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Neonatal transfusion practices

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Neonatal transfusion practices

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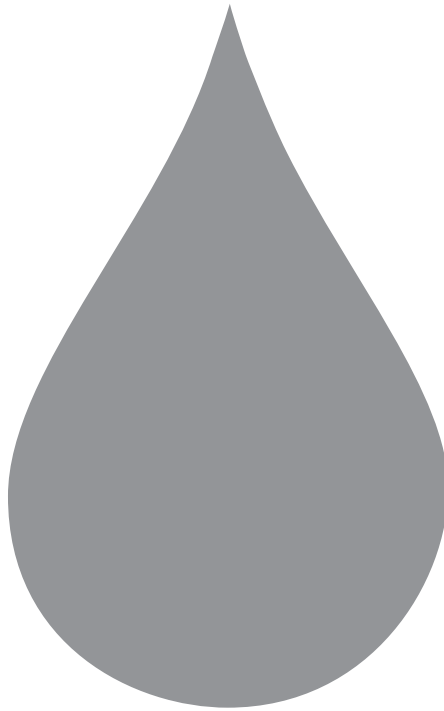
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Chapter 1

General introduction and outline of the thesis



General introduction

Neonatology is a fast evolving field in medicine. In recent years great improvements have been made, such as new respiratory support strategies which have greatly improved pulmonary outcome by reducing acute lung injury. Many drugs and therapies used in neonatal medicine have not been tested in randomized trials on young and small infants but are used based on extrapolations of the effects in older children and adults. A treatment mode neonatology can't do without is the use of blood products, especially red blood cell (RBC) transfusions. Over the past few decades the survival rate of (extremely) premature and low birth weight infants has increased, which has led to more emphasis on the long-term outcome in relation to the treatment modalities used during their stay in the neonatal intensive care unit.

Transfusion medicine is a field which has received little interest from pediatricians and neonatologists and it seems difficult to recruit clinical specialists for research on blood products in their patients despite blood products being a life saving treatment.¹

Red blood cells

In the younger gestational ages blood products are frequently used. Nearly all infants with a gestational age below 28 weeks or <1000 grams will need at least one red blood cell transfusion in the first few weeks of life.²⁻⁴

Concerns about the effects on the developing immune system, risk of increased oxidative stress and questions about the effect of extra volume loads on the circulation and cerebral perfusion during transfusion have been addressed.⁵⁻⁷

It is increasingly known that one of the major reasons for anemia of prematurity is blood drawn for laboratory investigation or loss during procedures. This increased awareness has led to micro-blood testing, a critical view on the amount of testing done and indwelling lines for blood drawing purposes leading to less blood loss.⁸⁻¹⁰ Despite these measures many RBC transfusions are still given in clinical practice.

When to transfuse and what volume to transfuse remain important questions, although these have been addressed in various reports. Research has been done to try and establish transfusion thresholds using hemoglobin (Hb) or hematocrit level and transfusion volume, but these studies advise different values and are difficult to compare due to differences in patient groups, used transfusion volumes, transfusion thresholds, transfusion products and outcome measures.

Results of various studies also show conflicting short-term outcome.¹¹⁻¹³ As far as long-term outcome is considered only one study has been done, showing possible detrimental effects of a lower RBC transfusion threshold.¹⁴

Donor exposure due to transfusion of blood products is a major concern. Effects on the developing immune system, transfer of infectious agents and graft-versus-host disease are only a few of the possible risks. Efforts to reduce the donor exposition comprise amongst others delayed cord clamping, umbilical cord 'milking', critical approach to the necessity of blood testing, micro-blood tests, single-donor programs, and defining a safe lower transfusion threshold.

Platelets

Another issue in transfusion medicine in neonatology is the need for platelet transfusions in newborns with thrombocytopenia (defined as a platelet count below $150 \times 10^9/L$). Research has been done to find a safe lower threshold for platelet transfusion using various platelet levels for different clinical conditions. However, the severity of thrombocytopenia does not seem to correlate with the severity of the detrimental effects, such as a major bleeding. The negative effects of a low platelet count can be so disastrous that no one dares to suggest a very low transfusion threshold. The question however is not, 'how low should we go?' but 'how low can we go?'. Most international protocols are based only partially on firm evidence. Many guidelines are also expert-based or based on (years of) experience.

Thrombocytopenia occurs in 1–7% of all newborn infants. Among infants admitted to the neonatal intensive care units this percentage is much higher (up to 35%).¹⁵⁻¹⁷ In adults and in children and newborn infants the effects of a low platelet count are not well known. Thrombocytopenia is usually the result of an underlying disease which can also cause deleterious effects. Platelets, next to clotting factors, are needed for the blood clotting process. How many thrombocytes one at least needs remains a question. Severe hemorrhages have been described with a completely normal platelet count and only superficial petechiae in extreme thrombocytopenic conditions as idiopathic thrombocytopenic purpura.

Study objectives

- a. To summarize the international literature on the use of red blood cell and thrombocyte products in peri- and neonatal medicine.
- b. To find a way to reduce donor exposure in the most vulnerable group of patients by harvesting umbilical cord blood and processing this to an autologous red blood cell product.
- c. To reduce donor exposure by transfusing autologous red blood cell products.

- d. To compare the short-term and long-term outcome of two different red blood cell transfusion volumes.
- e. To study the risk of hemorrhage in thrombocytopenic newborns.
- f. To evaluate the erythrocyte and thrombocyte transfusion protocols of the neonatal intensive care units in the Netherlands and compare them to the national and international guidelines.

Outline of the thesis:

In **chapter 2** an overview of the literature on the use of perinatal blood products is given.

Part I describes our studies concerning umbilical cord blood.

In **chapter 3** we describe our pilot study to lower donor exposure by harvesting RBCs after birth from the placenta and umbilical cord of premature born infants and turning this into a red blood cell product for autologous transfusion to be used in the first few weeks of life.

In **chapter 4** our feasibility study on the use of autologous umbilical cord blood RBCs is described.

Part II focuses on red blood cell transfusions in (premature) newborns.

Chapter 5 describes the short-term outcome in two groups of infants born before 32 weeks gestation treated with different RBC transfusion volumes.

Chapter 6 describes the long-term outcome of two groups of extremely premature infants treated with different RBC volumes.

In **chapter 7** we describe the current protocols used in the 10 Dutch neonatal intensive care units (NICUs) regarding RBC transfusions in newborns and compare them to the national guideline 2004, international literature and the revised guideline 2011.

Part III focuses on platelet transfusions in newborns.

The results of our retrospective study on all admitted newborns to our NICU with thrombocytopenia during a three year period are presented in **chapter 8**.

Chapter 9 shows the outcome of two different platelet transfusion strategies on hemorrhage in thrombocytopenic extreme premature neonates admitted to two Dutch NICUs.

Chapter 10 describes the current protocols used in the 10 Dutch NICUs regarding platelet transfusions in newborns and compares them to the national guideline 2004, international literature and the revised guideline 2011.

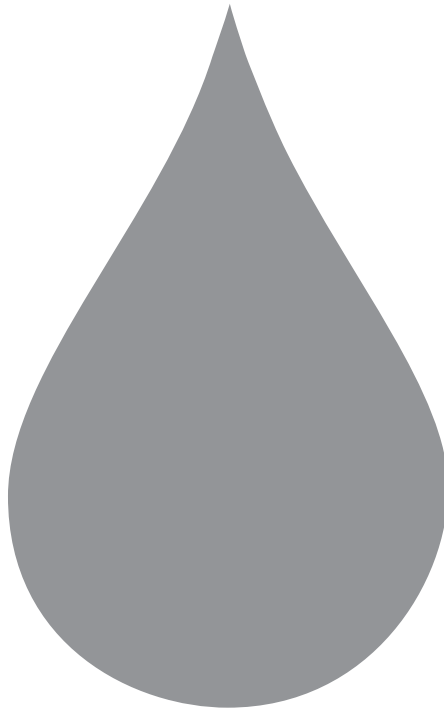
The results of this thesis are described in **chapter 11** and future research questions are addressed.

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Chapter 2

The use of blood products in perinatal medicine



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Summary

Fetal and neonatal medicine is a field with many new procedures and techniques. An increasing number of centres worldwide give intrauterine transfusions, which is considered to be standard-of-care treatment for severe fetal anaemia. The survival of very prematurely born neonates, in particular of a gestational age of <28 weeks, has greatly improved the last decade but almost all these children need transfusions. Although in many cases such blood transfusions are life saving, randomized controlled studies investigating appropriate indications, transfusion volume and type of blood product, have not been performed. Most of the protocols used are expert based.

Introduction

Fetal and neonatal medicine is a field with many new procedures and techniques. In many fetal and neonatal conditions, transfusions of blood products are life saving. However, randomized controlled studies into the appropriate indications, transfusion volume or type of blood product are needed. Studies on red-cell transfusions in adult patients reveal that, depending on the indication, too few or too many transfusions are associated with a worse outcome. Moreover, the efficacy of platelet transfusion triggers is currently under question, as the prevention of bleeding is scarcely demonstrated. There is no reason to assume that this should not apply to fetuses and newborns. Currently most of the protocols used with fetuses and newborns are expert based.

This paper presents an overview of the literature, transfusion guidelines and recommendations for further research for the most common transfusion indications.

Intrauterine transfusion

Ultrasound-guided umbilical cord puncture and transfusion is possible after the 17th week of gestation. In experienced centres, morbidity and mortality is 1–2 % per procedure,¹ although the complication rate is higher if the gestational age is <20 weeks or the fetus has thrombocytopenia.^{1,2} The major indication for treatment by intrauterine transfusion (IUT) is anaemia caused by maternal antibodies. Other, more rare, indications for fetal blood transfusion include human parvovirus B19 infection, severe fetomaternal haemorrhage, twin-twin transfusion syndrome and homozygous α -thalassemia. The decision to treat fetal thrombocytopenia with intrauterine platelet transfusions (IUPT) must be balanced against the risk of the procedure.

Transfusions of red blood cells

Haemolytic disease of the newborn (HDN) due to maternal immunoglobulin G (IgG) antibodies directed against an incompatible paternal antigen expressed on the child's erythrocytes is a major cause of in-utero anaemia. Active maternal IgG transport takes place from the 12th gestational week and, at birth, the IgG levels in the child are higher than in the mother. The severity of HDN depends on the haemolytic potential of the antibodies, which is determined by the expression of the antigen on mature fetal/neonatal red cells or on erythroid precursor cells.

Major ABO incompatibility occurs in 20–25% of pregnancies, often affecting the first-born child. Anaemia is rare because ABO antigens are not fully expressed at birth. HDN due to

anti-Rh-D or other red-cell antibodies generally presents with clinical symptoms in the second or later pregnancies and tends, unlike ABO antagonism, to be aggravated in the next child.

In Caucasian populations, severe HDN is caused predominantly by Rh-D, followed by anti-K1 and anti-c antibodies. Incidentally, antibodies against very rare (private) antigens or against antigens present in the majority of the population (public) cause HDN.¹

When clinically relevant antibodies are identified, pregnancy management by an experienced team is warranted. This includes non-invasive estimation of the degree of anaemia by ultrasound flow measurement of the peak systolic velocity of the middle cerebral artery. It is important to start IUT before hydrops has developed, as survival decreases from 95% to <70% in cases of severe, irreversible hydrops.¹ As deduced from small series, IUT is also beneficial for hydrops arising from parvovirus B19 infection.³

If possible, fetal red cells obtained by the first umbilical puncture should be extensively typed, because after the second transfusion the fetal erythropoiesis is often completely depressed. Red cells used for IUT should fulfil the same testing criteria for transmittable infectious diseases as standard transfusions. In addition, guidelines recommend the following criteria for the product used:⁴⁻⁶

- blood groups are compatible with maternal antibodies
- it does not transmit cytomegalovirus (CMV) or – preferentially – parvovirus B19
- there is a long erythrocyte survival
- it does not cause transfusion-associated graft versus host disease
- it is devoid of substances that might be toxic for the fetus
- it has a haematocrit (Ht) that is as high as viscosity allows
- it is of a temperature that is sufficient to avoid cold exposure

To fulfil these criteria, the unit of blood used for IUT is generally O, Rh-DCE negative (unless anti-c), Kell negative and, if applicable, compatible with other maternal antibodies. The unit must be free of clinically significant red-cell antibodies.⁵ A compatibility test with new maternal serum is necessary because approximately 25% of woman receiving IUT develop additional red-cell antibodies, particularly after transplacental puncture.^{7,8} Leucocytes must be removed by filtration to prevent CMV transmission, although many centres additionally select CMV-seronegative donors,⁵ and some also use parvovirus-B19-safe blood.⁴ The red cells used for IUT are generally stored for less than 3–5 days to offer a long erythrocyte survival.⁴⁻⁶ To prevent transfusion-associated graft versus host disease (TA-GVHD), the red cells are irradiated with 25 Gy.⁴⁻⁶ Because the transfused volume is huge (in relation to the fetoplacental blood volume), the transfusion speed is fast (often accomplished within 30

min) and the intrahepatic or intracardiac transfusion route may be used, much attention has been paid to the level of potassium and to the blood temperature as possible causes of fetal bradycardia and cardiac arrest.^{9,10} Both irradiation and the packing of red cells by centrifugation to obtain a haematocrit of $80 \pm 5\%$ enhance hyperkalemia. Galligan and colleagues compared washing (to remove adenine and potassium) in three different solutes: Ringers' lactate, NaCl 0.9% and plasma.¹⁰ Although plasma washing resulted in the lowest potassium levels, after washing of 3–5-day-old red cells with NaCl 0.9% and subsequent storage for up to 4 h, the potassium level remained below 12 mmol/L, which was considered safe for IUT. This emphasizes the importance of limiting the number of centrifugations and of aiming for a short interval between blood processing/irradiation and transfusion, although the allowed ranges mentioned in guidelines vary by up to 24 h.⁵

An alternative source of erythrocytes for IUT, and an inevitable source in case of HDN due to an antipublic antibody, are washed maternal red cells. Even major ABO-mismatched cells have been used. Healthy females, supplemented with iron, folate and vitamins can donate up to six units during pregnancy.¹¹ A theoretical advantage is an estimated 50% reduction of maternal new antibody formation.⁸ One study compared maternal and donor transfusions for IUT and observed, towards the end of pregnancy and in the neonatal period, a reduced transfusion requirement after maternal blood. This was attributed to increased reticulocytosis after repeated blood donation.¹²

