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A clinical study on the feasibility of autologous cord blood transfusion for anemia of prematurity

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This trial is registered as PUCB-trial ISRCTN01566504.

Abstract

Background: To investigate the use of autologous red blood cells (RBC) derived from umbilical cord blood (UCB), as an alternative for allogeneic transfusions in premature infants admitted to a tertiary neonatal center.

Study design and Methods: UCB collection was performed at deliveries <32 weeks of gestation and processed into autologous RBC products. Premature infants requiring a RBC transfusion were randomized to an autologous or allogeneic product. Primary endpoint was a ≥50% reduction in allogeneic transfusion needs.

Results: 57% of the collections harvested enough volume (≥15mL) for processing. After processing, autologous products (≥10mL/kg) were available for 36% of the total study population and for 27% of the transfused infants, and could cover 58% (range 25-100%) of the transfusion needs within the 21 day product shelf life.
Availability of autologous products depended most on the gestational age. Infants born between 24 and 28 weeks had the lowest availability (17%). All products however would be useful in view of their high transfusion needs. Availability was highest (48%) for the total group of infants born between 28 and 30 weeks. For 42% of the infants with transfusion needs in this group, autologous products were available. For the infants born between 30 and 32 weeks, autologous products were available for 36% of the infants. Transfusion needs in this group were however much lower compared to the other gestational groups.

Conclusion: Autologous RBC derived from UCB could not replace 50% of allogeneic transfusions due to the low UCB volumes collected and subsequent low product availability.

Introduction

The combination of an impaired erythropoietin response to anemia[1-2], a shorter life span of hemoglobin F bearing erythrocytes and high phlebotomy losses[2-4] results in a rapid decline in hemoglobin concentration in premature infants. More than 50% of these infants receive allogeneic blood transfusions early in life[5-8], placing them at risk to presumed negative transfusion effects, such as transmittance of infectious agents and immune suppression.[9] Umbilical cord blood (UCB) has often been suggested as a source for autologous transfusions. [10-12] Due to increasing use of cord blood for transplantation purposes, progress has been made on aseptic collection of UCB and processing of small bloodvolumes.[13-15]

Approximately 20 mL of UCB per kg of body weight can be harvested irrespective of birth weight[13] and several infants have been transfused with autologous UCB safely.[16-20] We previously investigated the potential coverage of autologous UCB in transfusion needs in a preclinical pilot study. This study suggested that UCB collection could be efficient for infants born between 29 and 31 weeks of gestation.[21] However, feasibility of the clinical application of autologous UCB remained to be investigated. To answer this question we performed a randomized clinical trial on the use of autologous cord blood red cell transfusions in premature infants. Our objective was to assess the practical and economical feasibility of autologous red blood cell transfusion and to investigate whether this application could result in a meaningful reduction in the use of allogeneic blood products.

Materials and Methods

This double-blind randomized controlled trial was conducted between January 2005 and October 2006 at the Leiden University Medical Center. The Medical Ethical Committee of the Leiden University Medical Centre approved the study protocol. This trial is registered as PUCB-trial ISRCTN01566504 (www.trialregister.nl).

Study population

Informed consent for study participation was obtained from (one of) the parents before delivery. Infants included were expected to be born before 32 weeks of gestation. According to Sanquin Blood bank guidelines for release of blood products[22], maternal blood was tested for TPHA (treponema pallidum agglutination assay), anti-HBV, anti-HTLV, anti-HIV and anti-HCV antibodies and nucleic acid testing on HIV and HCV. In the weekends STAT testing was performed. Exclusion criteria were maternal antibodies against minor blood group antigens, hemoglobinopathies, maternal infections (HIV, HBV, HCV, HTLV, and syphilis), premature rupture of the membranes and fever that were treated with antibiotics, and congenital infections (CMV, Parvovirus B19, and Toxoplasmosis).

Outcomes

Primary outcome parameter was a reduction of ≥50% in the use of allogeneic RBC products in the first 21 days after birth. Secondary outcome parameters were composite neonatal complications (bronchopulmonary dysplasia, retinopathy of prematurity, intraventricular hemorrhage), length of hospitalization, mortality and cost efficacy.

