J. of Obst. & Gyn. of India Vol. 52, No. 1 : January/February 2002 Pg 92-96

Blood Collection for Umbilical Cord Stem Cell Transplant: Optimized Methods

Deepika Deka, Bonilla Nayar, Kamal Buckshee, Deep Takkar, Vinod Kochupillai, Vinod Kumar Paul

Dept. of Obst and Gyn, Paediatrics and Medical Oncology, All India Institute of Medical Sciences, New Delhi – 110029.

Summary

Umbilical "waste" Cord Blood is a very rich source of progenitor stem cells. Collection needs to be done in an efficient and timely manner in amounts sufficient for engraftment at Umbilical Cord Blood Stem Cell transplant.

The aim of the study was to establish the optimized methods of cord blood collection, and to estimate the volume of blood collection.

Umbilical cord blood was collected after delivery in 60 cases (open method – 30 cases and closed method – 30 cases). Umbilical cord was clamped and cut immediately after delivery of baby, after 20 seconds or on cessation of cord pulsations. Mean volume of blood collected were 67.8 ± 22.7 ml and 55.7 ± 21.0 ml with open and closed methods respectively. Maximum volume of blood was obtained when cord was clamped immediately after delivery of baby (77.0 ± 22.5 ml).

Introduction

Many hematological diseases like aplastic anemia, B thalassemia, leukaemia originate from disorders of function or number of stem cells. They are potentially curable by replacing these stem cells with those from normal HLA matched donors. Traditionally, the hematopoietic progenitor cells necessary for engraftment have been obtained from bone-marrow for bone marrow transplant (BMT) (Thomas & Strol, 1975). However bone marrow harvesting is an invasive and painful procedure. The search for other sources of progenitor cells have led to development of stem cell transplant protocols from sources like fetal liver (Kochupillai & Sharma, 1991) and mobilized peripheral blood (Levin et al 1963). As early as 1974, Knudtzon demonstrated the presence of hematopoietic progenitor cells in umbilical cord blood (UCB) collected at delivery. It was Ted Boyse (unpublished work) who first proposed that cord blood could serve as a potential source of transplantable progenitor cells. Since then "in vitro" studies have shown that UCB is a rich source of hematopoietic stem cells. Enough stem cells appear to be present in a typical 60-100ml of cord blood that can be obtained after delivery of the infant, and reliably engraft by Umbilical Cord Blood Transplant (UCBT) a child weighing upto 40kg and possibly even adult patients (Broxmeyer et al 1992).

In this set up, the obstetrician plays a major role in collection of cord blood after delivery. Collection needs to be done in an efficient and timely manner without neglecting the needs of the mother or baby immediately after birth. Umbilical cord blood collection as a source of hematopoietic progenitor cells for umbilical cord blood transplant must be of amount sufficient for engraftment. For most obstetricians, there is little experience in collection of UCB other than for diagnostic testing where only 5-10ml blood is collected.

A term baby has around 300ml of blood, another 100-125ml in the placenta and cord, with a total volume

of 400-425ml in the Feto-Placental Unit. Most investigators are able to collect 40-125ml of blood (50-70ml on average), though using "optimized method" as much as 200ml has also been harvested. Studies by Broxmeyer et al (1989), Bertolini et al (1995), Cai et al (1996), O'Neil (1996) indicate that 50-200 ml of cord blood could be collected from "waste" cord and placenta. A 100 ml sample of cord blood is expected to contain 2 x 10^7 myeloid (CFU-GM) and 1 x 10^7 erythroid progenitor cells (Lunch et al 1989).

The present study was undertaken to establish the optimized technique of umbilical cord blood collection and to estimate the volume of cord blood collected.

Material and Methods

Umbilical cord blood collection at delivery was carried out in 60 cases. This group was further subdivided according to the method of collection and time of cord clamping:

a. **Open method:**
   Immediate clamping of cord after delivery of baby – 10 cases
   Cord clamping after 20 seconds – 10 cases
   Delayed clamping after cord pulsations ceased – 10 cases
b. **Closed method:**
   Immediate clamping – 10 cases
   After 20 seconds – 10 cases
   Delayed – 10 cases

Informed consent was taken. Age, parity, period of gestation at delivery and pregnancy complications were noted. Weight of newborn, sex, time of birth and apgar score were recorded. Cases with antepartum haemorrhage and Rh isoimmunization were excluded. Blood collection bags/sterile bottles containing anticoagulant were kept ready before delivery.