Many centres perform a combination of intravascular and intraperitoneal transfusions; the latter are less effective in case of hydrops. IUT aims to bring the post-transfusion Ht to 45% (or lower in severely anaemic hydropic fetuses) and several formulae made by the pioneers in this field are still in use.^{13,14} After the first IUT, a second transfusion is generally needed after 1–2 weeks. Subsequent IUTs require a 3–4 week interval, as there is a 1–2% daily decay of transfused red cells. IUT exerts several acute vascular dynamic effects related to changes in blood volume, viscosity, pH and 2,3-biphosphoglycerate-related oxygen delivery. There is often a transient decrease in cardiac output. Immediately after IUT, platelets decrease by more than 50% because of haemodilution and – possibly by induction of stress factors and demargination – numbers of monocytes and granulocytes increase relative to platelets.¹⁵ Iron overload is a long-term effect of both haemolysis and transfusion. It can play a role in neonatal cholestasis and hepatitis.¹⁶ In Rh-D HDN associated with hydrops, there is a 10–30% risk of concomitant thrombocytopenia below $50 \times 10^9/L$ and it is recommended to have platelets available for transfusion when umbilical vascular puncture is performed in hydropic children.^{17,18}

Intrauterine platelet transfusion

In contrast to the existing consensus for IUT in fetal anaemia, the practice of IUPT for fetal thrombocytopenia is more controversial. Most threatening thrombocytopenia occurs in fetal/neonatal alloimmune thrombocytopenia (FNAITP). In Caucasian populations, maternal anti-human platelet antigen (HPA)-1a is the dominant cause, followed by anti-5b-antibodies.¹⁹ In 15–20% of FNAITP cases, non-HPA-1a/non-HPA-5b antibodies are present or antibodies cannot be detected.²⁰ Thirty percent of women possess anti-HLA class I antibodies at delivery, which can be detected in cord blood, but there is no clear relationship between the presence of HLA antibodies and neonatal thrombocytopenia.²¹

The indication for IUPT is prevention of severe in-utero bleeding. From a prospective study in the UK, it was estimated that anti-HPA-1a antibodies caused intracranial haemorrhage (ICH) in 1 out of 12.000–25.000 pregnancies; the majority occurring in-utero between 30 and 35 weeks of gestation and in 50% affecting the firstborn.²²

Bussel et al. were the first to report that weekly maternal high-dose intravenous immunoglobulin (IVIG, 1.0 g/kg maternal weight) was effective at elevating the fetal platelet count and preventing ICH.²³ IVIG is now first-line treatment of FNAITP in subsequent pregnancies, eventually adjusted with corticosteroids and IUPT. The management of FNAITP is based on the history of the previous affected child. After the birth of a child with ICH, the risk for ICH in the next pregnancy for a child carrying the offending antigen is approximately 80%.²⁴ Although this high recurrence rate clearly requires intervention, we have found that the high rate of procedure-related fetal complications and the boosting of antiplatelet antibodies by IUPT has gradually resulted in less invasive treatment in our centre.²⁵

In case of a posterior cord insertion, IUPT has an increased risk. Withholding invasive treatment should be considered. In 60%–75% of cases, maternal IVIG results in improvement of the fetal platelet count. Moreover, even if the platelet count remains low, IVIG can protect against ICH.^{21,25} In cases of a previous child with thrombocytopenia but without ICH, the complication rate of IUPT and the need to repeat transfusions every 7–10 days outweighs the 7–13% risk of ICH in the next child.^{24,26}

In maternal autoimmune thrombocytopenia there is virtually no indication for IUPT because in-utero ICH has not been reported.²⁷ In hydropic fetuses with severe Rh-D HDN or parvovirus B19 infection, occurrence of severe intrauterine bleeding or ICH is rare. To prevent bleeding from the IUT procedure to correct anaemia, it is recommended to correct the platelet count as well. In particular, in parvovirus B19 infection 50% of affected fetuses have thrombocytopenia below $50 \times 10^9/L$ (range $4\text{--}49 \times 10^9/L$), which further decreases after transfusion of red blood cells (RBC) alone.^{3,15}

IUPT products are generally obtained by aphaeresis from selected donors. In case of FNAITP, the donors are HPA-1a and/or HPA-5b negative; in case of other antagonisms, washed maternal platelets must be used. Leucocytes are removed from the platelets to prevent CMV transmission and washing removes anti-HPA and other unwanted (maternal) antibodies. After washing, the platelets can be resuspended to the required concentration in AB-citrate plasma, in NaCl 0.9% or in platelet additive solution. For indications other than FNAITP, blood group O, Rh-D-negative donors are mostly selected. To prevent clinically relevant red-cell and leucocyte antibodies eventually causing haemolysis or transfusion-related lung injury, it is advised to limit the donor pool to males without a history of blood transfusion and with a low titre of ABO antibodies. The same donor safety requirements as for IUT with red cells are needed, including CMV and, preferentially, also parvovirus-B19-safe donors, irradiation of the product with 25 Gy to prevent TA-GVHD and a storage interval less than 24–48 hours after withdrawal to provide a long platelet survival. The aim of IUPT is to increase the fetal platelet count above $300 \times 10^9/L$ to ensure a platelet level above $30 \times 10^9/L$ for at least 1 week. This can be achieved by preparing leucocyte-reduced high-concentration platelet products ($1\text{--}3 \times 10^{11}$ in 50–60 mL) by aphaeresis systems.²⁸

Platelet transfusions can also be complicated by fetal bradycardia, and, besides haemodynamic causes, soluble factors (e.g. CD40 ligand) in platelet products can play a role.²⁹

Neonatal transfusions

Three categories of patients are at high risk of receiving neonatal transfusions: infants with (allo)immune anaemia and thrombocytopenia, neonates needing extracorporeal membrane oxygenation or surgery and infants born preterm.

Transfusion of red blood cells

Exchange and massive transfusions

Exchange transfusions (ET) are mostly indicated for hyperbilirubinaemia. The bilirubin trigger ranges between 175 and 450 $\mu\text{mol/L}$ and depends on comorbidity, such as pre- or dysmaturity, septicaemia, asphyxia and the speed of bilirubin increase. Polycythaemia and hyperkalaemia are also indications for ET, although evidence for the benefit of this practice is lacking.³⁰ RBC used for ET should be compatible with mother and child, and in general should be O-Rh-D, K negative, leucoreduced, and stored for less than 5 days because of 2,3-bisphosphoglycerate levels. According to guidelines, either whole blood from donors lacking high-titre anti-A and anti-B and irregular red-cell antibodies or reconstituted red cells to a Ht of circa 40% with AB-citrate plasma (fresh frozen plasma) are used.^{31,32} Plasma from male donors

lacking leucocyte antibodies that may cause transfusion-related lung injury is recommended. ET for premature infants or for neonates after IUT is irradiated with 25 Gy. The exchange volume is 160 mL/kg. ET blood contains high levels of glucose and sodium, and lacks ionized calcium. The speed of exchange is maximally 2mL/kg per minute and blood pressure, heart rate and breathing must be monitored. ET reduces the platelet count by 50% and it is advised to maintain the platelet level above $50 \times 10^9/L$, in particular in preterm infants and in the first few days after delivery. After ET, glucose should be controlled for rebound hypoglycaemia. In sick children, ET is associated with considerable (10%) morbidity. A mortality rate of 0–2% is reported, mainly as a result of hypocalcaemia and thrombocytopenia.³³ In case of massive transfusions, such as for extra corporeal membrane oxygenation or surgery for children less than 3 months old, similar product specifications and monitoring as for ET are advised.

Top-up transfusions

Despite changes in transfusion practices, prematurely born infants, especially extremely and very low birth weight infants, are still heavily transfused.

Blood drawn for laboratory investigation is an important cause of the need for RBC transfusion in these infants, next to erythropoietin (EPO) deficiency and critical illness anaemia. Microanalysis and point-of-care instrumentation, as well as indwelling lines, have reduced the amount of blood needed for testing but a critical approach to every test requested is still necessary.³⁴

Over the past two decades, restrictive guidelines and greater awareness have resulted in a 70% decline in the volume of transfused erythrocytes, as well as 54–80% reduction in the number of donors to which infants are exposed.^{35,36}

Most neonatal intensive care units use a transfusion protocol based on clinical condition, mechanical ventilation, gestational age, oxygen use and Ht or haemoglobin (Hb) level. However, with respect to Hb, there is no consensus on appropriate transfusion triggers and the volume of blood to be transfused.

Two randomized controlled studies have recently addressed this question.

In the large multi-centre Premature Infants in Need of Transfusion (PINT) study, more than 450 infants with a birth weight <1000 g and a gestational age <31 weeks were enrolled within 48 h of birth.³⁷ They were randomly assigned to a high or a low transfusion threshold. The attending physicians were free to give RBC for ‘clinical reasons’, bypassing the threshold. In all, 89% of infants in the low-threshold group received RBC versus 95% in the high-threshold group ($p = 0.037$); there was no significant difference in the total amount of transfusions in both groups. Because a single-donor program was not used, the low-threshold group was exposed to significantly fewer donors than the high-threshold group ($p = 0.035$). The compos-

its primary outcome (death or survival with major morbidity) and the secondary outcomes – including Hb levels, transfusion exposure, infections, growth rate, length of oxygen need and hospital stay in both groups – were similar, showing no negative effects when maintaining a lower Hb level.

A second, single-centre, study by Bell et al. showed an increase in intraparenchymal brain haemorrhage and periventricular leucomalacia in a group with a restrictive transfusion guideline compared with a liberal transfusion guideline;³⁸ the lower threshold group also had more apnoea. The number of donors to whom the infants were exposed was not significantly different, although fewer units were given in the restrictive transfusion group than in the liberal transfusion group (3.3 ± 2.9 versus 5.2 ± 4.5 ; $p=0.025$).

The unequivocal outcomes of these studies have been discussed extensively. As more liberal transfusion triggers failed to demonstrate deleterious effects, it might be prudent not to use low Hb/Ht transfusion triggers unless in clinical studies.

Guidelines need not to be generalized in the first 24 h after birth. Children born after (prolonged) intrauterine anaemia are accustomed to a lower haemoglobin level and may not need an RBC transfusion. However, newborns with severe intrapartum blood loss will need supplementation to make up for the lost blood and volume; their first Hb level shortly after birth will not show their true Hb count.

Table 1 summarizes thresholds for RBC as published in different articles. Nearly all centres take into account the postnatal age and whether a child is on ventilatory support or needs supplemental oxygen.

Although determined by the desired Hb/Ht after transfusion, the volume of RBC used for transfusion has not been much studied and varies from 5 to 20 mL/kg. A study by Paul et al., comparing 10 to 20 mL/kg of RBC, showed no negative effects on the pulmonary function with the larger volume.³⁹ Wong et al. showed that when giving 20 mL/kg instead of 15 mL/kg, fewer RBC transfusion events were necessary without any negative side effects.⁴⁰

Comparison of transfusion volumes is hampered by the different Ht of the products used. RBCs suspended in CPD-A usually have an Ht of around 40%, unless citrate phosphate dextrose adenine (CPD-A) plasma is reduced. In extended-storage media the Ht is $60 \pm 5\%$ and in some centres RBCs are centrifuged to a packed RBC concentrate of 80%.⁴¹ These differences in Ht should be taken into account when comparing different transfusion strategies but they are not always clearly mentioned in the reports, and even lacking in randomized studies.^{35,42} RBC used for young infants can be stored without causing negative effects when transfused. Strauss showed that small-volume transfusions (10–20 mL/kg), stored for up to 42 days,⁴¹ could be used safely. Because a small volume is given, there is hardly any rise in serum potassium or decrease in RBC 2,3-bisphosphoglycerate, and the potential negative influence of

Table 1. Transfusion thresholds for red blood cells

Study/centre	Endotracheal ventilation	NCPAP/IF	Breathing spontaneously	No respiratory support or supplemental oxygen
Leiden	Supplemental oxygen: Hb <12.8 g/dL No supplemental oxygen: Hb <11.2 g/dL		Preterm and term with SoA: Hb <9.6g/dL Preterm without SoA: ≤28 days Hb <9.6 g/dL >28 days Hb <8.0 g/dL	
Franz et al. ⁷⁴	FI _O ₂ >25% Ht _v <0.40 FI _O ₂ ≤25% Ht _v <0.30		Term without SoA: Hb <8.0 g/dL FI _O ₂ >25% or SoA present Ht _v <0.30	Ht _v <0.21
Kirplani et al. ³⁷	<1 week of age Hb _c <13.5/11.5 g/dL 1-2 weeks of age Hb _c <12.0/10.0 g/dL >2 weeks of age Hb _c <10.0/8.5 g/dL		<1 week of age Hb _c <12.0/10.0 g/dL 1-2 weeks of age Hb _c <10.0/8.5 g/dL >2 weeks of age Hb _c <8.5/7.5 g/dL	
Miyashiro et al. ⁷⁵	MAP ≥8 cm H ₂ O Ht <0.40 MAP 6-8 cm H ₂ O Ht <0.35 MAP <6 cm H ₂ O Ht <0.30	FI _O ₂ > 35% Ht <0.35 FI _O ₂ < 35% Ht <0.30	FI _O ₂ <35% by hood Ht <0.30	reticulocyte count <2% Ht <0.20
Fabres et al. ⁷⁶	Acute phase of RDS Hb <12 g/dL		Supplemental oxygen Hb <10g/dL	Hb <7 g/dL
Maier et al. ⁷⁷	Ht _v <0.40		FI _O ₂ >40% Ht _v <0.40 FI _O ₂ <40% and <2 weeks of age Ht _v <0.35 3-4 weeks of age Ht _v <0.30 >4 weeks of age Ht _v <0.25	Ht _v <0.25
Herman et al. ⁶	FI _O ₂ >35-40% or MAP >6-9 cm H ₂ O Ht <0.35-0.40 FI _O ₂ <35-40% or MAP <6-9 cm H ₂ O Ht ≤0.28-0.30		FI _O ₂ >40% or major surgery or SoA : Ht <0.28-0.30	Ht <20%
Bell et al. ³⁸	Ht <0.46/0.34	Ht <0.38/0.28	Ht <0.38/0.28	Ht <0.30/0.22

Hb=hemoglobin; Hb_c=capillary hemoglobin; Ht_v=venous hematocrit; IF=infant flow; MAP=mean airway pressure; NCPAP=nasal continuous positive airway pressure; RDS=respiratory distress syndrome; SoA=signs of anemia

the preservatives can be dismissed. These findings support the use of single-donor unit RBCs for multiple transfusions in extremely and very low birth weight infants. One donor RBC unit is divided into four or five smaller pedipacks, which can be reserved for one (or two) infant(s). The aim of a single-donor program is to expose infants to as few risks of transfusion-acquired infection, and, in particular, infections for which testing is not mandatory, as possible. White blood cell reduction is commonly used to prevent CMV transmission in RBC products as well as in platelet units. Other possible negative effects of white blood cells such as classic non-haemolytic febrile transfusion reactions, are very rare in neonates. Transfusions intended for neonates born before a gestational age of 32 weeks, or for a child who received IUTs, are irradiated with 25 Gy to prevent TA-GVHD.³⁻⁶

Alternative approaches to reduce red-cell transfusions

For optimal application of alternative approaches to reduce transfusions, it is important to make a reliable risk estimation for transfusion. In a study by Jansen et al.,⁴³ gestational age, birth weight and Apgar score at 1 minute were significant predictors for receiving an RBC transfusion in the first 30 days of life. Eighty-nine % of the infants with a gestational age <32 weeks, a birth weight below 1000 g and almost all infants with this weight and an Apgar score of <6 at 1 min needed at least one RBC transfusion.