UCB collection

A specially trained student team collected UCB. After a vaginal delivery, UCB was collected from the undelivered placenta. In case of a cesarean section, collection was performed after the placenta had been delivered. The collection system consisted of a 150 ml bag (Fresenius, Bad Homburg, Germany), a collection tubing system (Medisize, Hillegom, the Netherlands) with 5 mL CPDA-1 (Baxter, Illinois, USA) and a syringe (Medisize, Hillegom, the Netherlands) with 5 mL CPDA-1. The umbilical cord was sterilized with iodine, the cord vein was punctured and UCB was collected by gravity.

In monochorionic deliveries, UCB of both infants was collected in one bag for potential use for both infants. In dichorionic deliveries, two separate collection systems were used.

Blood processing by the Biosafe Sepax

A half mL whole blood was used to perform complete blood counts (Beckman Coulter Onyx, Miami, USA) and blood group typing (ABO-RhD, Diamed, Cressier, Switzerland). The remaining UCB was processed using a closed centrifuge circuit (Biosafe Sepax, Eysins, Switzerland). The procedure was started if total blood volume was at least 15 mL (excluding anti-coagulant). RBCs were separated from buffy-coat and plasma and adjusted with SAG-M to a red blood cell concentrate (RBCC) with a hematocrit between 55% and 65%. At least 10 mL of CPDA-1-plasma was used for aerobic and anaerobic bacterial culturing (BacTalert, Durham, USA).[23] UCB was not tested on contamination of maternal cells.

All processing was done within 24 hours after collection. Autologous RBC in SAG-M with a final volume of ≥ 10mL per kg of body weight of the child were stored for 21 days at 2-6°. Upon request, products were released with a negative bacterial screening or if request was within 7 days, with a negative culture up to date. The requested volume was transferred by sterile docking to a syringe (Medisize, Hillegom, the Netherlands). Autologous RBC products were not irradiated before transfusion, in contrast to the allogeneic donor products. All processes were performed under responsibility of the Sanquin Blood bank. Data regarding the product parameters (ATP, 2.3 DPG, hemolysis and pH) will be published separately, but were within allowed limits for standard packed cells stored for 35 days.

Randomization

All patients with parental consent remained in the study, even if delivery occurred beyond 32 weeks of gestation, to calculate total costs, including the extra costs for maternal virology. All live born infants were stratified according to gestational age, respectively 24-28, 28-30 and 30-32 weeks and were registered as "premature experimental product" (PEP) patients, each with a unique study number. Upon request for a transfusion, the request form was marked with a PEP label and sent to the hospital blood transfusion service. An employee of the blood transfusion department drew a sealed envelope in the correct gestational age group. The infants were randomly assigned to a standard or an autologous product. If an autologous product was not available, a standard product (irradiated leukodepleted packed RBC in SAG-M with a hematocrit between 55% and 65%) was delivered. Both types of transfusion products were delivered in a syringe (Medisize, Hillegom, the Netherlands) to guarantee blinding of the clinical staff of the neonatal intensive care unit.

Transfusion protocol

All infants were transfused according to the Dutch consensus guidelines for blood transfusion.[24] This is the same protocol that was used before start of the study. Transfusion volume according to the guideline was 10-15 mL per kg body weight. Allogeneic blood products were cross-matched with maternal serum and irradiated with 25 Gy. All transfusions were monitored and untoward reactions recorded, according to the hospital hemovigilance protocol.

Clinical data collection

Information on gestational age, gender, Apgar scores, birth weight, umbilical arterial pH, method of delivery, singleton or multiple birth, CRIB II score (Clinical Risk Index for Babies)[25], length of stay, mechanical ventilation and supplemental O_2, were systematically registered in clinical request forms. Data on RBC transfusions ≤ 21 days and > 21 days after birth and neonatal complications (infections, use of antibiotics, retinopathy of prematurity[26], bronchopulmonary dysplasia[27], intraventricular hemorrhage) were obtained from the clinical charts and computerized medical records. Six months after birth, standardized information on medical complications was collected from the parents and from the secondary hospital after transfer from our center.

