

Autologous umbilical cord blood transfusion

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Abstract

The purpose of this study was to examine some aspects of umbilical cord blood collection for autologous transfusion in premature infants. All 120 microbial cultures (aerobic and anaerobic) of cord blood samples as well as 30 cultures of mycoplasma were treated. Cord prothrombin fragment (F 1+2) concentrations were quantified at one and 10 minutes after clamping of the cord. F 1+2 concentrations assessed on 25 newborn infants were similar and no linear association with time of clamping could be drawn. This means that cord blood thrombosis is not activated for at least 10 minutes following clamping of the cord. As far as is known, the first newborn infant to benefit from this method of transfusion is reported here. The premature infant received two portions of autologous blood (on days 5 and 7). No untoward effects were noted.

Blood, collected from the umbilical cord, is a safe source for autotransfusion, provided that bacteriological testing has been carried out.

(Arch Dis Child 1995; 73: F181–F183)

Keywords: anaemia of prematurity, cord blood, autologous transfusion.

Studies have shown that very low birthweight infants receive about 50 ml of blood per kg from as many as eight to 10 adult donors during the first month of life.¹ The use of adult donor blood poses risks for these infants, including transmission of viral agents such as hepatitis B, cytomegalovirus, and HIV, as well as graft versus host disease and other complications.^{1,2} Placental vessels contain a quarter to a third of the newborn blood volume – blood which is currently discarded. Following preliminary studies on collection and storage of cord blood,^{1,3} we have further expanded the experiments on sterility of the collected blood and its coagulation system.

To examine the possibility of thrombin activation caused by clamping of the cord, we quantified the concentration of prothrombin fragments (F 1+2) one and 10 minutes after clamping the cord. We report the first premature infant who benefited from cord blood transfusion following storage of the blood.

Methods

All the studies were carried out in the Edith Wolfson Medical Center, Holon, Israel, with the approval of the institutional committee of ethics. Some of the preliminary results have

been published elsewhere.³ The salient features of the preliminary work done before the autologous umbilical cord blood transfusion (AUCBT) was performed are summarised briefly below.

The collection of umbilical cord blood was initiated immediately following delivery. After clamping the cord a distal portion of the cord was cleansed with betadine and alcohol 70% for five seconds (the same procedure was performed following caesarean sections). A 21 gauge needle of a quadruple blood collection set (Travenol Co) was introduced into the umbilical vein. The collecting bag contained CPDA-1 in the recommended ratio of about 1:7 with blood. Cord blood filled the bag by gravity and by uterine contractions.

Sterility was tested by culturing the blood in a pair of Bactec NR 730 bottles (aerobic and anaerobic), incubating it for seven days at 35°C, and checking it daily.⁴ Other samples were cultured for genital mycoplasma (*Mycoplasma hominis* and *Ureaplasma urealyticum*). To investigate the coagulation profile of the frozen plasma, fresh cord plasma and plasma stored at –29°C for 45 days were tested. Prothrombin time (PT), partial thromboplastin time (PTT), thrombin time (TT), fibrinogen, and factors V, VII, VIII, IX, X, XI, XII, and XIII were determined (reagents were from Sigma Chemical Co, USA). All tests were performed using the automated coagulation timer (Electra 800 USA) and Dade (Switzerland). The coagulation screening tests (PT, PTT, and TT) were performed using routine microtechniques.⁵

Factor assays were based on mixing known human deficient plasma with 10 µl to 30 µl of each infant's cord plasma. Von Willebrand factor and plasminogen activities were also determined.⁶ To verify that thrombin activity had not been generated following clamping, concentrations of prothrombin fragments (F 1+2) were determined as follows: at one and 10 minutes after clamping a 21 gauge needle was inserted into the umbilical vein and a 2 ml blood sample collected. The syringe contained 0.4 ml anticoagulant solution consisting of 38 mmol/l citric acid, 75 mmol/l sodium citrate, 136 mmol/l dextrose, 6 mmol/l EDTA, 6 mmol/l adenosine and 25 µ/ml heparin.⁷ The ratio of anticoagulant to blood was 0.2:1.0 (vol/vol). The blood was centrifuged and the plasma frozen at –20°C until batch analysis of the specimens. Concentrations of prothrombin F 1+2 were determined with the ELISA Thrombonostica F 1+2 (Organon, Teknika Corporation) using a spectrophotometer (Molecular devices Co) at 450 nm.