Umbilical cord blood was collected after 44 vaginal and 16 caesarean section deliveries. At vaginal delivery either open or closed method was used alternately, while at caesarean section collection was made by closed method only.

Open Method

Cord blood was collected with placenta-in-utero in bottles containing anticoagulant. After delivery of the baby, cord was doubly clamped and cut between clamps 5-7 cms. in way from the baby (one clamp towards maternal side and second clamp towards fetal side) at the specified time in the protocol and baby handed to pediatrician. With placenta still in utero, cord was cleaned with betadine and unclamped over sterile, wide mouth bottle containing anticoagulant – 20ml of citrate phosphate dextrose or heparin (2ml, 5000 IU, ml). Collection was made till the blood flow ceased, gently shaking the bottle to mix the anticoagulant. Milking of cord was done to enhance blood flow.

Closed Method

After birth of the baby, the cord was clamped at the specified time. Cord blood collection was begun after delivery of the placenta in standard blood collection bags. After delivery of placenta (placenta ex utero), it was held at its edges and suspended from a specially designed frame with the fetal surface and cord downwards. Cord was cleaned with betadine. The umbilical vein was punctured with the needle of sterile blood collection bags and blood was allowed to flow by gravity into the bag which was rotated gently continuously to mix blood with anticoagulant. Milking of cord was done to enhance blood collection. The blood bags contained anticoagulant (20ml) citrate phosphate dextrose. After the blood flow stopped, the tubing of blood bag was clamped and needle removed. Engorged vessels over the fetal surface of placenta were cleaned and aspirated with needle and syinge and volume obtained noted.

Measurement of Volume of collected cord blood

The volume of collected cord blood was measured using 1 or 2 100ml graduated cylinders. The volume of anticoagulant was subtracted and volume of collected cord blood was calculated.

Descriptive statistics, student's t test, one way ANOVA test and chi-square test were used for analysis of the results.

Results

At 44 vaginal and 16 caesarean section deliveries, umbilical cord blood was collected. Open method was used in collection of cord blood at 30 vaginal deliveries while closed method was used in collection of cord blood at 14 vaginal and 16 caesarean section deliveries.

After immediate clamping of the cord, the volume (mean ± SD) of cord blood collected by open method was 89.9 ± 18.5 ml while by closed method it was 63.2 ± 18.5ml The total volume (mean ± SD) of cord blood collected was 77.0 ± 22.5 ml (Tables I,II). Cord clamping after 20 sec of delivery, volume of cord blood obtained was 61.1

Table I: Relationship between volume of cord blood collected with method of collection and time of cord clamping

Time of cord clamping	Open method		Closed method		p-value	Significance
	No. of Cases	Volume (ml) Mean ± SD	No. of Cases	Volume (ml) Mean ± SD		
Immediate	10	89.9 ± 18.5*	10	63.2 ± 18.5*	0.01	S
After 20 sec	10	61.1 ± 12.9	10	60.6 ± 24.5	0.10	NS
Delayed	10	52.5 ± 10.1	10	43.4 ± 16.7	0.40	NS
Total	30	67.8 ± 22.7	30	55.7 ± 21.0	0.08	NS

± 12.9 and 60.6 ± 24.5 ml by open and closed methods respectively. The total volume (mean ± SD) of cord blood collected after 20 sec of cord clamping was 60.8 ± 19.1 ml. The volume (mean ± SD) of cord blood collected was 52.5 ± 10.1 and 43.4 ± 16.7 ml by open and closed methods respectively after delayed clamping. Total volume (mean ± SD) of cord blood after delayed clamping was 48.0 ± 14.0ml.

Table II: Time of cord clamping and volume of cord blood harvested

Time of cord clamping	Sample size	Volume of cord blood (ml) Mean ± SD
Immediate	20	77.0 ± 22.5*
After 20 sec	20	60.8 ± 19.1*
Delayed	20	48.0 ± 14.0*
Total	60	62.03 ± 20.84
		* $p < 0.05$

The volume of cord blood collected after immediate clamping was 26.6% and 60.4% larger than volumes collected after 20 sec and delayed clamping respectively. This result was statistically significant. The volume of cord blood collected after 20 sec clamping was 26.6% larger than that collected after delayed clamping and this was also statistically significant (Table II).