Sample size

We anticipated a reduction of ≥ 50% in the use of allogeneic RBC products to be worth the effort. To detect this reduction, with a power of 90% and α <0.05, two groups of 85 eligible transfused premature infants had to be included. Taking into account that ± 40% of the infants will not require any RBC transfusion and an expected mortality of 10-15%, a total of 325 infants would need to be included.

Statistical and data analysis

Data are presented as mean ± SD unless indicated otherwise. SPSS (version 12) for Windows was used for data analysis. Data were analyzed on an intention to treat basis and a secondary analysis was performed comparing autologous and allogeneic transfused neonates (according to treatment (ATT) analysis). Continuous variables were analyzed using independent t-tests. Other variables were analyzed using the Fisher's exact test or non-parametric tests. A *p*-value <0.05 was considered significant. Interim analysis was planned 1.5 year after the start of the study to confirm feasibility.

Results

UCB collection and processing

From January 2005 until September 2006, 427 women were admitted with imminent preterm delivery. One hundred and eighty women delivered in our hospital before 32 weeks of gestation. Three women were excluded directly post partum because of sepsis and one infant was excluded because of a congenital CMV infection. A total of 176 women (and 215 infants) met the study criteria for UCB collection (Figure 1). Within this group, 195 collections (from 132 singleton, 24 mono- and 20 dichorionic deliveries, in one dichorionic delivery was only one collection due to intra-uterine mortality) were possible. In 176 cases an attempt was made to collect UCB (Table 1). In 19 cases, there was no collection attempt due to several factors: student team not called in (n=12), umbilical cord torn off (n=3), placenta not intact (n=2) or reason not recorded (n=2). In 58 cases UCB was collected from an intra-uterine placenta and 98 times from an extra-uterine placenta (of which 76 after cesarean sections). In 19 cases an intra-uterine attempt was followed by an additional extra-uterine collection. In one case the record on the placenta was missing.

Of the 176 attempts, 101 (57.4%) harvested a UCB volume of ≥ 15 mL, suitable for Sepax processing. Total volumes, including CPDA-1, from the processed UCB in the three groups according to gestational age were respectively, 32 ± 7.7 mL (16 collections for 18 infants born between 24 and 28 weeks), 44 ± 27.4 mL (32 collections for 36 infants born between 28 and 30 weeks) and 33 ± 13.3 mL (53 collections for 58 infants born between 30 and 32 weeks).

Harvested UCB (excluding anti-coagulant) was respectively 16 ± 15 mL/kg for infants with a birth weight <1000g; 18 ± 18 mL/kg for infants weighing between 1000-1250g and 20 ± 23 mL/kg for infants with a birth weight >1250g. Collected UCB volume was not well correlated with birth weight (r^2=0.24).

Thirty-four blood processing procedures did not result in an approved autologous product (technical failure n=4; final volume too small n=27; other reasons n=3). In total 67 autologous products remained (66.3%, of 101) of which three were withdrawn due to a positive bacterial culture (Coagulase-negative staphylococci n=2, Gram-negative rods n=1). All three cultures were positive within 24 hours. Thus after processing and quality control, 64 suitable autologous products were available for transfusion (36.4% of all 176 collections) (Table 1).

