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Comparison of Three Methods in Umbilical Cord Blood Collection for Hematopoietic Stem Cell Transplantation

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ABSTRACT

Objective This study was undertaken to compare three methods of collection of human umbilical cord blood.

Design Randomized clinical trial.

Setting Department of Obstetrics and Gynaecology, King Chulalongkorn Memorial Hospital.

Subjects Sixty women who underwent vaginal deliveries were collected cord blood between October 1, 2001 and January 31, 2002.

Methods Sixty women with uncomplicated vaginal deliveries were randomized equally into three groups. One of three cord blood collection methods was applied to each woman. Method 1 was collection of cord blood by hanging method after delivering placenta. Method 2 was collection of cord blood into a standard blood bag while placenta is still in utero. Method 3 was collection of cord blood into a standard blood bag while placenta is still in utero, followed by flushing with sodium chloride solution mixed with 10% ACD anticoagulant through catheterized umbilical artery then collected placental blood from umbilical vein.

Main outcome measurement Analysis the quality and quantity of cord blood collection by comparison among the three groups of volume collected, total white blood cells, total $CD34^+$ progenitor cells and sterility.

Results Cord blood collection by the hanging method (method 1) resulted in a mean blood volume of 50.2±23.2 ml, mean total WBC count of $7.2\pm4.7x10^8$ cells, and mean total $CD34^+$cells of $2.3\pm2.1x10^6$cells.With collection of method 2, resulted in a mean blood volume of 56.9±20.6 ml, mean total WBC count of $8.0\pm4.0x10^8$ cells, and mean total $CD34^+$cells of $4.1+4.9x10^6$ cells. The method 3 resulted in a significantly higher ($P< 0.001$) of mean volume at 148.7±40.0 ml, mean total WBC of $15.9\pm11.0x10^8$ cells, and mean total $CD34^+$ of $6.7+8.6x10^6$ cells. Significant correlation was found between the volume and total white blood cells ($P<0.001$,$R=0.664$), the volume and total $CD34^+$ cells ($P=0.01$,$R=0.331$), and also between the total WBC and total $CD34^+$cells ($P<0.001$, $R=0.758$). No correlations were found with maternal age, gestational age, birth weight, placental weight, placental diameter, and cord length. No bacterial or fungal contamination was found in all cases.

Conclusion The syringe-assisted sodium chloride solution flush and drain collection method with a blood bag (method 3) was found to be the most effective method for human umbilical cord blood collection. This method gave the highest volume, the total white blood cells and total $CD34^+$ cells but the procedure was more complicated and needed more personals involved. The sterility test in this study was acceptable in all three methods.

Key words : Collection methods, human umbilical cord blood transplantation

Since bone marrow transplantation was first successfully done in 1968 on two children with immunodoficiencies,[1,2] the field of bone marrow transplantation (BMT) has advanced considerably. Bone marrow transplantation has been used to treat a variety of malignancies, hemoglobinopathies, immune deficiencies and congenital metabolic defects. For greatest chance of success, the HLA antigens of both donor and host must be fully matched.[3] However many patients requiring a bone marrow transplant are unable to find a related or an unrelated volunteer donor.[4]

Over the past decade, umbilical cord blood, a previously discarded material has been found to be efficacious as an alternative source of hematopoietic stem cells. Beginning in the early 1980s, it was shown that umbilical cord blood (UCB) contained high levels of hematopoietic progenitor cells.[5-7] The first umbilical cord blood transplantation (UCBT) was done in 1988 on a child with Fanconi's anemia from his HLA identical sibling.[8] Since the first successful umbilical cord blood transplantation, it was world wide used especially in children and low body weight recipient. Most of these patients have engrafted without severe graft-versus-host disease, even in transplants with one or two HLA antigen mismatches. Cord blood transplantation present several potentail advantages over bone marrow transplantation. Umbilical cord blood was abundantly available, and because it was an otherwise discarded material, its use obviously present no risk or discomfort for the donor. There was no need to find and evaluate the donor or to surgically remove the graft.[9] The risk of transmission of cytomegalovirus to the recipient was low because < 0.1% of healthy neonates were CMV positive, compared with 10-60% of adult volunteer donors. In addition, umbilical cord blood transplantation were associated with less graft-versus-host diseases than born marrow transplantation.[10]