Comparison of the results of the coagulation tests and of the cord blood and adult F 1+2 concentrations was determined using Student's *t* test.

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Accepted 8 August 1995

Results

The following results are based on 120 collections: the mean volume of the cord blood obtained was 86 ml (range 62–105) from full term and 44 ml (range 31–58) from premature infants (31–34 weeks' gestation).

All microbacterial cultures (aerobic and anaerobic) were negative, as were all 30 cultures examined for the presence of mycoplasma.

All the blood coagulation results, performed on 10 samples either before or after storage were within normal limits for this age group (data not shown). There were no significant differences in all the paired variables tested. The mean (SD) concentrations of Von Willebrand factor (n=26) and plasminogen (n=26) were 131.8 (32.9)% and 57 (10.7)%, respectively – that is in the normal range for this age group.

Thirty three samples of cord blood were examined for F 1+2 concentrations. The samples were obtained from 25 cords of full term infants (14 boys and 11 girls) whose birth-weights ranged from 3030 to 3970 g. Seventeen samples were collected at varying times after clamping while another eight sample pairs were withdrawn at one and 10 minutes after clamping. The results of F 1+2 determinations are shown in fig 1. The mean (SD) cord blood F 1+2 concentration was 0.53 (0.35) nM. The F 1+2 concentrations at one and 10 minutes after clamping were statistically similar, and no linear association with time of clamping could be drawn (r=0.29) (fig 2). All these results encouraged us to apply AUCBT in the infant reported below.

CASE REPORT

The third in a set of triplets born by caesarean section at 31 weeks' gestation, the baby weighed 1250 g. Following delivery, the placenta was placed on a sterile surface and cord blood was collected from the umbilical vein, as described. Thirty five millilitres of venous blood were collected within two minutes. A sample of the blood was sent for bacteriological testing; the remainder was stored in the blood bank in three separate bags.

Subsequently, the infant, whose haematocrit and haemoglobin concentrations at delivery had been 46% and 153 g/l, respectively, developed hyaline membrane disease and was

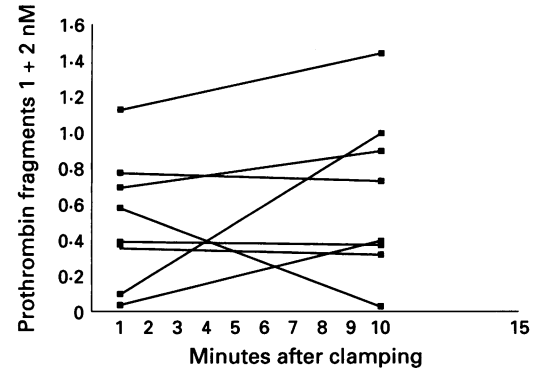


Figure 2 F 1+2 concentrations at one and 10 minutes after clamping.

mechanically ventilated. On day 5, the haematocrit dropped to 35% and the haemoglobin to 117 g/l. The collected umbilical blood was compatible with the mother's serum, and the cultures were negative. Following approval by the committee of ethics and the mother's written consent having been obtained, 10 ml of the packed cells were transfused into the infant. No untoward effects were observed. Blood gas analysis, as well as concentrations of sodium, potassium, calcium and glucose were all within normal limits. The packed cell volume (PCV) increased to 42%. Two days later, the PCV dropped to 37%, so an additional 10 ml of packed cells were transfused. Again, vital signs, blood gas analysis, and all biochemical tests were normal. Two days later, the patient was extubated. At the time of writing, the infant was 3 months old and healthy.