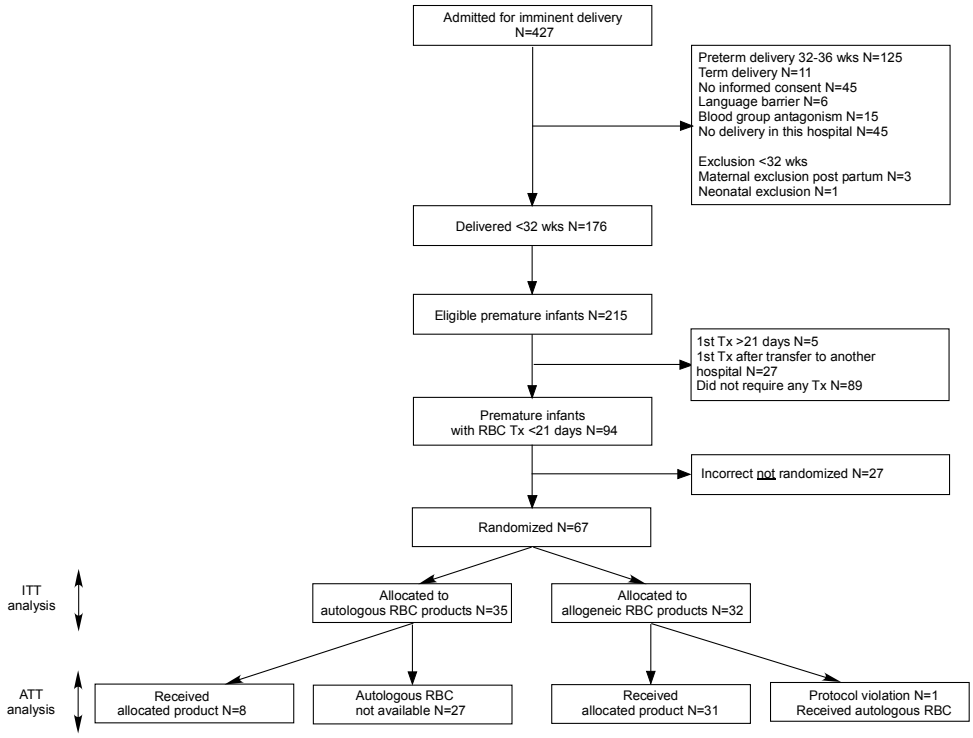

Figure 1: Flow chart – Patient Selection and Randomization scheme
Abbreviations: Tx: transfusion; ITT: intention to treat analysis; ATT: analysis according to transfusion; RBC: red blood cells

Table 1: Collected UCB and autologous RBCC processing

	Total	24-28 weeks	28-30 weeks	30-32 weeks
Eligible infants born <32 weeks, n	215	45	72	98
Possible collections, n	195	41	64	90
No collection attempt, n	19	6	8	5
Collection attempts, n	176	35	56	85
UCB volume < 15 ml	75 (43%)	19 (54%)	24 (43%)	32 (38%)
UCB volume ≥ 15 ml & processed	101(57%)	16 (46%)	32 (57%)	53 (62%)
Autologous RBCC after processing	67	6	27	34
Products with a positive bacterial culture	3	0	0	3
Available autologous RBCC for the total study population	64 (36%)	6 (17%)	27 (48%)	31 (36%)

Abbreviations: UCB: umbilical cord blood; RBCC: red blood cell concentrate

Study population

In the study period, 215 infants were born before 32 weeks of gestation. Of these, five infants required RBC transfusions after 21 days, 27 received their first RBC transfusion after transfer to another hospital, and 89 infants required no transfusions at all. Ninety-four infants required transfusions within 21 days after birth and should have been randomized upon transfusion request. However 27 infants that met the entry criteria for randomization were missed (Figure 1). For these infants, the standard RBC product, a pedi-pack of 65 ml irradiated leukodepleted packed RBC, was requested instead of a study product. In 9 of these cases the urgent transfusion indication within 24 hours after birth could have been the reason for these violations. For the other 18 protocol violations no specific cause was found. The 27 eligible, but non-randomized infants were compared with the two randomized groups to exclude possible confounding factors. All transfused patients remained included in the follow-up analysis, except one infant in the non-randomized group who was lost to follow-up. Baseline demographic and clinical characteristics of all transfused patients were comparable (Table 2). Sixty-seven randomized infants remained, 35 in the autologous arm and 32 in allogeneic arm (Figure 1).