Successful transplant outcomes depend on the quality and quantity of cord blood. One of the major determinants of the rate and speed of engraftment is the dose of cells provided to the transplant recipient. This can be expressed as the total number of nucleated cells CFU-GM or CD34-positive cells transplanted per kg of recipient weight.[11-13]

The optimal method for collection of cord blood is not known. The most widely used approach is to wait until delivery of placenta and then to place the placenta in a sterile supporting structure with the umbilical cord hanging through the support (Hanging method). An alternative method involves collecting the umbilical cord blood after the delivery of the child while the placenta is still in utero[3] (Aspiration from in utero placenta). Various cord blood collection methods should be compared regarding of safety, quality and quantity. Recently, Urial Elchalal et al. had proposed a method which was syringe-assisted with sodium chloride solution flush and drain with a blood bag comparing between open system and semi-closed system. They found that this method was effective but open method was not acceptable due to high contamination rate. Comprehensive studies are therefore greatly needed to evaluate the efficiacy of various collection methods. So the aim of this study was to compare between these three methods of closed/semi-closed system: (1) hanging method after delivering plaenta (closed system), (2) collecting from in utero placenta (closed system), (3)Collecting from in utero placenta, followed by syringe-assisted sodium chloride solution flush and drain with blood bag (semi-closed system).

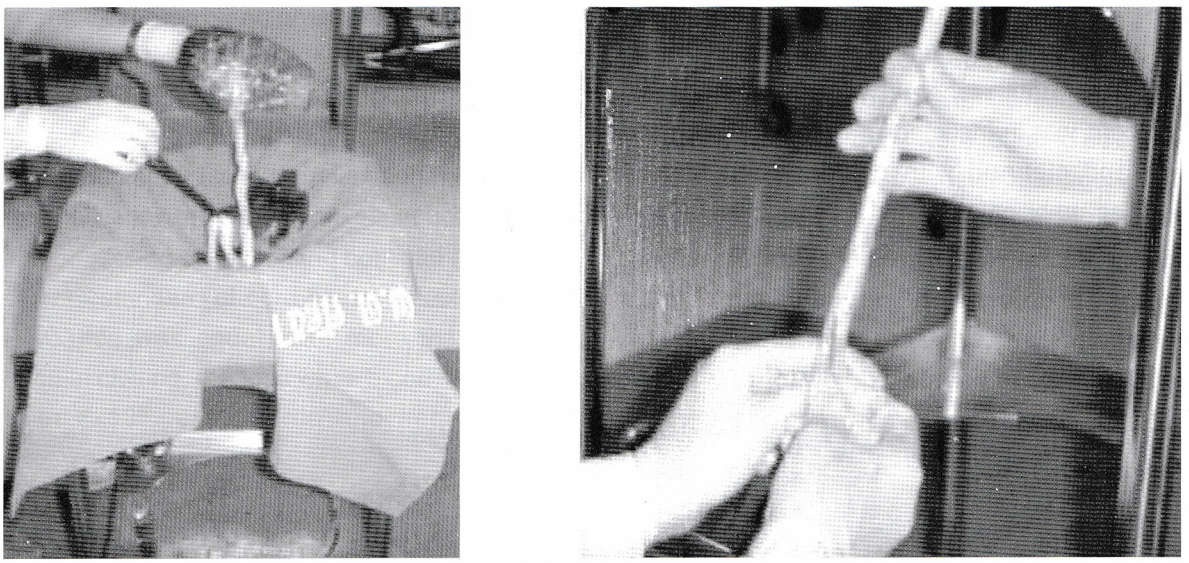
Material and Methods

Patient population. The collection of cord blood was performed in the delivery room of the Department of Obstetrics and Gynecology at King Chulalongkorn Memorial Hospital, Thailand, during October 1st,2001 to January 31st, 2002 by an OB-GYN resident trained in cord blood collection. Sixty pregnant women age 15-41 years old who underwent spontaneous normal vaginal delivery participated in the study. All women were interviewed regarding medical history including inherited hematologic diseases (such as thalassemia, hemophilia, etc), sexually transmitted diseases, history of blood transfusion by medical counsellor. Inclusion

criteria included the followings: singleton pregnancy, gestational age ≥ 37 weeks, birth weight ≥ 2500 gm. Exclusion criteria included the followings: any known genetic disease; positive serologic result for human immunodeficiency virus; hepatitis B virus, syphilis, maternal fever ≥ 37.8°C or received any antibiotics during or immediately before delivery; ruptured of membranes ≥ 18 hours or prolonged labor ≥ 24 hours before delivery; severe obstetric complication; still birth or congenital anomalies. This study was approved by the Ethical Committee of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand, and all participants gave informed consent.