In a study designed to predict which infants would benefit from erythropoietin, i.e. need an RBC transfusion after the second week of life, gestational age, 5-min Apgar score, transfusion during the first week of life and patent ductus arteriosus ligation were predictive factors for needing at least 2 transfusions. Gestational age <30 weeks alone or combined was the best overall predictor.⁴⁴ Alternatives should be sought for this young age group.

Delayed cord blood clamping

In 2007, Rabe and colleagues performed a systematic review of delayed cord clamping in prematurely born infants; the review was based on ten studies and included a total of 454 infants.⁴⁵ There was a lower transfusion need ($p=0.004$) and a lower incidence of ICH ($p=0.002$) in the delayed clamped group than in the early clamped group. Subsequently, Strauss published a randomized controlled trial in 105 neonates between 30 and 36 weeks gestation but found no difference in RBC transfusion needs and clinical outcomes between the groups. Children of <30 weeks gestation could not be included because of technical problems – including immediate resuscitation needs – with delayed cord clamping.⁴⁶

Autologous cord blood transfusion

Brune et al. took umbilical cord blood (UCB) from 290 term and premature newborns.⁴⁷ Of this group, 52 infants received an autologous placental blood transfusion; 30 because of anaemia of prematurity (AOP), the others for surgery directly after birth or in the first month of life. The amount of harvested blood was sufficient to cover all transfusion needs in about 40% of the infants with a birth weight between 1000 and 2500 g; all extremely low birth weight infants (<1000 g) needed additional allogeneic RBC.⁴⁷ Brune et al. concluded that, for term infants requiring surgery in the first few weeks of life, and for premature infants with a birth weight >1000 g and a gestational age <35 weeks, autologous cord blood can be an alternative source of RBC to allogeneic products.⁴⁷ Several other studies confirmed the use of autologous cord blood for infants requiring surgery for antenatal diagnosed birth defects. In 50–64% of these mostly term-born infants, the harvested cord blood red cells were sufficient to cover all transfusion needs.^{48,49}

Blood processing problems and a bacterial contamination rate of 8.6% were identified for cord blood transfusion of small premature infants with collected volumes of less than 30 mL. In the small number of children in whom an autologous cord blood red cell transfusion was possible, there was a similar rise in Hb/Ht as compared to donor RBC transfusions.⁵⁰

We performed a double-blind, randomized controlled to assess the feasibility of the use of autologous cord blood for AOP for infants born at a gestational age of <32 weeks (unpublished data). Due to low collected volumes, autologous products were available for only 27% of the infants needing transfusions, replacing 75% of allogeneic RBC for these children born between a gestational age of 27–30 weeks. Technically, UCB collection and processing are possible, but the costs are high.

Recombinant erythropoietin

Newborns do not produce EPO for the first two weeks of life and RBC transfusion further depresses the EPO response to anaemia.⁵¹ Several Cochrane studies have investigated the use of low- or high-dose EPO administered early (within the first week of life) or late (after the first week of life). These studies have evaluated exposure to RBC transfusions, the number of blood donors, clinical complications (retinopathy of prematurity) and hospital stay as endpoints, but have found no evidence for the use of EPO.⁵²

Platelet transfusion

A thrombocyte count below $150 \times 10^9/L$ in newborn infants is pathologic. The risk of severe thrombocytopenia ($<50 \times 10^9/L$) is haemorrhage, specifically an ICH with the danger of severe

neurological damage or death.⁵³⁻⁵⁶ To correct an abnormal platelet count, the cause of the thrombocytopenia should be taken into account.

Causes include maternal pathology such as HELLP (hypertension, elevated liver enzymes, low platelets) syndrome or (pre-)eclampsia with placental insufficiency, maternal idiopathic thrombocytopenia, fetal or neonatal alloimmune thrombocytopenia (FNAITP), sepsis, drugs and diffuse intra vascular coagulation. Whereas early thrombocytopenia often has a maternal reason, late thrombocytopenia – developing 3 days after birth or later – generally has a nosocomial cause. Newborns who receive platelets to treat thrombocytopenia have a higher mortality rate than neonates with a normal platelet count. The severity of the underlying disease explains the higher probability of death; thrombocytopenia and bleeding is not the cause of excess death rates.^{55,57,58}

In cases of platelet transfusions it is important to distinguish between alloimmune thrombocytopenia and other causes; first, because newborns with FNAITP, irrespective of prematurity, have a high risk for massive ICH; and second, because HPA-compatible platelets are warranted. The frequency of FNAITP in Caucasian populations is 1:1200 newborns. Prompt transfusion with HPA-1a-negative or HPA-5b-negative platelets is effective in 85–95% of cases.⁵⁹ Transfusion is generally undertaken during a planned delivery in women treated antenatally with IVIG. Often, just one HPA-matched transfusion is sufficient to maintain a stable platelet count above $100 \times 10^9/L$.⁶⁰ However, in 50% of the cases in which NAITP presents unexpectedly in a firstborn, HPA-compatible platelets might not be available and production and testing of washed (to remove antibodies) maternal platelets will cause a delay of 12–24 h. In a retrospective study in 27 newborns with FNAITP, Kiefel and colleagues observed a moderate to good increment in 24 children after random platelet transfusions and, moreover, no adverse effects.⁶¹ Random platelet transfusions can thus be used, awaiting matched platelets or effect of other therapy (i.e. IVIG), although the benefits of concomitant IVIG have not yet been shown.^{60,61}

Another maternal cause of neonatal thrombocytopenia is idiopathic thrombocytopenia. The occurrence of ICH during and after delivery is estimated 0–1.5%, and prompt random platelet transfusions, in conjunction with corticosteroids or IVIG, are indicated in case of severe bleeding.²⁷ Neonatal thrombocytopenia associated with maternal eclampsia and HELLP resolves spontaneously the first week after birth and needs no treatment.⁶⁰

A study by Bonifacio et al. found that two-thirds of the cases of severe thrombocytopenia ($<50 \times 10^9/L$) occurred beyond 72 hours after birth in a group of very prematurely born infants with a gestational age <30 weeks.⁶² No correlation was found between the severity or the time of onset of the thrombocytopenia and the occurrence of ICH. Platelets were administered in 85% of the cases of severe thrombocytopenia and in 65% of the moderate cases (platelet

count $50\text{--}100 \times 10^9/\text{L}$) without a reduction in mortality in the transfused group compared to the non-transfused group.

Murray et al. conducted a retrospective study in their own neonatal intensive care unit, evaluating all patients with a platelet count $<50 \times 10^9/\text{L}$ over a three-year period.⁵⁸ Six percent of the admissions developed a severe thrombocytopenia, most often after the first few days of life, thus after the critical period for developing severe ICH.

A randomized controlled trial conducted by Andrew et al. compared no-treatment for a platelet count between 50 and $150 \times 10^9/\text{L}$ with prophylactic platelet transfusion. They found a comparable short-term neonatal outcome irrespective of the child's clinical condition at time of thrombocytopenia.⁵³

The trigger for platelet transfusions varies among guidelines. Most neonatal units use a protocol taking platelet count, disease severity, indwelling drains, artificial ventilation or any recent surgery into account. Table 2 summarizes various protocols to show that – except in cases of major bleeding, ICH and surgery (for which a trigger of $100 \times 10^9/\text{L}$ is recommended) – most centres consider platelet transfusions in infants in whom the platelet count is $<50 \times 10^9/\text{L}$; in stable infants an even lower platelet count can also be considered.^{5,55,63} The different protocols also stress the importance of aggressive treatment of the underlying condition causing the thrombocytopenia. As stated by Calhoun et al., guidelines are based on consensus of practice, and not meant to be strict rules. Rather, they should be seen as aids to making decisions in one's own practice.⁶³

There are no studies to recommend whether thrombocytes should be given as a concentrate or as a suspension. Concentration of platelets causes approximately 15% platelet loss and, once in a syringe, the shelf life is reduced to a maximum of 6 h.⁶⁴ Currently, aphaeresis devices can collect such highly concentrated platelets, which can be stored for 1–3 days.²⁸ Calhoun et al. advise giving 10–15 mL/kg CMV-negative standard platelet suspension (containing circa 1×10^9 platelets/mL), which should raise the platelet count by approximately $100 \times 10^9/\text{L}$.⁶³ In our centre, we transfuse platelet concentrates of 5 mL derived from a single donor (containing circa 10×10^9 platelets/mL) and transfuse 10×10^9 thrombocytes/500 g infant weight (up to 2500 grams, above which we use 10 mL).

Platelet transfusions are not without risks; these include bacterial contamination, transmission of viral agents and transfusion-related lung injury.^{55,65} Consequently, adequate indication, the best product and quantity of platelet products and alternative treatments should be investigated. Even when we put the infants at risk for haemorrhage, without randomized controlled trials we will never know the safe thresholds for transfusion.

Table 2. Thrombocyte transfusion triggers

Study	Trigger stable	Trigger unstable	Definition unstable	No transfusions
Calhoun et al. ⁶³	<25	50	surgery or within 5 days after surgery, cardiovascular instability, respiratory instability, <72 hours after seizure, DIC, <1500 gram and <7 days	
Roberts et al. ⁵⁵ Murray et al. ⁷⁴	<30	30-49	consider if <1000 gram and <7 days, bleeding, clinically unstable, previous major bleed (IVH 3-4 or pulmonary bleeding), current minor bleeding (petechiae, oozing from puncture sites, stained endotracheal secretions), current coagulopathy, necessary surgery or exchange transfusion	
		50-99	if bleeding	>100
Leiden	<30	30-49	if <1000 grams and <1 week, previous major bleeding, exchange transfusion, planned surgery, platelet count expected to drop below 30	
		50-100	if bleeding	>100

Numbers are platelet counts x 10⁹/L, unless otherwise stated

DIC=diffuse intravascular coagulation; IVH=intraventricular haemorrhage

Alternative approaches to thrombocyte transfusions

Virtually no alternatives exist for bleeding neonates with thrombocytopenia.⁵⁵ IVIG, corticosteroids and thrombopoietin do not increase the platelet count instantly, and results with recombinant factor VIIa, used in children and adults, are lacking in neonates.

Granulocyte transfusion

The most common causes of leucocytopenia in the neonatal intensive care unit are sepsis, maternal hypertension, and endotoxemia.⁶⁶ Alloimmune maternal antibodies are a very rare cause of neonatal neutropenia. Neutropenia associated with maternal hypertension resolves spontaneously within hours to days and needs no treatment.

Septic newborns with granulocytopenia have a higher mortality rate than infants with a normal or raised white blood cell count upon infection.⁶⁷ Neutrophil transfusions are not useful in the prevention of infections but can be administered to help clear infections in infants with severe neutropenia being treated with antibiotics.⁶⁸

Granulocyte transfusions are used infrequently in neonatal sepsis⁶⁶ and there are no evidence-based studies on the use of granulocytes in newborns. A dose of 1–2 x 10¹⁰/L granulocytes per kg is suggested for effective treatment and this must be repeated daily or on alternative days for 1–2 weeks.⁶⁹ Granulocytes can be prepared on demand from (pooled, blood group O-Rh-D negative) buffy coats, but these contain fewer neutrophils than granulocytes obtained by aphaeresis of G-CSF stimulated donors and, in adults, are less effective in reducing mortality.⁷⁰

It is possible that the infusion of buffy coat granulocytes has more side effects than granulocyte colony stimulating factor (G-CSF) mobilized aphaeresis leucocytes. Wheeler et al. treated four infants with buffy coat granulocytes. In 2 infants the respiratory problems worsened after the infusion, possibly due to leucoagglutination, as described in adults.⁷¹ In a study by Baley et al. two of 13 infants had a decrease in P_aO_2 of more than 30 mmHg during their third granulocyte transfusion. This resolved spontaneously but no more leucocyte transfusions were given to these children.⁷²

A Cochrane review on the effect of granulocyte transfusion in septic, neutropenic infants being treated with antibiotics compared to placebo or no transfusion showed no significant difference in all cause mortality. Compared with IVIG, there was a borderline statistical difference in favour of the granulocyte transfusion in a study with 35 neonates. No long-term outcome was reported.⁷³

In leucocytopenia caused by maternal antibodies, G-CSF should attain a quicker response than IVIG.⁶⁶

Multi-centre, randomized controlled studies are necessary to achieve adequate power and sufficient patients enrolled in an acceptable time span, to be able to conclude on the effect of granulocyte transfusions.

Practice points

- Intrauterine RBC transfusions induce additional maternal erythrocyte antibodies in 25%, therefore compatibility tests must be performed with fresh maternal serum before every transfusion.
- RBC used for IUT should be manipulated as little as possible and there should be a short interval between processing/irradiation and transfusion because of potassium load.
- Intrauterine platelet transfusion is controversial and the high risk of transfusion-related complications must be balanced against the risk of ICH.
- The first-line treatment for intrauterine alloimmune thrombocytopenia is maternal IVIG.
- Delayed cord clamping should be considered for very low birth-weight infants if clinical condition permits.
- HPA-1a-negative and HPA-5b-negative donor platelets should always be available for neonates known with NAITP.
- For neonates suspected for FNAITP, random platelets are not contra-indicated while awaiting compatible (maternal) platelets.