Table 2: Demographic and clinical variables of infants with transfusion indication ≤21days (n=94)

	Autologous group n=35	Allogeneic group n=32	Non-randomized N=27
Birth weight, g, mean ± SD	1050 ± 357	1080 ± 317	1045 ± 358
Gestational age, weeks (range)	28^{+2} (25^{+0}-31^{+5})	28^{+1} (25^{+2}-31^{+6})	28^{+4} (25^{+3}-31^{+5})
-24-28 weeks, n	13	15	11
-28-30 weeks, n	16	12	8
-30-32 weeks, n	6	5	8
Male n, %	26 (74.3%)*	20 (62.5%)*	13 (48.1%)*
Live born infants from twin pregnancy, n	15	12	11
- Monochorionic	8	8	6
- Dichorionic	7	4	5
Vaginal delivery/Cesarean section, n	18:17	21:11	14:13
AS 1′ median (IQR)	6 (4-8)	6 (4-7)	7 (3-8)
AS 5′ median (IQR)	8 (7-9)	8 (7-8)	8 (6-9)
Arterial pH, mean ± SD	7.27 ± 0.1 (n=21)	7.27 ± 0.1 (n=24)	7.28 ± 0.1 (n=18)
Hematocrit after birth %, mean ± SD	44 ± 7	41 ± 10	42 ± 6
Cord clamping time, sec, mean ± SD	7 ± 4 (n=28)	8 ± 4 (n=27)	8 ± 6 (n=22)
CRIB II, median (IQR)	9 (8-11)	10 (7-12)	10 (7-12)
Infants with supplemental O_2, n	33	31	24

Abbreviations: CRIB II: clinical risk index for babies, AS: apgar Score; IQR: interquartile range *most unequally distributed variable p=0.11

Primary outcome

Transfusion triggers in the groups were comparable (Table 3). In the autologous group 2.5 ± 1.4 RBC transfusions were given, 2.7 ± 2.2 transfusions in the allogeneic group (p=0.65) and the non-randomized group received 2.3 ± 1.6 standard pedi-packs. Intention to treat analysis showed that the total number of allogeneic transfusions given was 2.3 ± 1.4 in the autologous group and 2.7 ± 2.2 transfusions in the allogeneic group. This mean difference of 0.4 allogeneic units (15%) in the autologous group was not significant (p=0.38). A reduction of allogenic RBC of 25% was present in infants born between 28 and 32 weeks of gestation (Table 3).

Table 3: Transfused RBC units and transfusion parameters

	Autologous group N=35	Allogeneic group N=32	*p**	Non-randomized n=27
Total transfusions ≤21days Mean ± SD	2.5 ± 1.4	2.7 ± 2.2	0.65	2.3 ± 1.6
-24-28 weeks	3.1 ± 1.1	3.1 ± 2.4	0.98	2.6 ± 1.0
-28-30 weeks	2.5 ± 1.7	2.8 ± 2.2	0.74	2.0 ± 2.1
-30-32 weeks	1.3 ± 0.5	1.6 ± 0.9	0.58	2.4 ± 1.9
Pre hematocrit, % mean ± SD	35.5 ± 4.2	34.4 ± 6.4	0.99	36.1 ± 4.0
Post hematocrit, % mean ± SD	45.7 ± 6.1	44.5 ± 6.5	0.49	44.5 ± 5.2
1st transfusion, day, median (IQR)	4 (2-9)	3 (2-9)	0.25	4 (2-10)
Interval till next transfusion, days, median (IQR)	3 (2-6)	4 (2-8)	0.93	4 (2-6)
Allogeneic RBC				
Allogeneic transfusions ≤21days mean ± SD	2.3 ± 1.4	2.7 ± 2.2	0.38	
-24-28 weeks	3.0 ± 1.1	3.0 ± 2.4	1.00	
-28-30 weeks	2.1 ± 1.6	2.8 ± 2.2	0.41	
-30-32 weeks	1.2 ± 0.4	1.6 ± 0.9	0.36	

Abbreviations: RBC: red blood cells; IQR: interquartile range
* autologous versus allogeneic group

Analysis according to autologous or allogeneic transfusion

Eight infants (23%) in the autologous group received the allocated product. For 27 (77%) infants an autologous product was not available. One infant in the allogeneic group received erroneously an autologous product. After transfusion of the autologous RBC products (n=9) the average hematocrit increase was 9.8% ± 5.5 %, comparable with the increase after an allogeneic transfusion of 10% ± 5.2 %. Median interval until the next transfusion in these infants was 3 days, comparable to the infants who only received allogeneic products. For two infants the available autologous RBC volume only covered 1 of the 4 transfusions they required in the first 21 days. In three infants available autologous RBC covered half of the required transfusions. Three infants received autologous RBC products only (Table 4). No adverse effects were observed.

