001 ตามลำดับ) เลือดสายสะดือทารกจำนวน 18 รายที่มีปริมาตรรวมกับสารกันเลือดแข็ง (CPD-A) แล้วมีเกิน 100 มิลลิลิตรจะนำมาปั่นด้วยความเร็ว 1500 รอบ/นาที นาน 10 นาทีและแยก buffy coat ด้วยเครื่อง Optipress I ปีบแยก buffy coat ได้เฉลี่ย 70 $\pm$ 3 มิลลิลิตร มีค่า % recovery ของจำนวน WBC, เซลล์ CD34+, BFU-E และ CFU-GM เท่ากับ 89, 95, 109 และ 102 ตามลำดับ จึงนับว่าวิธีนี้มีประสิทธิภาพสูงมีการสูญเสียเซลล์น้อย เป็นการเก็บเลือดและเตรียมเลือดด้วยระบบปิด สะดวก รวดเร็วสามารถเตรียมเลือดเสร็จภายใน 1 ชั่วโมงหลังจากได้รับถุงเลือด

คำสำคัญ : เลือดสายสะดือทารกแรกคลอด, เซลล์ต้นกำเนิดเม็ดเลือด, CD34+

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