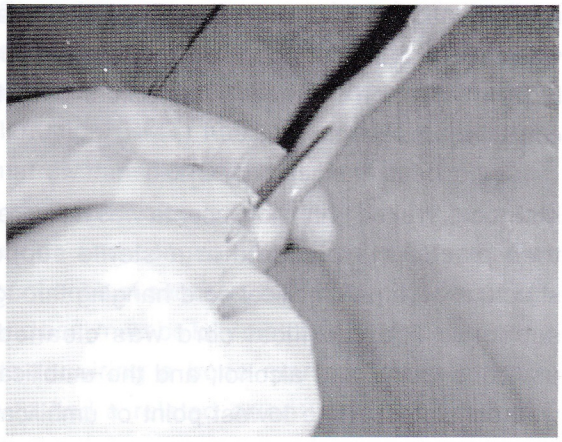
Collection of cord blood: Sixty participants were systematically allocated into three groups by block randomization. After delivery of the baby, umbilical cord was clamped and cut about 2-3 centimeters from the baby then each method of umbilical cord blood collection was performed.

Method 1 Waited 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 cleaned with povidone-iodine and alcohol, and the umbilical vein was canulated at the lowest point of umbilical cord using a needle connected to a standard blood collection bag with Citrate Phosphate Dextrose Anticoagulant (CPD-A) solution. The umbilical cord blood was then collected by gravity drainage.



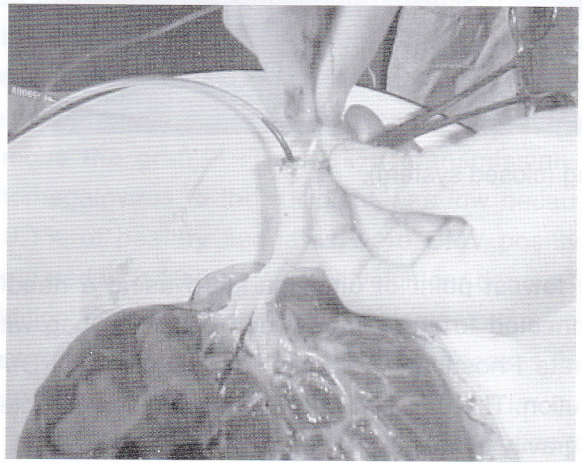
Picture 1. Method 1 - Hanging (closed system).

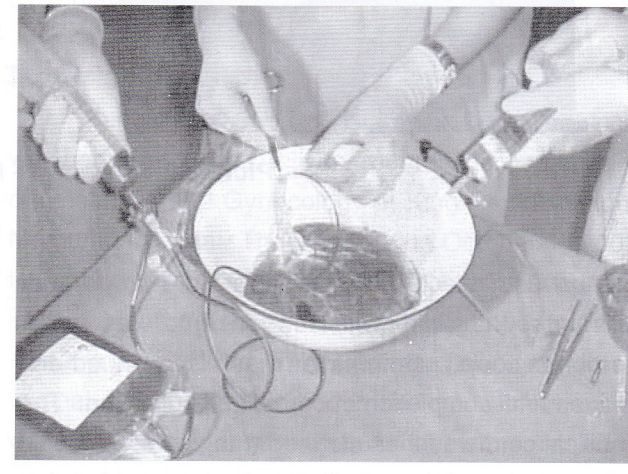
Method 2 Collected the umbilical cord blood after delivery of the child while the placenta was still in utero. Oxytocin augmentation was discontinued and delayed until completion of cord blood collection. The umbilical cord was then cleaned with povidone-iodine and alcohol, and the umbilical vein was canulated at the lowest point of umbilical cord using a needle connected to a standard blood collection bag with Citrate Phosphate Dextrose Anticoagulant (CPD-A) solution. The umbilical cord blood was then collected by gravity drainage.



Picture 2. Method 2 - Placental in utero (closed system).

Method 3 Collected the umbilical cord blood as in method 2 then until placental was delivered, then umbilical cord was transected at 5 centimeters from placenta and cleaned with povidone-iodine and alcohol. The umbilical vein and one umbilical artery were canulated with feeding tube number 8 and 5 respectively. A 100 ml aliquot of isotonic sodium chloride solution mixed with 10% ACD-A was injected through umbilical artery and draw the remaining placental blood via umbilical vein using three-way connector connecting into the previous standard blood collecting bag.