Because the study was randomized, 11 autologous products were available for infants assigned to allogeneic RBC and 6 products for non-randomized infants. The potential transfusion coverage of these products was estimated. If this had not been a randomized study, the available products would have covered 58% (range 25-100%) of the transfusion needs ≤ 21 days per infant (Table 5).

Table 4: Transfused autologous RBC products and reduction in allogeneic transfusion

Patient # (stratum)	Total Tx ≤21 days (n)	Received Autol Tx (n)	Received Allog Tx (n)	Reduction in Allog Tx (n)
1 (24-28 weeks)	4	1	3	25%
2 (24-28 weeks)	2	1	1	50%
3 (28-30 weeks)	4	1	3	25%
4 (28-30 weeks)	2	1	1	50%
5 (28-30 weeks)	2	1	1	50%
6 (28-30 weeks)	2	1	1	50%
7 (28-30 weeks)	1	1	0	100%
8 (28-30 weeks)	1	1	0	100%
9 (30-32 weeks)	1	1	0	100%
Pre hematocrit, % mean ± SD	37.5 ± 5.2	36.0 ± 6.0	39.0 ± 4.0	
Post hematocrit, % mean ± SD	46.5 ± 7.3	45.8 ± 8.3	47.2 ± 6.5	

Abbreviations: Tx: transfusion; Autol: autologous; Allog: allogeneic, IQR: interquartile range

Table 5: Premature infants with available autologous RBC product in relation to the actually received transfusions

Stratum	Tx needs ≤21 days, n	Infants, n	Autologous RBC ml/kg, mean ± SD	Coverage in Tx needs (%)
24-28 weeks	No tx	0	-	-
	1 tx	1	14 ± 0	100%
	2 tx	2	13 ± 3	50%
	>2 tx	3	11 ± 3	25-33%[1]
28-30 weeks	No tx	12	16 ± 4	-
	1 tx	7	27 ± 12	100%
	2 tx	5	18 ± 8	50-100%[2]
	>2 tx	3	30 ± 11	25-71%[3]
30-32 weeks	No tx	27	17 ± 8	-
	1 tx	3	19 ± 9	100%
	2 tx	1	11 ± 0	50%
	>2 tx	0	-	-

Abbreviation: Tx: transfusion
[1]: 2 infants required 4 transfusions and 1 required 3 transfusions; coverage was respectively 25% and 33%
[2]: 4 infants with 50% coverage, 1 with 100%
[3]: Infants required respectively 3, 4 and 7 transfusions with coverages of respectively 33%, 25% and 71%

Secondary outcomes

Clinical outcome variables are shown in Table 6. Length of stay was registered in our center. Neonatal complications were registered during the complete hospital stay and the six-month follow-up period. The most unequally distributed variable in intention to treat analysis was length of stay (p=0.19), in favor of the allogeneic group and the composite mortality and morbidity (p=0.20) in analysis according to transfusion, in favor of the autologous transfused infants. Analysis of the outcome variables showed no significant differences between the groups.

Table 6: Neonatal Mortality and Morbidity

	ITT		ATT		Non-randomized (N=27)
	Autol (N=35)	Allog (N=32)	Autol (N=9)	Allog (N=58)	
Composite morbidity and mortality, n, %	27 (77%)	24 (75%)	5 (56%)§	46 (79%)§	19 (70%)
Mortality n	3 (9%)	4 (13%)	0 (0%)	7 (12%)	3 (11%)
IVH n	10 (29%)	14 (44%)	2 (22%)	22 (38%)	9 (33%)
≥ grade 3	4 (11%)	4 (13%)	0 (0%)	8 (14%)	1 (4%)
ROP n	8 (23%)	5 (16%)	1 (11%)	12 (21%)	2 (7%)
≥ grade 3	1(3%)	1 (3%)	0 (0%)	2 (3%)	1 (4%)
BPD n	20 (57%)	10 (31%)	4 (44%)	26 (45%)	14 (52%)
≥ grade 2	11 (31%)	5 (16%)	1 (11%)	15 (26%)	6 (22%)
Length of stay, Median (IQR) days	30 (15-48)*	23 (13-39)*	30 (14-37)	28 (15-44)	21 (9-48)
in NICU	19 (14-30)	20 (10-30)	18 (11-31)	20 (11-29)	16 (8-30)