By open method the volume of cord blood collected was 89.9 ± 18.5 ml, while that collected by closed method was 63.2 ± 18.5 ml when cord was clamped immediately and this was statistically significant. There was no significant difference in volume of cord blood retrieved by open/closed methods when cord was clamped after 20 sec or at delayed clamping.

Discussion

In our study the mean volume of cord blood collected was 62.03 ± 20.84 ml., (range – 28-123ml). Paxson (1979) showed that an average of 70 ml of cord blood was obtained following a single needle puncture of the umbilical vein by open method. On three occasions difficulty in collection of cord blood was encountered and only 6, 20 and 25ml were obtained. Broxmeyer et al (1992) collected 101 samples of cord blood at delivery after immediate clamping of the cord by open method. The average blood volume collected was 56.3 ± 2.4ml.

In validation studies by O'Neil (1996) 161 cord blood units were collected by 20 obstetricians at term deliveries using closed method. The cumulative mean volume was 67.66 ± 25ml (range 20-140ml). In a study by Auerbach et al (1990) – cord blood samples were collected in 4 cases. Cord was immediately clamped after delivery. Blood was recovered with the placenta in utero and also after delivery of the placenta to optimize the collection.

One of the major determinants of blood volume collection is placental transfusion in the time interval between the delivery and cord clamping. Studies have shown that delayed clamping is associated with a reduced volume of residual placental blood (DeMarsh et al 1942, Nyberg & Westin 1958, Whipple et al 1957). One-quarter of placental transfusion or about 40 ml of blood enters the infant within 15 secs, and one half (80 ml) within 60 secs after birth (Lind, 1965). Delayed clamping allows placental transfusion to occur hence the residual blood volume was less whereas after immediate clamping there is no/little transfer of placental blood to newborn and residual placental blood

Table III: Volume of umbilical cord blood collected at delivery

Study	No. of cases	Mean volume (ml)	Range (ml)
Broxmeyer et al (1992)	101	56.3 ± 2.4	-
Auerbach et al (1990)	4	-	99-283
O'Neil (1996)	161	67.6 ± 25	20-140
Present study	60	62.0 ± 20.8	28-123

volume and hence cord blood harvest was larger. Bertolini et al (1995) also harvested greater cord blood volume after 20-30 sec clamping than after delayed clamping as also reported by Yao et al (1969) and Philip (1971). Bertolini et al (1995) made a comparative study of different procedures for collection and banking of umbilical cord blood. Data collected in the study indicate that both the open and the closed systems allow an average collection of 75ml UCB. Timing of umbilical cord clamping influenced the volume of collection. When cord was clamped within 30 sec after delivery, 77 ± 23ml CB was collected whereas this volume was reduced to less than one-half when cord clamping was delayed.

In our study, after immediate clamping, the volume of cord blood collected by open method (placenta in utero) was 42% larger than by closed method (placenta ex utero). This is because uterine contractions during third stage exert a squeezing action and force greater volume of placental blood to drain into collecting bottles (Apperley, 1994). With closed method (placenta ex utero) the effect of uterine contractions are lost. After 20 sec and delayed clamping similar volumes of cord blood were harvested by either open or closed method. This may be explained by the fact that uterine contractions had already forced sufficient placental blood into infant circulation before the cord was clamped.

Because the onset of blood clotting in umbilical cord and placental veins is relatively slow a slight delay in retrieving the blood is permissible. Therefore, collection could be accomplished outside the delivery room without disturbing mother and infant care activities. Open method was found to be easy but interfered with the obstetrician's work though the collection was completed within 5 min. O'Neil (1996) reported collection time of 2-4 minutes. The closed method required procurement of blood bags from blood banks, and did not interfere with the obstetrician's work.

Using "optimized method" collection of 150-300 ml has been described. In many instances gentle "milking" of the cord was done. Blood was collected patiently for as long as the flow lasted. When it ceased, the umbilical cord was transected 2 cms above the free end and gently "milked" until flow stopped. After the placenta was delivered, placental blood was obtained by gentle needle aspiration of engorged vessels on the fetal surface until vessels collapsed.