Picture 3. Method 3 - Syringe-assisted sodium chloride solution flush and drain (semi-closed system).

While collecting of cord blood in each method, the sterile blood bag must be swung continuously for prevention of blood clot.

Cord blood collection time required about 5 minutes by method 1, 10 minutes by method2 and 15 to 20 minutes by method 3. All step of cord blood collection was done by aseptic technique. After umbilical cord blood collection was completed, the weight of infant, placental weight, placental diameter and cord length were collected. Cord blood bag were sent to the National blood bank for processing, freezing and storage in order to collecting data of mean collected cord blood volume (calculated from volume of collected cord blood subtracted with volume of anticoagulant), differential count, mean total nucleated cells (by automated cell counter), $CD34^+$ cells (by flow cytometry) and viability (by trypan blue). In addition, blood sample for sterility test were sent for bacterial culture (aerobic and anaerobic bacteria) and fungal culture. The microbiologic bacterial culture media were tryptic soy broth (aerobic culture), thioglycollate broth without indicator (anaerobic culture) and then processing by incubating bottle for 7 day. For fungal culture media, we used sabouraud broth and processing by incubating bottle for 1 month. The minimum standard of cord blood collected were that the volume was equal to or more than 50 ml without partial clotting, the total white blood cells were more than $4x10^8$ cells, sterile and negative for transmissible infectious markers.

Statistical methods: With twenty women in each group, a test with a significant level of $\alpha = 0.05$ would detect statistical difference. The comparison between values of the quantitative variable in the three groups was done by an analysis of variance test or Kruskal-Wallis test. After the differences existed, we used Bonferroni test or Mann-Whitney test to perform pairs 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. The data were analyzed with the SPSS version 7.5.

Results : The study included 60 cord blood collections that allocated systematically into three groups. Each method described above was applied to each group. The maternal age range was 15 to 41 years. Gestational age at delivery was 37 to 42 weeks. Birth weight was 2500 to 4100 g. The characteristics among the three groups were similar as shown in table1. The three groups were found to be similar with respect to maternal age, gestational age at delivery, birth weight, placental weight, placental diameter, and cord length in this study. No significant correlations were found between the volume of cord blood collected and maternal age, gestational age at delivery, birth weight, placental weight, placental diameter, and cord length in this study.

Table 1. Characteristics of 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	3107.5 ± 234.0	560.5 ± 97.9	19.2 ± 2.8	51.8 ± 12.7
2	23.85 ± 7.8	38.6 ± 1.6	3057.3 ± 417.3	591.5 ± 108.7	20.3 ± 2.9	53.0 ± 9.2
3	23.65 ± 4.5	38.6 ± 1.4	3063.0 ± 300.1	575.0 ± 94.5	18.9 ± 2.5	47.7 ± 9.3

Results are presented as mean ± SD.

In the method 1 and method 2, most collected cord blood volume (100% and 95% respectively) was less than 100 ml. There were 9 cases (45%) in the method 1 and 8 cases (40%) in the method 2 that collected volume was less than 50 ml. Conversely, most collected cord blood volume (75%) in the method3 ranged 100-200 ml, and none had cord blood volume less than 50 ml (Table 2).

Table 2. Volumes of cord blood collected by each method

Method	< 50ml	51-100 ml	101-150 ml	151-200 ml	>200 ml	Range (ml)
1(n=20)	9	11	0	0	0	10-95
2(n=20)	8	11	1	0	0	25-109
3(n=20)	0	3	7	8	2	69-202

All results except range are numbers of patients.

For calculating volume of each method, anticoagulant (30ml) and weight of blood bag (35 gm) were subtracted.

The method 1 in this study yielded the smallest volume of cord blood collected, with a mean (± SD) of 50.2 ± 23.2 ml (range 10-95 ml). Similarly, in the method 2, the mean (± SD) of cord blood volume collected was 56.9 ± 20.6 ml (range 25-109 ml). No statistical significant differences in volume of cord blood collection between method 1 and 2 (P=1.000), (Table 3). The method 3 succeeded in obtaining volumes of umbilical cord blood significantly higher than that obtained with method 1 and 2 (P<0.001), with a mean (±SD) of 148.7 ± 40.0 ml (range 69-202 ml), (Table 4).