- A critical approach to blood taking for investigation, next to alternatives to donor RBC, could reduce the number of allogenic transfusions to neonates, especially in those born <30 weeks gestation.

Research agenda

- Clinical studies on appropriate transfusion thresholds, volumes and products.
- Identification of patients who can benefit most from alternatives to allogeneic RBC transfusions.
- Prophylactic platelet transfusion studies using bleeding as the endpoint.
- Multicentre collaborative studies into the safety and efficacy of granulocyte transfusions.
- To improve care without taking risks, a study by an international consortium should compare differences in transfusion policy.

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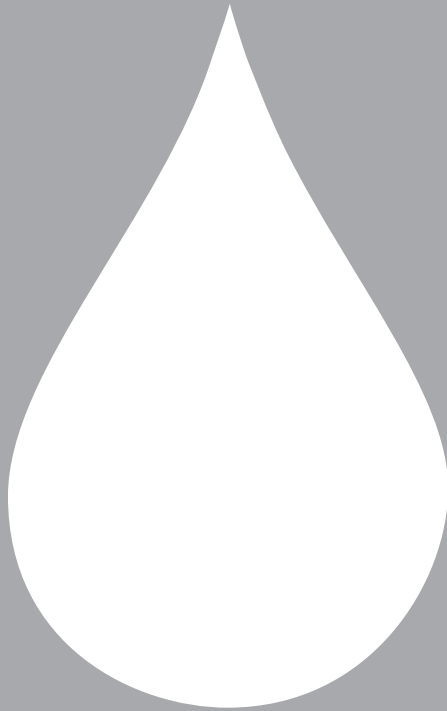
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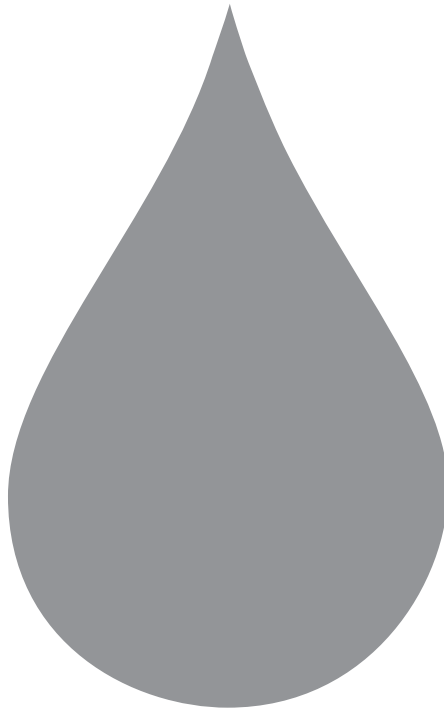
Part I

Umbilical cord blood



Chapter 3

Potential use of autologous umbilical cord blood red blood cells for early transfusion needs of premature infants



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Abstract

Background This prospective study investigated whether the odds of receiving a red blood cell (RBC) transfusion in premature infants can be predicted at birth and for whom of these infants harvesting of umbilical cord blood (UCB) for autologous transfusion within 30 days after birth would be worthwhile.

Study design and methods Characteristics were evaluated from 288 premature infants with a gestational age between 24 and 36 weeks and who were admitted to our neonatal center. In 144 (63%) of these infants UCB collection was attempted and the early transfusion needs could be compared with the amount of UCB available for transfusion.

Results Sixty-nine of 114 (61%) inborn infants with a gestational age of less than 32 weeks received one or more RBC transfusions of 10 mL per kg within 30 days after birth. Apgar score at 1 minute of less than 6 and gestational age of less than 32 weeks were independently associated with the chance of receiving a transfusion in this group. In 31 of 69 (46%) infants, at least 15 mL of UCB per kg of birth weight was collected and in 28 of 69 (41%) this would have been sufficient to cover their early transfusion needs.

Conclusion The decision to collect UCB for postnatal transfusion can be made just after labor, based on Apgar score and gestational age. The collection of UCB is most effective and efficient for premature infants between 29 and 31 weeks of gestation. For infants less than 29 weeks of gestation, the technical aspects of UCB collection need improvement. This pilot study requires a prospective clinical study to evaluate the proportion of premature infants that can be fully or substantially supported with autologous UCB.

During the first weeks of life, premature infants often develop anemia and need allogeneic red blood cell (RBC) transfusions.^{1,2} The number and amount of transfusions for premature infants and the donor exposure have decreased over the past decennia.²⁻⁴ Nonetheless, these infants are exposed to risks inherent to allogeneic transfusions, such as transmission of infectious agents.^{5,6} Use of autologous umbilical cord blood (UCB) may reduce these complications. Several investigators have demonstrated the feasibility of the collection, storage, and use of UCB RBCs for autologous transfusion in newborn infants. Approximately 20 mL of UCB per kg of body weight can be harvested,^{1,6} and a linear association has been found between collected blood volume and birth weight with a greater relative blood volume from placentas of smaller newborns.^{7,8} Few data are available on the question of which infants will need RBC transfusions.^{1,9} We investigated whether the risk of receiving a RBC transfusion during the neonatal period can be predicted at birth, to estimate for which premature infants UCB should be harvested, tested, processed, and stored as a RBC product. We evaluated the characteristics and need for RBC transfusions of 288 infants born between 24 and 36 weeks of gestation. In addition, we report on the amount of UCB collected in relation to the transfusion needs.

Materials and methods

Patients

Informed consent to collect UCB was obtained from the parents before delivery if the delivery took place in the Leiden University Medical Center. The Medical Ethical Committee of the Leiden University Medical Center approved the study protocol. Neonates included in this study were all premature infants born in 2002 at a gestational age between 24 and 36 weeks and admitted immediately or within 1 day (without any phlebotomy losses) to the neonatal center. Infants were included irrespective of disease severity, duration of hospitalization, and the level of care provided. As a cutoff point in following the course of premature infants, we used a period of 30 days, corresponding to the expected shelf life of the cord RBCs. Exclusion criteria for participation in this study were diseases for which autologous blood transfusion was contraindicated, such as active blood group antagonism (hydrops fetalis) and HPA antagonism. Nonviable premature infants were excluded. The existence of twin-to-twin transfusion syndrome was not considered as an exclusion criterion.

During a 3-month period, 22 consecutively (in)born infants at a gestational age of less than 32 weeks, who were admitted to the neonatal center, were closely monitored for a period of 14 days to estimate the amount of blood loss for diagnostic purposes and to correlate the amount of phlebotomy losses and RBC transfusions.

Collection of UCB

In vaginal deliveries, the placenta was still in utero when blood was harvested. No drugs were given to increase uterine muscle tone. In cesarean section deliveries, the placenta was removed from the uterus before blood was harvested. The umbilical cord was double clamped, transacted, and cleansed. UCB was collected with an adapted collection system including a 150-mL collection bag (Compoflex, Fresenius, Emmer Compascuum, the Netherlands) to which a collection system (Medisize 1992.002D, Macopharma, Langen Germany) was attached. The collection bag contained 5 mL of CPD-A. The collection system included two cannulas with needles to permit two punctures of the umbilical vein. To increase the volume of blood collected after the ceasing of spontaneous blood flow, a syringe (volume of 20 mL) containing 5 mL of CPD-A could be attached to a three-way tap. A buffy coat-depleted packed RBC suspension was prepared from the UCB by centrifugation in a Sepax device (Biosafe, Switzerland) and adjusted with SAG-mannitol to a hematocrit (Hct) of 55 to 65%. The supernatant plasma was used for bacterial culture (BacT/ALERT, bioMérieux, Hazlewood, MO) and the buffy coat was used for research purposes. The RBC products were stored for 30 days at 2 to 6°C. The quality control variables during storage will be reported separately. Because some UCB collections could not be processed due to technical and logistic reasons, the analysis is based on preliminary approved products (defined as a collection of at least 15 mL of UCB exclusive CPD-A, without visual clots) instead of finally approved blood products in all instances. None of the collected cord blood RBC products were transfused in this pilot study.

Patient data

Clinical data of infants and their mothers were obtained from the hospital chart and hospital and laboratory information systems. Information was recorded on gestational age, sex, Apgar scores, umbilical arterial pH, birth weight, placental weight, method of delivery, singleton or multiple birth, length of hospitalization, RBC transfusions, and death. Data collection specified the number and volume of RBC transfusions during the 30-day observation period. The indication for RBC transfusions was based on local transfusion guidelines, which consider age of the infant after birth, ventilatory status, and hemoglobin concentration. At each transfusion 10 mL of RBCs per kg was infused over 3 h.

Statistical analysis

The infants' clinical characteristics are presented as medians and their ranges, numbers, and percentages. The differences between the subgroups of infants are presented as p values and were calculated with the two-tailed t-test for continuous variables and the chi-square test for the categorical variables. The differences between the four groups in Table 3 were analyzed

with the independent samples t-test. To investigate the correlations between different variables, Spearman's correlation coefficient rho was calculated (except birth weight, none of the variables followed a Gaussian distribution).

The chance of an infant receiving at least one RBC transfusion within the first 30 days of life and the variables influencing this chance were calculated by backward stepwise logistic regression analysis with the dependent variable "transfused within 30 days" (no=1, yes=2) and several variables (gestational age, birth weight, sex, Apgar scores, placental weight, and hospitalization unit). The selection of the variables was based on the significant differences of these variables between transfused and nontransfused infants ($p < 0.05$). Logistic regression analysis was used to correct for mutually connected confounders.

Results

During the 12-month observation period, 288 premature infants with a gestational age of less than 36 weeks were admitted to the neonatal center, of whom 16 met the exclusion criteria. A total of 140 of the 272 infants included were inborn with a gestational age of less than 32 weeks. Clinical characteristics of the study population are shown in Table 1.

RBC transfusions

The number of RBC transfusions administered during hospitalization was recorded up to 30 days after birth. Ninety-six of the 272 (35%) premature infants received one or more RBC transfusions. Premature infants with a gestational age of less than 32 weeks received more transfusions than infants with a gestational age between 32 and 36 weeks, respectively, 82 of 140 (59%) and 14 of 132 (11%). There was only a weak correlation, however, between the number of transfusions and gestational age within the group of less than 32 weeks of gestation ($n=140$, $r=-0.553$, $p=0.000$).

The median birth weight of all infants less than 32 weeks ($n=140$) was 1244 g (range, 538–2155 g) and of the 82 infants who received transfusions, 1082 g (range, 538–1895 g). Among the infants with a birth weight of less than 1500 g ($n=104$), 69% ($n=72$) received at least one transfusion; the median number of transfusions was 2 (range, 1–9). The large majority (89%) of infants with a birth weight of less than 1000 g received at least one transfusion with a median number of 3 (range, 1–9).

Likelihood of transfusion

In premature infants of less than 32 weeks of gestational age, Apgar scores at 1 and 5 minutes, birth weight, placental weight, duration of hospitalization, and death influenced the chance of receiving a RBC transfusion (all $p < 0.05$ in univariate analyses). In the logistic regression analyses,

Table 1. Characteristics of included infants (n=272)

	Gestational age (weeks)				
	24-28	28-30	30-32	32-34	34-36
Number	34	46	60	40	92
Birth weight (g)					
<1000	25	10	2	1	0
1000-1449	9	30	28	7	4
1500-1999	0	6	29	20	23
2000-2499	0	0	1	12	40
>2500	0	0	0	0	25
1-min Apgar score					
<6	22	19	22	17	14
≥6	11	26	38	23	78
Unknown	1	1	0	0	0
Neonatal unit					
Intensive care	34	46	57	32	34
Special care	0	0	3	8	58
Umbilical cord pH					
≤7.0	20	2	3	2	4
>7.0	3	33	43	25	66
Not available	11	11	14	13	22
Delivery					
Vaginal	28	26	31	19	61
Cesarean section	6	20	29	21	31
Placental weight (g)					
<200	2	2	0	0	0
200-499	22	29	44	19	51
500-749	2	5	3	5	21
≥750	0	0	0	0	1
Unknown	8	10	13	16	19
Hospitalization (days)					
<4	3	5	12	9	37
4-7	4	6	10	16	28
8-14	8	12	23	10	19
15-30	8	18	10	4	7
>30	11	5	5	1	1
Deceased					
Yes	16	10	3	0	1
No	18	36	57	40	91

duration of hospitalization and death were not considered suitable as variables because these data are not available at birth. The Apgar score at 1 minute after birth was selected because of the strong correlation between these scores at 1 and 5 minutes.

The sensitivity and specificity of the Apgar score were 63.8%(95% CI 0.532–0.743) and 79.3%, respectively. In the three gestational age subgroups (24–28, 28–30, and 30–32 weeks), respectively, 93.9%, 62.2%, and 35.0% of the infants received transfusions. In the three birth weight subgroups (<1000, 1000–1499, and 1500–1999 g), respectively, 88.9%, 57.6%, and 27.8% received transfusions. Logistic regression analyses showed that birth weight and Apgar score at 1 minute were independently associated with the odds of receiving a RBC transfusion ($p=0.033$ and $p=0.000$, respectively).

Infants born at a gestational age between 24 and 28 weeks had 8.88 times the odds of receiving a transfusion as infants born at 30 to 32 weeks of gestation ($p=0.016$), but the risk of receiving a transfusion at a gestational age between 28 and 30 weeks was not significantly greater than for those born between 30 and 32 weeks of gestation ($p=0.138$). Infants of less than 1000 g had 7.71 times the odds of receiving a transfusion compared to infants of 1500 to 1999 g ($p=0.014$). Likewise, the odds for infants with a birth weight between 1000 and 1499 g and those with a birth weight between 1500 and 1999 g were similar ($p=0.078$). Premature infants with an Apgar score of less than 6 had 7.25 times the odds of receiving a transfusion as those with an Apgar score of at least 6 ($p=0.000$).

The predicted odds of receiving at least one RBC transfusion during the first 30 days of life are shown in Fig. 1. In all three gestational age subgroups, with a corresponding Apgar score, the odds of receiving a transfusion diminished with increasing birth weight. This association

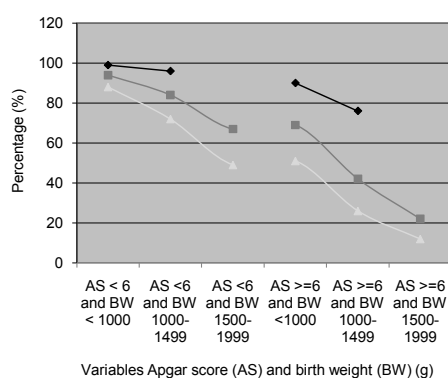


Fig 1. Percentage chance of receiving at least one RBC transfusion within 30 days after birth in infants with a gestational age of less than 32 weeks
 (♦)Gestational age 24 to 28 weeks, n=33; (■) gestational age 28 to 30 weeks, n=45; (▲) gestational age 30 to 32 weeks, n=60

is most obvious in infants with a gestational age of greater than 28 weeks. Irrespective of gestational age, almost all infants with an Apgar score of less than 6 and a birth weight of less than 1000 g received transfusions, whereas only a few infants with an Apgar score of at least 6 and a birth weight of 1500 to 1999 g needed any transfusion.