Harris (1996) analysed 5 methods of cord blood collection. Human umbilical cord blood (UCB) samples were obtained from normal full term vaginal deliveries. Techniques analyzed for blood collection were: (1) drawing as much blood as possible from the umbilical vein with two or three, 50 ml syringes while the placenta was in uterro (Syringe method); (2) cannulation of the umbilical vein in a standard blood donor set after delivery of the placenta (Blood Bag method); (3) collection with syringes with the placenta in utero followed by injection of heparinized saline (20-50 ml) into the delivered placenta and withdrawing additional blood by syringe (Syringe/Flush method); (4) the same method as in (3) except that after injection of heparinized saline the end of the cord was cut and the cord allowed to drain into a sterile container (Syringe/Flush and Drain method); and (5) the same method as in (3) except that after injection of the heparinized saline the umbilical vein was cannulated with a standard blood donor set for additional blood collection (syringe/Flush/Blood Bag method). Prior to collection of umbilical cord blood by any of these methods the cord was wiped with alcohol, followed by betadine to ensure sterility of the collections. Efforts were made to obtain maximal volumes for each collection.

In conclusion, obstetricians may soon be called upon to collect adequate umbilical cord blood samples which we now consider 'waste' for umbilical cord blood stem cell transplant programmes. Therefore, an understanding of the feto placental unit and expertise in techniques of collection of umbilical cord blood in

Table IV: Relationship between time of cord clamping and volume of cord blood harvested.

Study	No. of Cases	Time of cord clamping	Volume of cord blood (mean)
Yao et al (1969)	12	5 sec	126.6 ml
	17	30 sec	95.8 ml
Philip (1973)	28	5 sec	103 ml
	29	94 sec	45 ml
Bertolini et al (1996)	378	30 sec	77 ± 23 ml
	67	35-180 sec	31 ± 18 ml
Present study	20	< 5sec	77.0 ± 22.5 ml
	20	20-30 sec	60.8 ± 19.1 ml
	20	35-180 sec	48.0 ± 14.1 ml

optimal amount is very essential. Immediate clamping of cord with "optimized" methods enables maximum collection of cord blood necessary for stem cell transplant and engraftment.

References

1. Auerbach AD, Lui Q, Ghosh R, Broxmeyer HE. 30: 682, 1990.
2. Apperley JF. 14: 187, 1994.
3. Bertolini F, Lazzari L, Lauri E, Corsini C, Castetli C, Gorini F and Sirchia G. Journal of Hematotherapy 4: 29, 1995.
4. Broxmeyer HE, Douglas GW, Hangoc G, Cooper S, Bard J, English D, Army M. Proc Natl Acad Sci 86: 3828, 1989.
5. Broxmeyer HE, Hangoc G, Cooper S, Riberio R, Graves V. Proc natl Acad Sci 89: 4109, 1992.
6. Cai WZ, Yu XL, Chen LH. Chung-Hua-Li-Tsa-Chih 31: 5, 1996.
7. De March QB, Windle WF, Alt HL. Am J Dis Child 63: 1123, 1942.
8. Harris DT. J Hematother 5: 1238, 1996.
9. Knustzon S. Blood 43: 357, 1974.
10. Kochupillai V, Sharma S. Eur J Hematol 47: 319, 1991.
11. Levin RH, Whang J, Tjio JH. Science 142: 1305, 1963.
12. Linch DC, Knott LJ, Rodeck CH. Blood 59: 976, 1982.
13. Lind J. Canad Med Assoc J. 93: 1091, 1965.
14. Nyberg R, Westin B. Scand 47: 350, 1958.
15. O'Neil Brien. Curr Probl. Obstet Gynecol Fertil 19: 65, 1996.
16. Paxson CL. Am J Obst Gyn 134: 708, 1979.
17. Philip GSA. Obstet. Gynecol; 42; 334, 1973.
18. Thomas ED, Strob R. N Engl J Med 292: 832, 1975.
19. Whipple GA, Sisson TRC, Lund CT. Obstet Gynecol 10: 603, 1957.
20. Yao AC, Moinian M, Lind J. Lancet 2: 871, 1969.