Table 3. Cord blood data

Data	Method 1	Method 2	Method 3	Statistical significance
Volume (ml)	50.2±23.2	56.9±20.6	148.7±40.0	P = 0.001*
Total WBC (x10^8 cells)	7.2 ± 4.7	8.0 ± 4.0	15.9 ± 11.0	P < 0.001*
Total CD34 (x10^6 cells)	2.3±2.1	4.1±4.9	6.7±8.6	P = 0.05**

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

*** Kruskal Wallis Test (The mean difference is significant at the 0.05 level)*

Table 4. Comparisons of the cord blood data

Data	Method		95% Confidence Interval		Statistical significance
			Lower	Upper	
Volume	1	2	-29.48	16.08	P = 1.000*
	2	3	-114.58	-69.02	P < 0.001*
	3	1	75.72	121.28	P < 0.001*
Total WBC	1	2	-6.49	4.90	P = 1.000*
	2	3	-13.59	-2.20	P = 0.003*
	3	1	2.99	14.38	P = 0.001*
Total $CD34^+$	1	2	18.27	22.73	P = 0.229 **
	2	3	17.10	23.90	P = 0.066 **
	3	1	14.40	26.60	P = 0.001 **

* Bonferroni (The mean difference is significant at the 0.05 level)

** Mann-Whitney Test (The mean difference is significant at the 0.013 level)

The total white blood cells for method 1, 2, and 3 were 7.2 ± 4.7 x 10^8 cells, 8.0 ± 4.0x10^8 cells, and 15.9 ± 11.0 x10^8 cells, respectively, which is statistically significant comparing method 3 with other two methods (P = 0.001 and P = 0.003, respectively), (Table 4). Significant correlations were found between the volume of cord blood collection and the total white blood cells (P<0.001, R=0.664) as shown in Fig. 1.

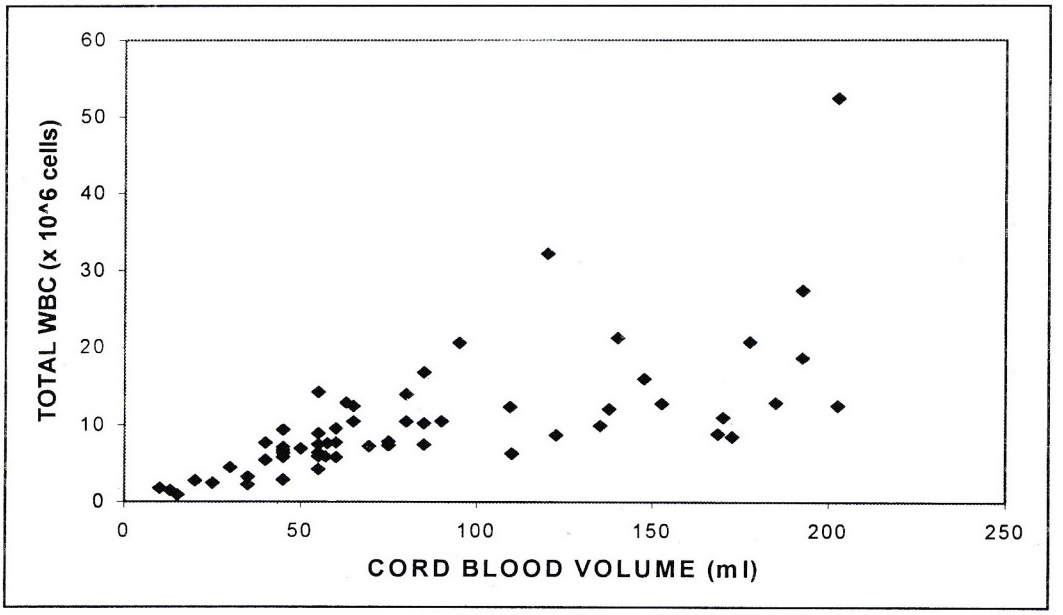


Fig. 1. The relation between total WBC and cord blood volume (R = 0.664).