Logistic regression analyses were also performed on all 272 infants born at less than 37 weeks. Only gestational age ($p=0.007$) and Apgar score at 1 minute ($p=0.000$) were independently associated with the chance of receiving a RBC transfusion. Infants with an Apgar score of less than 6 had 4 times the odds of receiving a transfusion compared to infants with an Apgar score of at least 6.

UCB collections

As shown in Fig. 2, 227 of the 288 premature infants admitted to the neonatal center were born in our hospital, of whom 96 (48 deliveries) were twins. An attempt to harvest UCB was performed in 111 deliveries and resulted in 144 UCB collections, of which 91 (63%) were greater than 15 mL (excluding CPD-A). After processing and bacterial culture of these 144 UCB collections, 88 (61%) resulted in a product suitable for potential transfusion. In 114 infants with a gestational age of less than 32 weeks, 69 attempts to collect cord blood resulted in 32 (46%) in a collected volume of greater than 15 mL, of which 30 (43%) were approved for transfusion. UCB was tested for aerobic and anaerobic bacterial contamination (Bact/ALERT). From all processed collections, 6 of 91 were contaminated (7%).

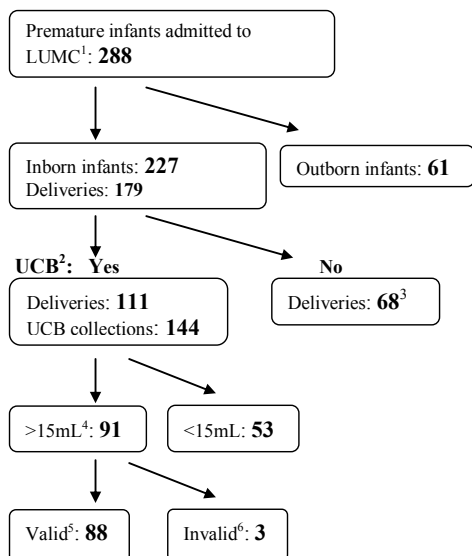
After all processing was completed (i.e., with RBCs at a Hct level of $60 \pm 5\%$ in storage medium), the volume of the units available for transfusion was approximately 70% of the harvested blood volume. The mean volume of the 88 approved blood products was 22 mL/kg of birth weight (range, 7–68 mL/kg) and of the 30 collections from infants less than 32 weeks of gestation 23 mL/kg of birth weight (range, 10–55 mL/kg).

In premature infants of less than 32 weeks, no correlation was found between the amount of UCB per kg of birth weight harvested and gestational age ($n=69$, $r=0.219$, $p=0.071$), birth weight ($r=0.150$, $p=0.218$), placental weight ($r=0.077$, $p=0.531$), or method of delivery ($r=0.244$, $p=0.064$).

Missed UCB collections

The reasons why no attempts were done to collect UCB at 68 deliveries are summarized in Table 2. Only in a few cases a clear explanation for the failure could be identified. In most cases there were no complicating circumstances (19 deliveries), and failures were often secondary to an urgent spontaneous delivery or cesarean section ($n=16$); only three times was no consent given for collection of UCB.

A: Premature infants 24-36 wk



B: Premature infants <32wk

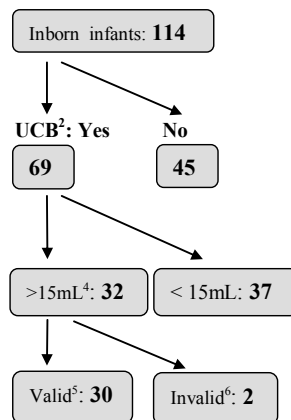


Fig 2. UCB collections

¹: All premature infants (24–36wk) admitted during the study period

²: (Delivery at which) UCB collection was performed

³: No UCB collection was or could be performed by complicating circumstances

⁴: Volume excluded CPD-A

⁵: Suitable for transfusion

⁶: UCB contained clots, cell counting problems, separation problems, positive bacterial culture

Potential coverage of transfusion needs with autologous cord blood

In, respectively, 63% and 54% of the 144 infants for whom UCB collection was attempted, at least 15 mL and more than 15 mL/kg of birth weight was available. For the 69 premature infants of less than 32 weeks of gestation for which collection was attempted, these percentages were 46% and 45%. This would have covered the total need for early transfusions in 28 of 69 (41%) premature infants of less than 32 weeks of gestation with an available UCB product (≥ 15 mL).

Dividing the infants of less than 32 weeks into four different groups according to “transfused” or “not transfused” and “cord blood product available” or “no cord blood product available” shows that for 24 of 39 (62%) of the premature infants who needed transfusions no UCB product was available (Table 3). For 11 of 39 (28%) premature infants who received transfusions who could receive autologous RBCs, the collected UCB would have covered their transfusion needs during the first 30 days after birth.

Table 2. Circumstances of deliveries (n=68) at which no attempt at cord blood collection was done

Circumstance	Singletons	Twins	Triplets
Forgotten	3		
Urgent delivery or cesarean section ¹	13	1	2
Primary cesarean section	4	1	
Allo-immunisation ²	3	2	
Respiratory problems ³	2		
GBS/PROM ⁴	2	2	
Corticosteroid study	1		
Placenta praevia	1		
Lasered TTTS		1	
Complications twins ⁵	2		
Not possible by broken placenta	1		
Delivery in emergency room via other hospital	2		
GBS	1		
Unknown	19	1	
PPROM		1	
No consent	3		

¹ Because of solutio placentae/maternal condition (preeclampsia, HELLP)/fetal condition

² Anti D/intracranial hemorrhage(ICH)/hydrops fetalis/twin-to-twin transfusion syndrome (TTTS) and severe ICH/intrauterine growth retardation and idiopathic thrombocytopenic purpura

³ Respiratory distress syndrome (RDS) III/hydrothorax/postdelivery pneumothorax

⁴ Group B streptococcus (GBS)/(premature) prolonged rupture of membranes (P)PROM/lasered TTTS and PROM and GBS/PROM and cord prolapse and sepsis (twins)

⁵ First infant born 2 days before second infant/first infant died: intrauterine fetal death

Phlebotomy losses

Twenty-two infants were divided in two groups according to gestational age: Group A (25 to <30 weeks) and Group B (≥30 to 32 weeks). The median gestational age of Group A was 27⁺⁵ weeks (range, 25⁺⁹–29⁺⁶ weeks), the median birth weight was 1125 g (range, 809–1699 g) and the median 1-minute Apgar score was 3 (range, 1–9). For Group B, these values were 31⁺⁰ weeks (range, 30⁺⁰–31⁺⁵ weeks), 1277 g (range, 558–1735 g) for birth weight and 8 (range, 2–10) for Apgar score.

After 14 days the infants of Groups A and B had median total phlebotomy losses of, respectively, 6.4 (5.2–10.7) and 5.7 (5.3–9.9) mL/ kg. During this period the infants of Groups A and B received, respectively, 15.0 (0–54.1) and 8.2 (0–19.6) mL/kg RBCs (Fig. 3). The volume of phlebotomy loss came close to the volume of transfused RBCs in infants of Group B. In infants of Group A, however, after 2 weeks of hospitalization, the volume of RBCs transfused exceeded the volume drawn for diagnostic purposes with approximately 10 mL/kg of birth weight.

Table 3. Transfusion volume during the first 30 days after birth and available UCB volume (excluding CPD-A) in infants <32 weeks gestation (n=69)

Group (n)	Tx	Product*	Median volume (mL)		Median product volume (mL/kg)	Median gestational age (weeks ^{+days})	Median birth weight (g)	Median Apgar score
			Tx†	UCB‡				
A (17)	No	Yes		39 (17-81)	23 (11-55)	31 ⁺⁰ (29 ⁺³ -31 ⁺⁶)	1500 (1198-1933)	8 (5-10)
B (24)	Yes	No	22 (8-73)			28 ⁺⁵ (25 ⁺⁰ -31 ⁺¹)	998 (536-1711)	5 (1-10)
C (15) [#]	Yes	Yes	17 (13-76)	26 (16-63)	22 (10-42)	30 ⁺² (25 ⁺⁴ -31 ⁺²)	1400 (812-1895)	4 (2-10)
D (13)	No	No				30 ⁺⁴ (28 ⁺⁰ -31 ⁺²)	1386 (1115-1840)	7 (1-9)

* At least 15 mL of UCB collected

† Allogeneous transfusions (mL) calculated as 10 mL per kg birth weight; Hct level of 0.60

‡ Collected UCB (minus CPD-A)

In 4 of 11 infants the cord blood was sufficient to support all of their transfusion needs

Tx=transfusion

Discussion

This observational study among 272 premature infants born at a gestational age between 24 and 36 weeks focused on the question whether the odds of receiving a RBC transfusion can be predicted at birth. The aim of the study was to estimate whether appropriate collection, testing, processing, and storage of autologous cord blood with the aim of transfusion and avoidance of waste of resources is feasible.

Various studies of premature infants investigated the possibilities for collection, storage, and use of UCB RBCs for autologous transfusion^{1,8,9}, but few epidemiologic data are available on

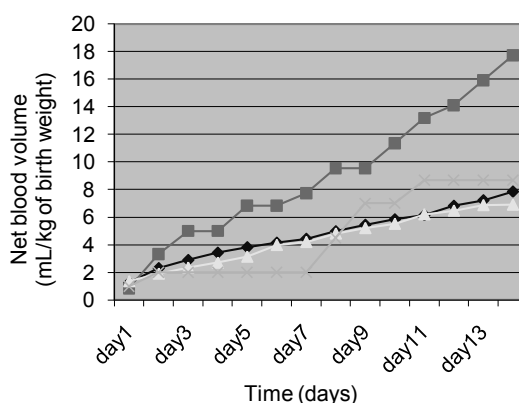


Fig 3. Drawn (mL/kg/day) versus transfused (mL/kg/day), cumulatively (◆) Cumulative drawn, Group A; (■) cumulative transfused, Group A; (▲) cumulative drawn, Group B; (X) cumulative transfused, Group B.

the contribution of autologous cord blood to circumvent allogeneic transfusions. This is the largest study regarding this subject in an unselected population of premature infants. Gestational age and Apgar score were significant predictors for the odds of receiving an RBC transfusion. In particular for infants born at more than 32 weeks of gestation, which as a group have a low risk for transfusion (11%), a 1-minute Apgar score of less than 6 increased the risk to receive a transfusion by 20%. Within the group of infants with a gestational age of less than 32 weeks, also birth weight significantly determined the odds of receiving a transfusion.

Only two previous studies addressed a similar question. Kling and coworkers investigated the occurrence of RBC transfusions in 47 premature infants of <1500 g, who were compared for demographic characteristics, phlebotomy blood loss, diagnosis, medications, and score for neonatal acute physiology (SNAP). They found phlebotomy loss and SNAP score at 7 days of life to be significant predictors of the number of transfusions.⁹ Brune and colleagues reported that almost 65% of premature infants with a birth weight of less than 1500 g receive at least one RBC transfusion during the first week of life.¹

The overall incidence of transfusions in our population is in agreement with previous reports showing that 45 to 60% of very low-birth-weight infants (<1500 g) received transfusions, although the number of units administered showed wide variation among studies.^{1,3,8,9} In our cohort, only 15% of the transfused infants needed more than three transfusions. Variations of clinical condition, transfusion policy, and phlebotomy loss in the nursery may explain the differences between the results of the different studies.

Several studies reported on the collection of UCB RBCs.^{1,5-8,10-13} Anderson and colleagues⁷ and Eichler and colleagues⁸ found a linear association between collected blood volume and birth weight with a greater relative blood volume from placentas of smaller newborns. Brune and colleagues collected approximately 20 mL of placental blood per kg of body weight irrespective of birth weight.¹ Surbek and colleagues collected a mean volume of 23.1 ± 1.1 mL cord blood per kg for infants with a mean gestational age of 32 ± 4 weeks and showed a correlation between collected cord blood volume and birth weight.⁶ Correspondingly, we collected a median volume of 23 mL per kg for infants of less than 32 weeks without a linear correlation between collected cord blood volume and gestational age, placental weight, or birth weight. Many collection attempts, however, were not successful because of difficulties to obtain vascular access and blood flow. Both Eichler and coworkers⁸ and Brune and coworkers¹ reported a poor correlation between the volume of cord blood collected and the transfusion requirements and thus often too little or too much RBC products were prepared. Therefore, Brune and coworkers¹ concluded that autologous blood seems not effective for treatment of anemia of prematurity because the collected amount is generally too small to cover transfu-

sion requirements, although for certain categories of newborn infants cord blood can replace or at least reduce allogeneic transfusions.¹⁰ To investigate whether UCB could cover the early transfusion needs, we compared the amount of UCB collected and the amount of allogeneic transfusions received during the first 30 days after birth. We could have provided 45% of the infants with a gestational age between 24 and 32 weeks with at least 15 mL of cord blood per kg of birth weight. Sufficient cord blood RBCs to supply all needs for transfusions during the first 30 days after birth were available for 41% of these infants. This implies that not enough cord blood was available to meet the (complete) early demand for blood in 59% of premature infants with a gestational age below 32 weeks. In contrast, it seems not useful to collect UCB for all premature infants with a gestational age over 32 weeks because only a minority will need RBC transfusions. If gestational age, birth weight, and Apgar score are taken into account, it can be decided to collect cord blood if the likelihood of transfusion is higher than 30 to 50%. This approach (and associated costs) should be prospectively tested in a study with sufficient patients to draw conclusions on the feasibility of autologous cord blood RBC transfusions.

Moreover, attempts to reduce allogeneic transfusions can be undertaken by strict indications for laboratory tests.^{3,9,14,15} Hume performed a retrospective review in premature infants and reported a diminishing number of transfusions if phlebotomy losses for diagnostic purposes were diminished and a more conservative transfusion policy was applied.³ We calculated phlebotomy losses in a subgroup and found that in premature infants of greater than 30 weeks of gestation, phlebotomy losses almost equal transfusion requirements.