Discussion

Premature infants are frequently subjected to blood transfusions. Most premature infants born at less than 32 weeks of gestational age will receive more than two transfusions after 2 weeks of age.¹ Serious medical complications may accompany the transfusions. They include infections such as AIDS,⁸ cytomegalovirus,⁹ and hepatitis¹⁰; sensitisation to plasma, red cell, or HLA antigens¹¹; errors in blood group or patient identification and graft-versus-host disease.¹² Placental vessels contain 75 to 125 ml of blood at birth or nearly one quarter to one third of the fetal blood volume.¹³ This study shows that the fetal blood left in the placental vessels may serve as a source of blood for autotransfusion. Horn *et al* found that cord blood cells can be stored in a CPDA-1 medium for at least 35 days.¹⁴ Bifano *et al* examined the feasibility of collecting and storing placental blood.¹ The authors concluded that placental blood can be used for autologous transfusion for the sick neonate. Apart from being 'self' donation, thus avoiding the above mentioned homologous transfusion associated complications, AUCBT has other advantages: (a) immediate availability; (b) high levels of haematopoietic progenitor cells, such as colony forming units – granulocyte macrophage (CFU-GM), as well as high levels of granulocyte macrophage-colony stimulating factor (GM-CSF) and granulocyte-colony stimulating factor (G-CSF). It has already been shown that

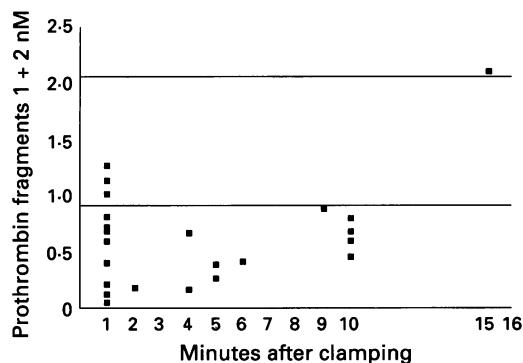


Figure 1 F 1+2 concentrations in 33 samples of cord blood.

cord blood stem and progenitor cells, may reconstitute marrow haematopoiesis.¹⁵⁻¹⁹ Umbilical cord blood, enriched with self-haematopoietic growth factors and progenitor cells, may be beneficial to premature infants who frequently suffer from leucopenia and thrombocytopenia caused by lack of marrow reserves.

Our study has shown that umbilical cord blood can be withdrawn without bacterial contamination. The coagulation assays performed in this study have proved the efficacy of stored cord plasma as a source of coagulation factors. We have also demonstrated that the clotting system is not significantly activated for at least 10 minutes after clamping.

The premature infant described here is the first (as far as is known) reported newborn to benefit from AUCBT following storage of the blood. The collected umbilical cord blood served for autologous transfusion twice. No side effects were seen. The idea of using placental blood for transfusion was initially proposed by Halbrecht.²⁰ He reported 220 cord blood transfusions (obtained from 520 placentas) given to anaemic children and adults. In recent years there has been renewed interest in the use of cord blood as a source of stem and progenitor cells for marrow reconstitution.⁹

Recombinant human erythropoietin (EPO) administration to premature infants is a relatively new strategy to reduce the risks of blood transfusion in nurseries.²¹ This mode of treatment has some disadvantages and side effects. Treatment with EPO, given subcutaneously, may increase the risk of infections, it depletes iron stores, the infants gain less weight than their controls and it is expensive.

We believe that blood, collected from the umbilical cord, is a safe source for auto-transfusion, provided that bacteriological testing has been carried out. Furthermore, any autologous transfusion programme should incorporate secure methods and safeguards in identification for collecting, storing, and dispensing the harvested blood. More clinical experience is warranted to consolidate all these concepts.

We thank Dr S Bar-Shany and Dr E Shinar from the Israeli Blood Bank for their professional assistance and Mrs D Ben-Porath for her cooperation.

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