The total $CD34^+$ progenitor cells were statistically different among the three methods of cord blood collections with a mean ($\pm$ SD) of 2.3 $\pm$ 2.1 $x10^6$ cells, 4.1 $\pm$ 4.9 $x10^6$ cells, and 6.7 $\pm$ 8.6 $x10^6$ cells, respectively (P= 0.005), (Table 3). The method 3 succeeded in obtaining total $CD34^+$ progenitor cells higher than that obtained by method 1 and 2. There was statistical significant in total $CD34^+$ progenitor cells between method 1 and 3 (P=0.001), (Table 4). Significant correlation was found between the volume of cord blood collection and the total $CD34^+$ progenitor cells (P= 0.01, R=0.331), (Fig. 2). Significant correlation was found between the total $CD34^+$ progenitor cells and total white blood cells (P<0.01, R=0.758), (Fig.3).

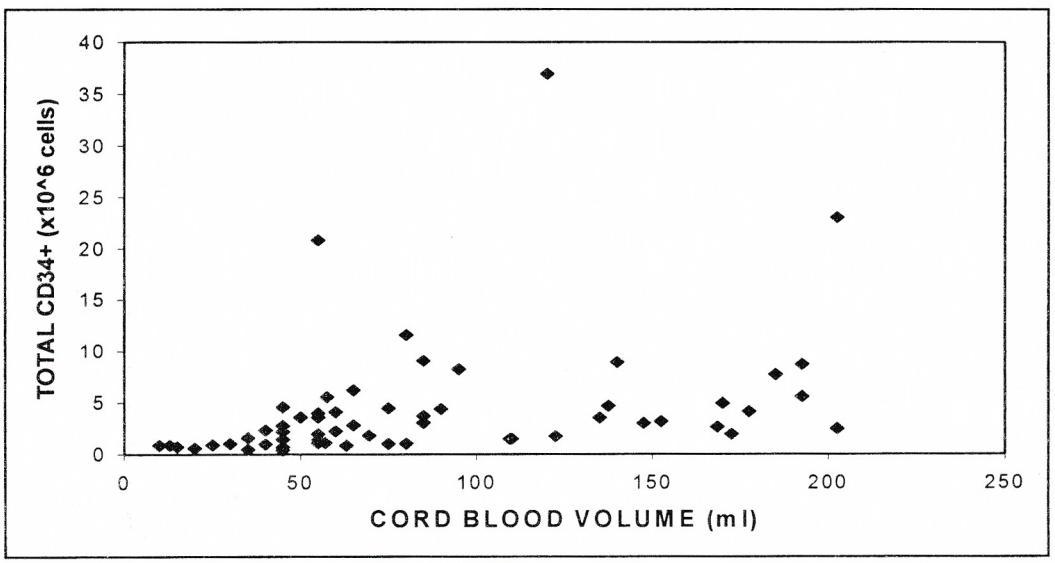


Fig. 2. The relation between total $CD34^+$ and cord blood volume (R = 0.331).

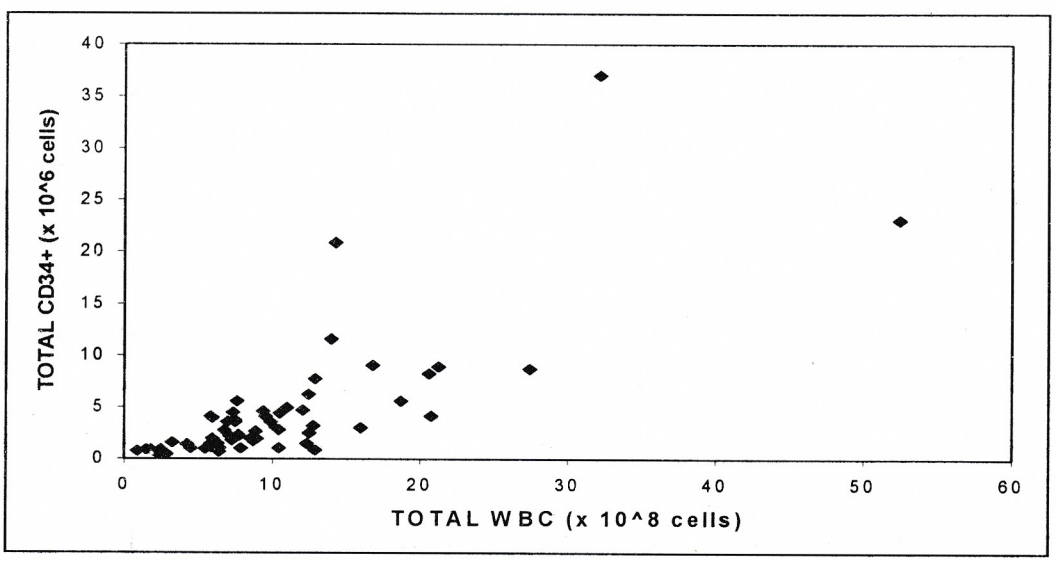


Fig. 3. The relation between total WBC and total $CD34^+$ (R = 0.758).