In this study we used a period of 30 days as a cutoff point in following the course of premature infants. The cutoff point corresponds to the expected shelf life of the umbilical cord RBCs of 30 days. This limits the scope of our study because we only considered the early transfusion needs of these premature infants, but not the need for "late" transfusions, which many very premature infants receive and cannot be met by placental blood.

In summary, the decision to collect cord blood with the intention for early transfusion can be made just after labor. The collection of UCB may be effective and efficient for premature infants between 29 and 31 weeks of gestation and a birth weight between 1000 and 1500 g. For infants born at less than 29 weeks of gestation, the organizational and technical aspects of the UCB collection need improvement to optimize the collected volume and reduce the proportion of premature infants that need allogeneic transfusions. As our study was a theoretical exercise, because collected and processed cord blood RBCs were not transfused, a clinical feasibility study is needed to test these assumptions.

Acknowledgements

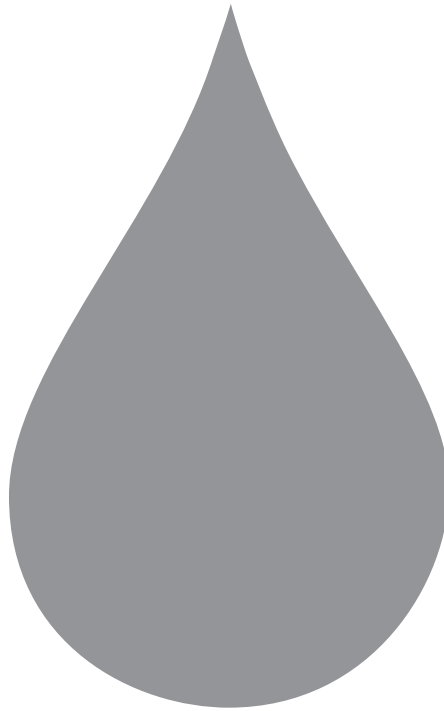
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Chapter 4

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



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Abstract

Background The objective was 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 of less than 32 weeks of gestation and processed into autologous RBC products. Premature infants requiring a RBC transfusion were randomly assigned to an autologous or allogeneic product. The primary endpoint was an at least 50% reduction in allogeneic transfusion needs.

Results Fifty-seven percent of the collections harvested enough volume (≥ 15 mL) for processing. After being processed, autologous products (≥ 10 mL/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 (87%) transfusion needs. Availability was highest (48%) for the 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 (19%) 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.

The combination of an impaired erythropoietin response to anemia,¹⁻² a shorter life span of hemoglobin (Hb) F-bearing red cells (RBCs) and high phlebotomy losses²⁻⁴ results in a rapid decline in Hb 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 Owing to increasing use of cord blood for transplantation purposes, progress has been made on aseptic collection of UCB and processing of small blood volumes.¹³⁻¹⁵

Approximately 20 mL of UCB per kg of body weight can be harvested irrespective of birth weight¹³ 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 Feasibility of the clinical application of autologous UCB remained to be investigated, however. To answer this question, we performed a randomized clinical trial on the use of autologous cord blood RBC transfusions in premature infants. Our objective was to assess the practical and economical feasibility of autologous RBC 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 (<http://www.trialregister.nl/trialreg/index.asp>).

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 syphilis (*Treponema pallidum* agglutination assay), hepatitis B virus (HBV) antibody, human T-lymphotropic virus (HTLV) antibody, human immunodeficiency virus (HIV) antibody and hepatitis C virus (HCV) antibody, 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 (e.g., rhesus D antagonism), hemoglobinopathies, maternal infections (HIV,

HBV, HCV, HTLV, and syphilis), premature rupture of the membranes and fever that were treated with antibiotics, and congenital infections (cytomegalovirus (CMV), parvovirus B19, and Toxoplasmosis).

Outcomes

The primary outcome variable was a reduction of at least 50% in the use of allogeneic RBC products in the first 21 days after birth. Secondary outcome variables 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 citrate-phosphate-dextrose-adenine (CPDA)-1 (Baxter, Deerfield, IL) and a syringe (Medisize) with 5 mL of 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 a closed centrifuge circuit

A quantity of 0.5 mL of whole blood was used to perform complete blood counts (Beckman Coulter Onyx, Miami, FL) 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 anticoagulant). RBCs were separated from buffy coat and plasma and adjusted with saline-adenine-glucose-mannitol (SAG-M) to a RBC unit with a hematocrit (Hct) level between 55 and 65%. At least 10 mL of CPDA-1 plasma was used for aerobic and anaerobic bacterial culturing (BacT/ALERT, bioMérieux, Durham, NC).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 at least 10 mL/kg of body weight of the child were stored for 21 days at 2 to 6°C. 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). 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 (adenosine triphosphate, 2,3-diphosphoglycerate acid, hemolysis, and pH) will be published separately, but were within allowed limits for standard RBCs 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 to 28, 28 to 30 and 30 to 32 weeks and were registered as “premature experimental product” patients, each with a unique study number. Upon request for a transfusion, the request form was marked with a premature experimental product 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 RBCs in SAG-M with a Hct level between 55 and 65%) was delivered. Both types of transfusion products were delivered in a syringe (Medisize) 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 to 15 mL/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, sex, 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 O₂ were systematically registered in clinical request forms. Data on RBC transfusions within 21 days and more than 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 at least 50% in the use of allogeneic RBC products to be worth the effort. To detect this reduction, with a power of 90% and $\alpha < 0.05$, two groups of 85 eligible transfused premature infants had to be included. Taking into account that $\pm 40\%$ of the infants will not require any RBC transfusion and an expected mortality of 10 to 15%, a total of 325 infants would need to be included.

Statistical and data analysis

Data are presented as mean $\pm$ SD unless indicated otherwise. Computer software (SPSS version 12 for Windows, SPSS, Inc., Chicago, IL) 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 analysis). Continuous variables were analyzed using independent t-tests. Other variables were analyzed using the Fisher's exact test or nonparametric tests. A p value of less than 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, a total of 427 women were admitted with imminent preterm delivery. A total of 180 women delivered in our hospital before 32 weeks of gestation. Three women were excluded directly post partum because of sepsis and 1 infant was excluded because of a congenital CMV infection. A total of 176 women (and 215 infants) met the study criteria for UCB collection (Fig. 1). Within this group, 195 collections (from 132 singleton, 24 monochorionic and 20 dichorionic deliveries; in 1 dichorionic delivery 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 1 case the record on the placenta was missing.

Of the 176 attempts, 101 (57.4%) harvested a UCB volume of at least 15 mL, suitable for closed centrifuge circuit 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

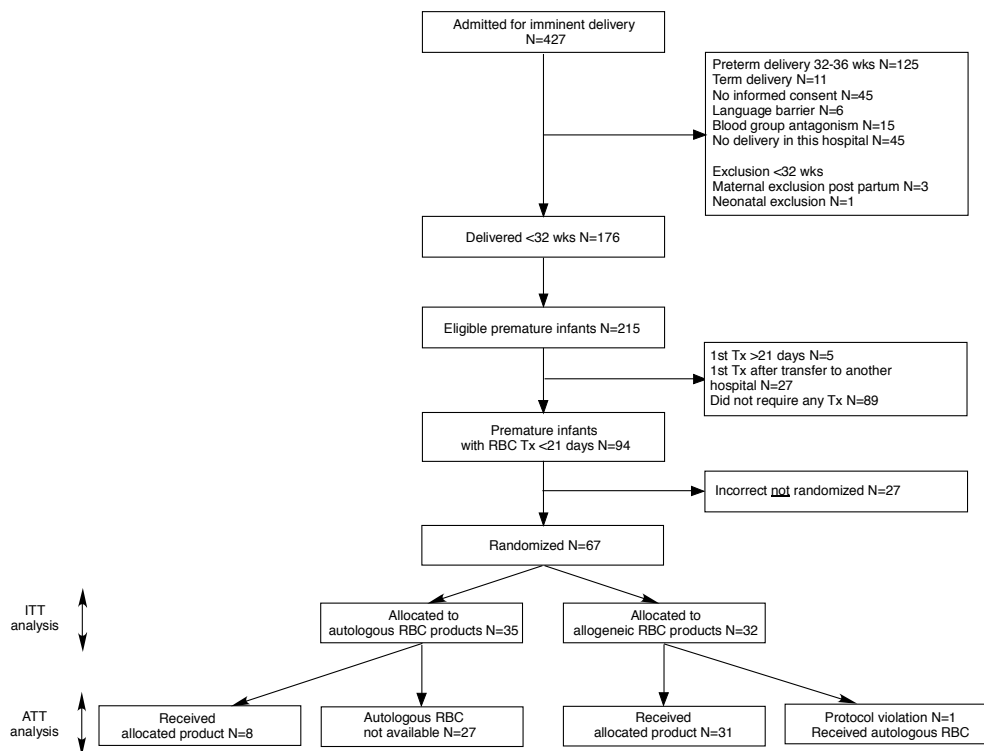


Fig 1. Flow chart: patient selection and randomization scheme

ITT=intention to treat analysis; ATT=analysis according to transfusion; Tx=transfusion

between 28 and 30 weeks), and 33 ± 13.3 mL (53 collections for 58 infants born between 30 and 32 weeks).

Harvested UCB (excluding anticoagulant) was respectively 16 ± 15 mL/kg for infants with a birth weight of less than 1000g, 18 ± 18 mL/kg for infants weighing between 1000–1250g and 20 ± 23 mL/kg for infants with a birth weight of more than 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 (out of 101, 66.3%) of which three were withdrawn due to a positive bacterial culture (coagulase-negative staphylococci, $n=2$, Gram-negative rods $n=1$). All 3 cultures were positive within 24 hours. Thus, after processing and quality control (QC), 64 suitable autologous products were available for transfusion (36.4% of all 176 collections; Table 1).

Table 1. Collected UCB and autologous RBC processing

	Total	24-28 weeks	28-30 weeks	30-32 weeks
Number of eligible infants born <32 weeks	215	45	72	98
Number of possible collections	195	41	64	90
Number of no collection attempt	19	6	8	5
Number of collection attempts	176	35	56	85
UCB volume <15 mL*	75 (43)	19 (54)	24 (43)	32 (38)
UCB volume ≥15 mL and processed*	101 (57)	16 (46)	32 (57)	53 (62)
Autologous RBC units after processing	67	6	27	34
Products with a positive bacterial culture	3	0	0	3
Available autologous RBC units for the total study population*	64 (36)	6 (17)	27 (48)	31 (36)

*Data are reported as number (%)

Study population

In the study period, 215 infants were born before 32 weeks of gestation. Of these, 5 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. Twenty-seven infants that met the entry criteria for randomization were missed, however (Fig. 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 nonrandomized infants were compared with the two randomized groups to exclude possible confounding factors. All transfused patients remained included in the follow-up analysis, except 1 infant in the nonrandomized 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 (Fig. 1).

Table 2. Demographic and clinical variables of infants with transfusion indication within 21days after birth (n=94)

	Autologous group (n=35)	Allogeneic group (n=32)	Nonrandomized (n=27)
Birth weight (g)*	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 †	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' §	6 (4-8)	6 (4-7)	7 (3-8)
AS 5' §median (IQR)	8 (7-9)	8 (7-8)	8 (6-9)
Arterial pH*	7.27 ± 0.1 (n=21)	7.27 ± 0.1 (n=24)	7.28 ± 0.1 (n=18)
Hct after birth %*	44 ± 7	41 ± 10	42 ± 6
Cord clamping time, sec*	7 ± 4 (n=28)	8 ± 4 (n=27)	8 ± 6 (n=22)
CRIB II§	9 (8-11)	10 (7-12)	10 (7-12)
Infants with supplemental O ₂ (n)	33	31	24

* Data are reported as mean ±SD

† Data are reported as number (%)

‡ Most unequally distributed variable, p=0.01

§ Data are reported as median (IQR)

AS= Apgar score; CRIB II = clinical risk factor for babies; IQR = interquartile range

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 allogeneic RBC of 25% was present in infants born between 28 and 32 weeks of gestation (Table 3).

Table 3. Transfused RBC units and transfusion variables*

	Autologous group (n=35)	Allogeneic group (n=32)	p Value [†]	Nonrandomized(n=27)
Total transfusions ≤ 21days	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
Pretransfusion Hct (%)	35.5 ± 4.2	34.4 ± 6.4	0.99	36.1 ± 4.0
Posttransfusion Hct (%)	45.7 ± 6.1	44.5 ± 6.5	0.49	44.5 ± 5.2
First transfusion (day)	4 (2-9)	3 (2-9)	0.25	4 (2-10)
Interval until next transfusion (days)	3 (2-6)	4 (2-8)	0.93	4 (2-6)
Allogeneic transfusions ≤ 21days	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	

* Data are reported as mean ± SD or median (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 $9.8 \pm 5.5\%$, comparable with the increase after an allogeneic transfusion of $10 \pm 5.2\%$. 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 one of the four transfusions they required in the first 21 days. In 3 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 within 21 days per infant (Table 5).