ITT: intention to treat analysis; ATT: according to treatment; Autol: autologous; Allog: allogeneic. *: most unequally distributed parameter in ITT analysis, p=0.19
§: most unequally distributed parameter in ATT analysis, p=0.20

Collection and Production efficacy

Overall successful UCB collection rate (≥ 15ml blood excluding anti-coagulance) was low (57%). Most of the collections were performed from an extra-uterine placenta. Analysis of harvested volume per collection method showed that collections from an intrauterine placenta resulted in higher UCB volumes (37.7 ± 20.2 ml) than collections from an extra-uterine placenta (30.6 ± 22.1 ml). Hence, the proportion processed UCBs after an intra-uterine collection was larger. We also analyzed production efficacy of the Sepax device. Of the 101 successful UCB collections, 64 autologous products remained after processing, mainly because of loss of RBC during the procedure. Efficacy per group was lowest in the 24 and 28 wks group, 16 productions resulted in only 6 RBCC with the required volume and hematocrit. However, each of these infants required transfusions. Processing of the UCBs from the 30 and 32 week group resulted in the highest number of products, but as the infants in this group had much lower transfusion needs, most of these products remained unused. UCB collection as well as processing was optimal for the infants

born between 28-30 weeks. In particular, 69% of the intrauterine collections in this group were large enough for processing to red cell concentrates and resulted all in autologous products. More than half of the available products in this group were intended for infants with transfusion needs and could cover approximately 75% of the transfusion needs in this group (Table 5).

Estimation of costs

Maternal virology testing was performed for 202 cases. For 26 cases testing was performed, but the delivery occurred beyond 32 weeks. In 18 cases UCB was processed in the weekend for which STAT testing was performed, resulting in a huge increase of the total costs. The costs for UCB collection disposables were made for 176 cases, the costs for Sepax processing for 101 cases and the costs for quality control for 67 products. Ultimately the direct cost for 25 indicated RBC products were almost 15 times higher than to the costs of a standard pedi-pack (Table 7). Without STAT testing, the costs would be 6.5 times higher.

Table 7: Autologous RBCC cost estimation

	N	€ per unit	Total costs
Maternal virology (regular)	202	€ 15.50	€ 3.131
STAT maternal virology (weekends)	18	€ 1.330	€ 23.940
UCB collection disposables	176	€ 14.17	€ 2.494
Sepax kit for processing	101	€ 117.09	€ 11.826
Quality control (ABO-RhD, BacTalert)	67	€ 7.31	€ 490
Available autologous products	64	€ 654	
Cost per indicated product	25	€ 1.675	
Cost per indicated product (excluded STAT)	25	€ 729	
Standard pedi-pack, irradiated		€ 114	

RBCC: red blood cell concentrate

Interim analysis

Interim analysis revealed low availability of autologous products due to a low number of successful UCB collections. This availability of < 40 % autologous products for all premature infants with collected UCB would preclude that the primary endpoint of a reduction of at least 50% in use of allogeneic RBC products would be attainable. Therefore we decided to stop enrollment in October 2006 after inclusion of 215 infants and 94 transfused infants.

Discussion

In this clinical trial we collected UCB after premature deliveries < 32 weeks and randomized premature infants who required a RBC transfusion to either an autologous or allogeneic product. From the start of the study we considered a reduction of ≥ 50% in use of allogeneic blood

worth the effort. Interim analysis revealed that for 27% of the transfused premature infants an autologous RBC product was available for at least one transfusion. Therefore this trial was stopped prematurely.