The viability of white blood cells were significantly high(>95%) in all of the three methods. No difference was found in viability between the method 1, 2, and 3.

All cord blood collected was analyzed for bacterial contamination (aerobes and anaerobes) and fungal. No contamination bacterial or fungal contamination was found in all cord blood in this study.

Comment

It is widely accepted that cord blood is a good alternative to bone marrow transplantation. Up to now, more than 2,000 patients world wide have undergone umbilical cord blood transplantation[14] and about 20 cases of related cord blood transplantation were done in Thailand.[15] Many diseases have been proved to be cured by umbilical cord blood transplantation.[14]

The success rate of umbilical cord blood transplantation mainly depends on quality and quantity of collected cord blood including sterility and negative for transmissible infectious markers. Patients who received more than $1.5x10^7$ nucleated cells/kg or $1.5x10^5$ $CD34^+$ cells/kg had favorable engraftment despite unrelated match or related mismatch cord blood.[16] For these reasons, the optimal method for cord blood collection is regarded. Currently, the methods of cord blood collection with closed/ semi-closed system include hanging method after delivering placenta, collecting from in utero placenta, syringe-assisted sodium chloride solution mixed10% ACD-A flush and drain with a blood bag.

The optimal method for cord blood collection is still not encountered. Recent study by Uriel Elchalal et al. found that syringe-assisted normal saline flush & drain with closed system was the optimal method for cord blood collection but contamination was still found in this method. Our study aimed to compare three methods which had not been compared previously.

Method 1 was simple and did not need high experience for collection but the success of this method was depend on the time to collect cord blood before it became clotted. Method 2 needed more experience in collection cord blood. Contraction might increase volume of collection by induction of blood in uteroplacental circulation but this method might reduce collected blood volume because of early placental seperation and decrease blood flow from placenta. Method 3 had advantage as it was not affected by timing of placental delivery because the 1st collection is done while the placenta was in utero and continue the 2nd collection after placental delivery. Normal saline flush & drain might also increase blood volume remained in placental circulation. But this method was more complicated and took more time than the other two methods, thus it had high risk for contamination and required high experience and well-trained team for cord blood collection.

In this study, method 1 (hanging method) resulted in relatively small volumes of blood collection, total WBC, and total $CD34^+$cells. Method 2 compared with the hanging method, was higher in result, but there was no statistical different in both methods. Method 3 resulted in larger volumes of cord blood collection (triple than method 1 and double than method 2), with double amount of total WBC and the largest numbers of total $CD34^+$cells, thus providing its superiority. Although the collection of cord blood was performed by only one team leaded by a well-trained physician, some pitfalls could not be rule out. There were a few cases in this study which volume was lower than expected. In method 1, when the placental delivery took longer time, so that the clotted blood occurred and decreased the volume collected. In contrast with method 2, although the oxytocin was discontinued, the placental separation might sometime occur earlier than expected and the blood collection could not be continued. These were not problems with method 3, which the first collection was done while the placenta in utero and the second collection was continued after the placenta was delivered, thus it was not effect by the timing of the placental delivery.

The sterility of cord blood collection was an additional important factor in selection the optimal method for cord blood collection. All the three methods were closed/semi-closed system and no bacterial or fungal contamination in all specimens. The

best sterility in this study was due to the meticulous control in all steps of collection by trained cord blood collection team. Although method 3 required more exposure and took more time in collection than method 1 and 2, no contamination was found in this method, similar to the other methods.

Because method 3 gave the highest cord blood volume, total WBC, total $CD34^+$ progenitor cells and good sterility, therefore our study suggested that the method 3 was the optimal method for collection of human umbilical cord blood.

In this study, there was no correlation between the cord blood collection with maternal age, gestational age, birth weight, placental weight and cord length. The correlation was found significantly between and total WBC and total $CD34^+$ progenitor cells. In other study, it was also found the correlation between cord biood volume and length of gestational, time from delivery of the infant to cord clamping and placental weight.[17] These factors which effect the cord blood collection should be further studied in the future in order to get the optimal method for umbilical cord blood collection.

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