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

Patient (stratum)	Total number of transfusions ≤21 days	Number of autologous transfusions	Number of allogeneic transfusions	Reduction in number of allogeneic transfusions (%)
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
Pretransfusion Hct (%)*	37.5 ± 5.2	36.0 ± 6.0	39.0 ± 4.0	
Posttransfusion Hct (%)*	46.5 ± 7.3	45.8 ± 8.3	47.2 ± 6.5	

*Data are reported as mean ± SD

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

Stratum	Number of transfusion needs ≤21 days	Number of infants	Autologous RBCs (mL/kg)*	Coverage in transfusion needs (%)
24-28 weeks	No transfusion	0		
	1 transfusion	1	14 ± 0	100%
	2 transfusions	2	13 ± 3	50%
	More than 2 transfusions	3	11 ± 3	25-33%†
28-30 weeks	No transfusion	12	16 ± 4	
	1 transfusion	7	27 ± 12	100%
	2 transfusions	5	18 ± 8	50-100%‡
	More than 2 transfusions	3	30 ± 11	25-71%§
30-32 weeks	No transfusion	27	17 ± 8	
	1 transfusion	3	19 ± 9	100%
	2 transfusions	1	11 ± 0	50%
	More than 2 transfusions	0		

* Data are reported as mean ± SD

† Two infants required four transfusions and one required three transfusions; coverage was, respectively, 25 and 33 percent

‡ Four infants with 50 percent coverage and one with 100 percent

§ Infants required, respectively, three, four, and seven transfusions with coverages of, respectively, 33, 25 and 71percent

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 6-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		Nonrandomized (N = 27)
	Autologous (N = 35)	Allogeneic (N = 32)	Autologous (N = 9)	Allogeneic (N = 58)	
Composite morbidity and mortality	27 (77%)	24 (75%)	5 (56%) [†]	46 (79%) [†]	19 (70%)
Mortality	3 (9%)	4 (13%)	0 (0%)	7 (12%)	3 (11%)
IVH	10 (29%)	14 (44%)	2 (22%)	22 (38%)	9 (33%)
≥ grade 3	4 (11%)	4 (13%)	0 (0%)	8 (14%)	1 (4%)
ROP	8 (23%)	5 (16%)	1 (11%)	12 (21%)	2 (7%)
≥ grade 3	1(3%)	1 (3%)	0 (0%)	2 (3%)	1 (4%)
BPD	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 (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)

* Data are reported as number (%) or median (interquartile range)

[†] Most unequally distributed variable in ATT analysis, $p=0.20$

[‡] Most unequally distributed variable in ITT analysis, $p=0.19$

ITT=intention to treat analysis; ATT=according to treatment; BPD=bronchopulmonary dysplasia;

IVH=intraventricular hemorrhage; ROP=retinopathy of prematurity; NICU=neonatal intensive care unit

Collection and production efficacy

Overall successful UCB collection rate (≥ 15 mL blood excluding anticoagulance) was low (57%). Most of the collections were performed from an extrauterine 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 extrauterine placenta (30.6 ± 22.1 mL). Hence, the proportion processed UCB units after an intrauterine collection was larger. We also analyzed production efficacy of the closed centrifuge circuit 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 to 28 week gestation group; 16 productions resulted in only 6 RBC units with the required volume and Hct. However, each of these infants required transfusions. Processing of the UCB units 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 RBC units 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 closed centrifuge circuit processing for 101 cases and the costs for QC 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 RBC unit cost estimation

Variable	N	Cost 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
Closed centrifuge circuit kit for processing	101	€ 117.09	€ 11.826
QC (ABO-RhD, BacT/ALERT)	67	€ 7.31	€ 490
Available autologous products	64	€ 654	
Cost per indicated product	25	€ 1.675	
Cost per indicated product (excluding STAT)	25	€ 729	
Standard pedi-pack, irradiated		€ 114	

Interim analysis

Interim analysis revealed low availability of autologous products due to a low number of successful UCB collections. This availability of less than 40 % autologous products for all pre-mature 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 of less than 32 weeks and randomly assigned 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 at least 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 posttransfusion Hct 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 cannot be fully explained. Analysis of the nonrandomized infants, however, showed comparable demographics and outcome, suggesting no selection bias.

Our collection methods resulted in 101 UCB units 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 nonrandomized 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 at least 15 mL of whole blood. After being processed, however, a smaller number of products remained available due to a higher mean birth weight of the infants in this group, which required a larger absolute volume of RBC product. In addition, transfusion needs in this group were much lower (19%, 19 of 98) compared to the other gestational groups (Tables 1 and 2). Taking this all together, UCB collection and autologous RBC production were most efficient for the infants born between 28 and 30 weeks compared to the other gestational groups. In this group, autologous RBCs were 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 closed centrifuge circuit processing. Most of the successful collections were harvested from an intrauterine placenta. Restriction of UCB collection to only intrauterine harvesting would increase production efficacy by 25%.²⁸ In addition, this resulted for infants born between 24 and 28 weeks, in a 10% lower RBC recovery compared to the other processed UCB units, 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 RBC volume loss. Furthermore, performing 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 closed centrifuge circuit 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 RBC recovery could result in higher volumes and available products expecting to cover more than 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 intraventricular in premature infants.^{32,35} The described time range of delayed clamping, however, was broad and varied from 30 until 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 RBCs 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 RBC units 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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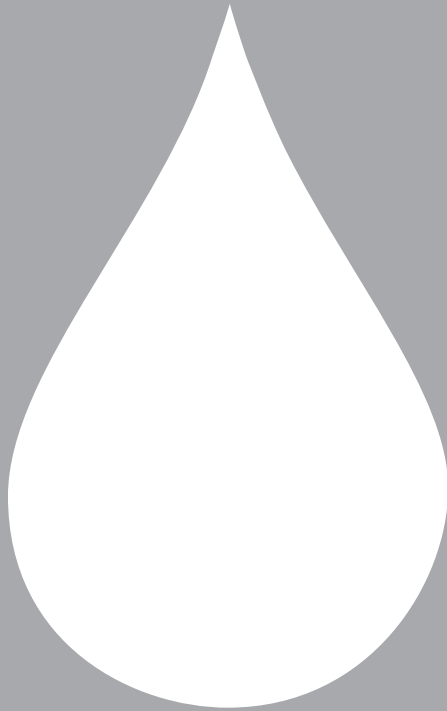
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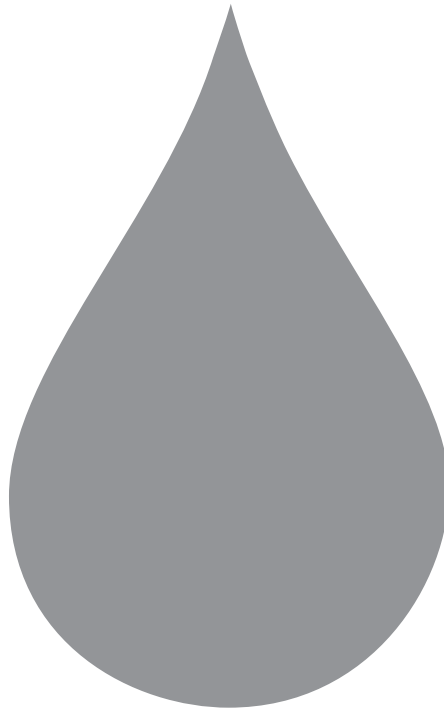
PART II

Red blood cells



Chapter 5

A comparative cohort study on transfusion practice and outcome in two Dutch tertiary neonatal centres



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Summary

The objective of this study was to investigate how a red blood cell transfusion volume of 15 or 20 mL/kg body weight affects the total number of administered transfusions and neonatal complications in premature infants born before 32 gestational weeks.

In this observational study, we analysed clinical data from two cohorts of 218 and 241 premature infants admitted to two neonatal centres which used the same transfusion guideline and product, but different transfusion volumes. Outcome parameters were the number of administered transfusions and the composite outcome of bronchopulmonary dysplasia, retinopathy of prematurity, intraventricular haemorrhage and mortality. The proportion of transfused infants was significantly lower (59 vs 77%) in the centre using a lower transfusion volume of 15 mL/kg. In infants born between a gestational age of 24 0/7 weeks and 27 6/7 weeks, a similar proportion received transfusions in both centres, with an equal number of transfusions per infant. In infants born between a gestational age of 28 0/7 weeks and 31 6/7 weeks, the proportion of transfused infants (49 vs 74%) was significantly higher in the centre using a larger transfusion volume. In these infants transfusion with 20mL/kg resulted, however, in a mean reduction of one transfusion episode per infant. The higher proportion of transfused infants was associated with a higher pre-transfusion haematocrit in less ill infants, suggesting the use of different triggers based on clinical grounds. Composite clinical complications were similar in both cohorts. Clinical neonatal outcome was similar disregard of a higher proportion of transfused patients and a higher total amount of RBC transfused in one of the centres. A larger transfusion volume of 20mL/kg prolonged the interval until next transfusion and can reduce donor exposure in infants born between a gestational age of 28 0/7 and 31 6/7 weeks.

The number of red blood cell (RBC) transfusions in premature infants has successfully declined the past decade due to the use of specific transfusion guidelines.¹⁻³ These comprise recommendations for transfusion triggers based on the post-natal age of the infant and the need for respiratory support, as well as a recommended transfusion volume range from 10 to 20 mL/kg body weight, an equivalent of 2–4 units of RBC in adults. These guidelines are, however, not evidence based. In addition, strategies to limit donor exposure by dedicating a blood product to one or two premature infants have been evaluated.^{4,5} Single-donor programs have been shown to be feasible⁶ and cost-effective,⁷ but are not standard practice in every neonatal centre. In spite of these guidelines, premature infants are still frequently transfused. Recently, the use of restrictive transfusion triggers has been addressed in several randomized controlled trials. Two randomized studies, in which a restrictive and a more liberal transfusion strategy were compared, showed that the use of restrictive transfusion triggers resulted in a lower number of transfusions per infant or fewer infants receiving transfusions.^{8,9} The results of the first study by Bell et al., suggested that infants under a more restrictive transfusion policy may experience a higher rate of neurologic sequelae.⁸ These adverse effects were not observed in the larger so called Premature Infants in Need for Transfusion (PINT) study by Kirpalani.⁹ Lowering the transfusion triggers may be feasible; however, this should still be conducted in well-designed clinical trials. Studies on the impact of a higher transfusion volume per kilogram body weight on the total number of transfusions per infant are scarce. Paul and colleagues compared transfusion with 10 and 20 mL/kg in 13 very low birth weight infants and found higher post-transfusion haematocrits when transfused with 20 mL/kg. Whether the higher volume resulted in a lower number of transfusions per infant could not be confirmed in this study.¹⁰ Wong et al compared 15 mL/kg with 20 mL/kg and reported significant higher post-transfusion haematocrits, but a higher transfusion volume did not result in a lower number of transfusions per infant.¹¹ It is difficult to compare transfusion volume reliably as the RBC volume is dependent on the product used. For instance, although the transfusion volume per kilogram in the studies by Bell and Kirpalani was similar, the transfused products were different. Bell et al transfused products that had a haematocrit between 80 and 85%.⁸ The products transfused in the PINT study were washed before transfusion and the actual haematocrit of these products was not mentioned.⁹ How the differences in RBC volume in transfusion products affect total transfusion needs and outcome is unknown.

In this study we compared clinical data from 2 of the 10 Dutch tertiary neonatal centres, using the same transfusion thresholds¹² and the same transfusion product, but a different transfusion volume per kilogram bodyweight. Our aim was to investigate whether a different transfusion volume affected the total number of received transfusions until discharge to home and clinical relevant morbidity in premature infants born before 32 weeks of gestation.

Material and methods

This study was approved by the medical ethical committees of the Leiden University Medical Center and the University Medical Center Utrecht. Informed consent was obtained from the parents of all included infants.

Subjects studied

In Unit A clinical data from premature infants born before 32 weeks of gestation were registered between December 2004 and October 2006. In Unit B, data collection was performed from March 2005 until October 2006. Infants suffering from allo-immune haemolytic disease, congenital infections or those in need of major surgery (i.e. gastro-intestinal or cardio-thoracic surgery) were excluded from analysis. All other infants were included. Data on gestational age, birth weight, gender, Apgar score, mortality, endotracheal ventilation, transfusion parameters (number of transfusions, increase in haematocrit, time interval until next transfusion and volume transfused) and the CRIB (Clinical Risk Index for Babies) II score¹³ from birth until discharge were collected on clinical request forms. The CRIB II score is a risk adjustment tool that comprises 5 items—gestational age, gender, birth weight, temperature and base excess at admission—and a score ranging from 0 to 27, with higher scores indicating a higher risk of mortality and morbidity. Data on neonatal complications, i.e. retinopathy of prematurity (ROP),¹⁴ bronchopulmonary dysplasia (BPD),¹⁵ and intra-ventricular haemorrhage (IVH),¹⁶ and length of stay until discharge home, were obtained from our hospitals or the referral hospitals.

Transfusion guideline

All infants were transfused according to the Dutch consensus for blood transfusion.¹² The recommended transfusion triggers vary with post-natal age and need for respiratory support.

1. In the first 24 hours after birth: Hb <8 mmol/L (13 g/dL) (haematocrit range 0.38–0.40 L/L) capillary (or <7 mmol/L arterial (11 g/dL)) (haematocrit range 0.32–0.35 L/L).
2. Stable infants with cardio-respiratory problems and/or mechanical ventilation: Hb 7 mmol/L (11 g/dL) (haematocrit range 0.32–0.35 L/L) capillary.
3. Infants with a postnatal age <4 weeks: Hb 6 mmol/L (10 g/dL) capillary (haematocrit range 0.27–0.3 L/L); infants with a postnatal age >4 weeks: Hb 4.5 mmol/L (7 g/dL) (haematocrit 0.2–0.23 L/L) capillary. In case of symptomatic anaemia, it is recommended that transfusion should take place at higher triggers.

Transfusion volume per kilogram body weight was different between the hospitals; 15 mL/kg in Unit A and 20 mL/kg in Unit B. These transfusion volumes were not specially chosen for this study, but were part of the standard practice in the neonatal intensive care units. The same transfusion product was used in both hospitals. All products consisted of pre-storage filtered RBC stored in additive solution SAG-M, with a haematocrit between 55 and 66%. The products were irradiated with 25 Gy less than 24 h before transfusion.

Outcome parameters

Primary outcome included mortality and a composite of clinical relevant morbidities (ROP, BPD and IVH). The total number of transfusions, transfused volume, transfusion triggers, post-transfusion haematocrit measured 24 h after transfusion and length of stay were specified as secondary outcome parameters.

Statistical analysis

All variables were analysed by univariate analysis for continuous variables and chi-square or Fishers exact probability test for nominal variables (SPSS 12). Backward stepwise logistic regression analysis was used for the independent effect of the Apgar score, CRIB II score, mechanical ventilation and hospital of admittance, on the transfusion needs. A p-value of less than 0.05 was considered significant.

Results

Study population

In the, respectively, 22- and 19-month study period, a total of 221 infants in Unit A and 248 infants in Unit B were born before 32 weeks of gestation. After admittance three and seven infants respectively were in need of major surgery, and were excluded from analysis. A total of 459 infants were included in this study, 218 infants in Unit A and 241 infants in Unit B. Both groups were comparable with regard to birth weight, gestational age, gender, median Apgar scores, median CRIB II scores and need for endotracheal ventilation (Table 1). The CRIB II score was incomplete in 27 infants from the Unit B cohort and these were indicated as missing in further analysis. All data on other variables were complete.