Eight out of 35 patients received autologous RBC in the group assigned to autologous products. Possible clinical benefits of these autologous transfusions could therefore not be evaluated, although post-transfusion hematocrit increment, interval until next transfusion, incidence of adverse events and composite neonatal complications were not at variance. This was in accordance with the results observed by other groups.[17-20] Unfortunately, 27 infants were not randomized. This high number of protocol violations can not be fully explained. However, analysis of the non-randomized infants showed comparable demographics and outcome, suggesting no selection bias.

Our collection methods resulted in 101 UCBs that could be processed, of which only 25 available products were intended for transfused infants. This implies that for each effective product three extra products were made unnecessary. Considering all products would have been used for patients with a transfusion indication (in case of a non-randomized study), 21 of the 25 products would have been large enough to cover at least 50 % of the transfusion needs per infant in the first 21 days after birth.

Despite the lower number of successful UCB collections for the infants born between 24 and 28 weeks, almost every available product would be useful, in view of the large number of infants (87%) with transfusion needs (39 out of 45). For the infants born between 28 and 30 weeks, UCB collection volumes were more often sufficient for processing. In this group of infants, 50% (36 out of 72) had transfusion needs. UCB collections for the infants born between 30 and 32 weeks were most successful according to our definition of ≥ 15ml whole blood. After processing however, a smaller number of products remained available. In addition, transfusion needs in this group were much lower compared to the other gestational groups (Tables 1 and 2). Taken this all together, UCB collection and autologous RBCC production was most efficient for the infants born between 28 and 30 weeks compared to the other gestational groups. In this group autologous RBC was available for 42% of the infants that required transfusions. Harvested UCB was not enough to cover the transfusion needs of the smallest infants and the autologous products made for infants born between 30 and 32 weeks remained unused due to lower transfusion needs.

Some possible improvements of efficacy need to be considered. Only 57% of all collection attempts resulted in a UCB volume large enough for Sepax processing. Most of the successful collections were harvested from an intrauterine placenta. Restriction of UCB collection to only intrauterine harvesting[28] would increase production efficacy by 25%. In addition, this resulted for infants born between 24 and 28 weeks, in a 10% lower RBC recovery compared to the other processed UCBs, because of relative higher cell losses in these smaller collections (data not shown). RBC separation by filtration has been described as an alternative.[29-30] However; the intrinsic volume of the filters could also result in absolute red cell volume loss. Furthermore, doing

regular maternal virology testing instead of expensive STAT testing can reduce the costs. In this study, the release of 18% of the UCB collections suitable for Sepax processing would be delayed for a maximum of 72 hours.

For future exploration of the possible clinical effects of autologous cord blood, UCB harvesting must be more efficient. Additional placental perfusion with heparinized saline after UCB collection has been described as an effective method for enlarging the total collected volume.[31] Whether this method is also useful in the smaller and more fragile placentas after premature deliveries remains to be investigated. The restriction to infants born before 30 weeks of gestation, the use of intrauterine collections only (also after cesarean sections) and a processing procedure with higher red cell recovery could result in higher volumes and available products expecting to cover > 50% of the transfusion needs. Another approach of autologous transfusion is placental transfusion by delayed cord clamping.[32-36] Recent studies show that delayed cord clamping is associated with reduced transfusion needs and a lower risk of IVH in premature infants.[32, 35] However, the described time range of delayed clamping was broad and varied from 30 till 120 seconds.[32] Although the results seem promising, it is desirable that they are confirmed by larger studies with a uniform definition and method of delayed cord clamping, in particular in critically ill children with a low Apgar score and a high risk of transfusion probability.[21]

In conclusion, autologous RBC derived from UCB could replace allogeneic transfusions for 27% of the infants in need of transfusions. Whether an autologous product was available for an infant with transfusion needs depended most on the gestational age. UCB collection and processing for infants born between 28 and 30 weeks was most efficient. In this trial a small number of autologous RBCC were actually transfused, therefore no clinical benefits could be determined. Whether premature infants may benefit clinically from autologous blood remains a question unanswered at this moment.

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