Administered transfusions and transfusion parameters

In Unit A 128 out of 218 (59%) infants born before 32 weeks of gestation received RBC transfusions, compared to 186 out of 241 (77%) infants in Unit B ($p < 0.001$). In the infants born between a gestational age of 24 0/7 weeks 27 6/7 weeks respectively 95% and 91% of the

Table 1. Clinical characteristics of the study population

	Unit A (n=218)	Unit B (n=241)
Birth weight, g (mean $\pm$ SD)	1256 $\pm$ 358	1261 $\pm$ 343
Gestational age weeks (mean, range)	29 3/7 (25 0/7–31 6/7)	29 3/7 (25 0/7–31 6/7)
24 0/7 weeks–27 6/7 weeks, % (n)	20% (44)	18% (43)
28 0/7–31 6/7 weeks, % (n)	80% (174)	82% (198)
Born from singleton pregnancy, % (n)	62% (134)	66% (159)
Born from multiple pregnancy, % (n)	38% (84)	34% (82)
Male gender, % (n)	57% (125)	56% (135)
CRIB II Score (median, range)	7 (1–16)	6 (1–14)*
AS 5' <7, % (n)	12% (27)	11% (26)
Cord clamping time, sec (median, range)	5 (0–30)	10 (0–120)
Endotracheal ventilation % (n)	48% (105)	42% (100)
Ventilation days, median (range)	4 (1–89)	6 (1–95)
Infants requiring transfusion, % (n)	59% (128) [†]	77% (186) [†]
24–28 weeks, % (n)	95% (42)	91% (39)
28–32 weeks, % (n)	49% (86) [†]	74% (147) [†]

AS: Apgar score

*Median CRIB II score of 214 infants, 27 scores in Unit B were incomplete

[†]p<0.01

infants received transfusions. In the group born between a gestational age of 28 0/7 and 31 6/7 weeks the proportion of infants receiving blood transfusions was respectively 49% (n=86) in Unit A and 74% (n=147) in Unit B (p<0.001) (Table 1). Because of this difference, we also analysed the cohorts according to gestational age. In the following two paragraphs the results from the infants born between a gestational age of 24 0/7 weeks and 27 6/7 weeks and from the infants born between a gestational age of 28 0/7 weeks and 31 6/7 weeks are shown separately.

In the group infants born between a gestational age of 24 0/7 weeks and 27 6/7 weeks the number of infants receiving transfusions was comparable: 42 out of 44 (95%) in Unit A and 39 out of 43 (91%) in Unit B (p=0.43). Also the mean number of transfusions per infant was similar, 5.1 $\pm$ 2.9 in Unit A and 5.2 $\pm$ 2.3 in Unit B (p=0.88, mean difference 0.1, (CI–1.2 to 1.1)). Consequently, this resulted in a significant difference in total transfusion volume of 77 vs. 104 mL/kg body weight. The median first day of transfusion was day 5 (range 1–33) in Unit A and day 3 (range 1–49) in Unit B (p<0.01). The median interval until the next transfusion was 7 days (range 0–44) in Unit A and 9 days (range 0–59) in Unit B (p=0.02). The pre-transfusion haematocrit and haematocrit increment after transfusion ($\pm$ 24 h) were not statistically different (Table 2).

The number of infants born between a gestational age of 28 0/7 weeks and 31 6/7 weeks that received transfusions was significantly higher in Unit B. The mean number of transfusions per transfused infant was respectively 3 ± 2.7 in Unit A and 2 ± 1.5 in Unit B ($p < 0.01$, mean difference 1.0, 95% CI 0.3–2.0). The mean volumes transfused per kilogram body weight were comparable (45 mL/kg in Unit A and 40 mL/kg in Unit B). The median first day of transfusion was comparable in Units A and B, day 8 (range 1–77) and day 8 (range 1–84) respectively but the interval until the next transfusion was 9 days (range 0–97) in Unit A and 14 days (range 0–65) ($p < 0.01$) in Unit B. Transfusion trigger and haematocrit increment after transfusion showed no differences between both groups (Table 2).

Table 2. Transfusion parameters in the transfused infants according to gestational age

	24 0/7–27 6/7 weeks			28 0/7–31 6/7 weeks		
	Unit A (n = 42)	Unit B (n = 39)	p-value	Unit A (n = 86)	Unit B (n = 147)	p-value
Total number Tx, mean $\pm$ SD	$5.1 \pm 2.9^*$	$5.2 \pm 2.3^*$	0.88	$3.0 \pm 2.7^*$	$2.0 \pm 1.5^*$	<0.01
Total volume transfused, mL/kg, mean $\pm$ SD	77 ± 44	104 ± 46	<0.01	45 ± 41	40 ± 30	0.20
First Tx day, median (range)	5 (1–33)	3 (1–49)	<0.01	8 (1–77)	8 (1–84)	0.99
Interval until next Tx, days median (range)	7 (0–44)	9 (0–59)	0.02	9 (0–97)	14 (0–65)	<0.01
Hematocrit trigger, %	34.9 ± 4.7	35.4 ± 2.9	0.18	34.6 ± 4.6	34.6 ± 3.6	0.99
Hematocrit increment [†] , %	10.1 ± 5.0	9.7 ± 4.3	0.38	10.1 ± 5.9	10.7 ± 4.2	0.33

Tx: transfusion

*Transfusion volume per kilogram: unit A, 15mL/kg and unit B, 20mL/kg

[†]Calculated $\pm$ 24hours after transfusion

Transfusion triggers in practice

Within the first 24 h of birth, an Hb of lower than 8 mmol/L (13 g/dL) is recommended as the transfusion trigger. Seventeen (8%) and 32 (13%) infants were transfused in Unit A and B respectively within 24 h of birth. The mean haematocrits before transfusion were respectively 34.3 ± 8.1 and 33.6 ± 5.8 ($p = 0.71$). After 24 h, the recommended trigger depends on whether an infant suffers from cardio-respiratory problems. We compared the proportion of transfused infants in the two units that also had endotracheal ventilation. These proportions were similar in both cohorts: 85 infants out of 218 in Unit A (39%) and 90 of 241 in Unit B (37%). The mean haematocrit before transfusion was respectively 35.1 ± 4.6 and 35.2 ± 3.1 in the two cohorts. The proportion of transfused infants without endotracheal ventilation was 43 out of 218 in Unit A (19%) and 96 out of 241 in Unit B (40%). The mean haematocrit before transfusion was respectively 32.1 ± 4.3 in Unit A and 34.4 ± 3.7 in Unit B ($p = 0.001$, mean difference 2.3, 95% CI –3.7 to –0.78).

Clinical outcome

Composite mortality and neonatal complications in both total cohorts as well were comparable when analysed according to gestational age (Table 3 and 4).

Table 3. Neonatal mortality and morbidity in the study cohorts

	Unit A (n=218)	Unit B (n=241)	p-value
Composite mortality, BPD, ROP and IVH, % (n)	47% (102/218)	41% (100/241)	p= 0.26 OR 1.2 (0.9–1.8)
Mortality, n (%)	5% (11/218)	5% (13/241)	p= 1.00 OR 1.1 (0.5–2.4)
Survived with BPD (total), n (%)	26% (53/207)	22% (51/228)	p= 0.43 OR 0.8 (0.5–1.3)
≥ grade 2, n (%)	10% (20/207)	12% (28/228)	p= 0.44 OR 1.3 (0.7–2.4)
Survived with ROP (total), n (%)	9% (18/207)	5% (11/228)	p= 0.12 OR 0.5 (0.3–1.2)
≥ grade 3, n (%)	4% (7/207)	2% (5/228)	p= 0.56 OR 0.6 (0.2–2.1)
Survived with IVH (total), n (%)	23% (47/207)	21% (47/228)	p= 0.64 OR 1.1 (0.7–1.8)
≥ grade 3, n (%)	3% (7/207)	3% (7/228)	p= 1.00 OR 0.9 (0.3–2.6)
Length of stay, days, median (IQR)	58 (45–76)	56 (47–69)	p=0.41

IQR=interquartile range

Table 4. Neonatal morbidity and mortality according to gestational age in the study cohorts

	24 0/7–27 6/7 weeks			28 0/7–31 6/7 weeks		
	Unit A (n=44)	Unit B (n=43)	p-value	Unit A (n=174)	Unit B (n=198)	p-value
Composite mortality, % (n)	77% (34/44)	81% (35/43)	0.79 OR 0.8 (0.3–2.2)	39% (68/174)	33% (65/198)	0.23 OR 1.3 (0.9–2.0)
Mortality, n (%)	20% (9/44)	16% (7/43)	0.78 OR 1.3 (0.4–3.9)	1% (2/174)	3% (6/198)	0.29 OR 0.4 (0.1–1.9)
Survived with BPD (total), n (%)	57% (20/35)	69% (25/36)	0.33 OR 0.6 (0.2–1.6)	19% (33/172)	14% (26/192)	0.16 OR 1.5 (0.9–2.7)
≥ grade 2, n (%)	31% (11/35)	39% (14/36)	0.62 OR 0.7 (0.3–1.9)	5% (9/172)	7% (14/192)	0.52 OR 0.7 (0.3–1.7)
Survived with ROP (total), n (%)	23% (8/35)	17% (6/36)	0.56 OR 1.5 (0.5–4.8)	6% (10/172)	3% (5/192)	0.19 OR 2.3 (0.8–6.9)
≥ grade 3, n (%)	9% (3/35)	11% (4/36)	1.00 OR 0.8 (0.2–3.6)	2% (4/172)	1% (1/192)	0.19 OR 4.5 (0.5–41)
Survived with IVH (total), n (%)	26% (9/35)	33% (12/36)	0.60 OR 0.7 (0.2–1.9)	22% (38/172)	18% (35/192)	0.36 OR 1.3 (0.8–2.1)
≥ grade 3, n (%)	11% (4/35)	6% (2/36)	0.67 OR 2.1 (0.4–12)	2% (3/172)	3% (5/192)	0.73 OR 0.7 (0.2–2.8)
Length of stay, days, median (IQR)	59 (46–71)	60 (48–72)	0.58	58 (45–77)	54 (47–68)	0.26

Predictors of receiving a transfusion

The variables Apgar score, mechanical ventilation, CRIB II score and hospital of admittance were included in a multivariate analysis. The hospital of admittance (OR 4.7, 95% CI 2.7–8.0), mechanical ventilation (OR 3.6, 95% CI 1.9–6.4) and the CRIB II score (OR 1.5, 95% CI 1.4–1.7) were independent predictors of transfusion needs ($p < 0.001$). Because the median CRIB II scores, the number of infants with mechanical ventilation and duration of ventilation were comparable between both cohorts (Table 1), differences in proportion of transfused infants and transfusion outcome could only be explained by differences in hospital transfusion practice.

Discussion

More than half of the infants born before 32 weeks of gestation received blood transfusions early in life. This is in accordance with published practice since the introduction of specified transfusion guidelines for premature infants.^{1,17} Postulated transfusion triggers and transfusion volumes per kilogram body weight in the current guidelines remain topics of discussion. In the Netherlands, all 10 perinatal centres use transfusion triggers that are recommended in the Dutch transfusion guideline. The transfusion volume per kilogram body weight, however, varies within a range of 10–20 mL/kg. In this observational study, we aimed to investigate whether a different standard transfusion volume per kilogram body weight had impact on the total number of administered transfusions and clinical outcome in two cohorts of premature infants born before 32 gestational weeks.

Although the demographic parameters were similar between both cohorts, we surprisingly observed a significant difference in the number of infants receiving RBC transfusions, 59% in Unit A and 77% in Unit B (Table 1). When we analysed the cohorts according to their gestational age at birth, almost all infants born between gestational age of 24 0/7 weeks and 27 6/7 weeks received RBC transfusions in both units. Transfusion with 20mL/kg did not reduce the total number of transfusions and increased the transfused RBC volume by 35%. As we observed no differences in haematocrit before and after transfusions between Unit A and B, we can only speculate about possible explanations for this difference. These may be due to larger phlebotomy losses in Unit B, or differences in fluid intake or less use of diuretics. The greater volume transfused in infants in Unit B and thereby a greater volume of haemoglobin A, was not associated with deleterious effects on the health status of these infants. In both units, these extreme low gestational age infants suffered from considerable mortality and such extensive morbidity that a possible deleterious effect of a larger transfused volume remains undetected (Table 4).

Analysis of the two cohorts born between a gestational age of 28 0/7 weeks and 31 6/7 weeks showed a significant difference in the proportion of transfused infants (49% vs. 74%) in both units. Although the number of administered transfusions per infant in Unit A was higher; when adjusted for the smaller transfusion volume per kilogram bodyweight; the transfused infants received comparable volumes of donor blood (Table 2). If a centre does not apply a donor reduction policy by reservation of a RBC unit for the same patient, transfusion with a lower volume can lead to a higher donor exposure. The first transfusion day was similar between both cohorts. The interval between transfusions, however, was significantly shorter in Unit A, which used a lower transfusion volume. Comparing the clinical outcome of both

cohorts with a gestational age of 28 0/7 weeks to 31 6/7 weeks, we observed no statistical differences. Although in Unit A fewer infants received transfusions, this was not associated with a poorer clinical outcome, compared to Unit B.

Surprisingly, we did not observe a higher haematocrit increase after transfusion with a larger amount of red cells. In the study performed by Paul et al.,¹⁰ the higher haematocrit increment after a larger transfusion volume was measured 8 h after transfusion instead of after 24 h as in our study. The use of furosemide or other diuretics given as a single-dose post-transfusion is not a standard practice in our centres. Nevertheless, we saw the benefit of the larger volume with respect to a longer interval to next transfusion.

In view of the difference in number of transfused infants that were born between gestational ages of 28 0/7 weeks and 31 6/7 weeks, we looked at the transfusion triggers that had been used in infants in relation to the recommended transfusion triggers, i.e. infants less than 24 hours after birth and infants with and without endotracheal ventilation. Only in infants older than 1 day and without endotracheal ventilation, we observed a higher pre-transfusion haematocrit in Unit B. This higher trigger could explain the larger number of transfused infants. The decision to transfuse could be influenced by a difference in clinical symptoms that were not recorded, e.g. apnea or arrhythmia or to a different perception of clinical symptoms by the physicians. In view of these possible differences, a scenario-based survey between the neonatal intensive care units could help to identify under which circumstances the decision to transfuse differs most.

Logistic regression analysis confirmed the hospital of admittance as an important independent predictor of the administration of transfusions. Since the median CRIB II scores and number of infants with mechanical ventilation in both units were comparable, differences in the proportion of transfused infants and outcome could only be explained by different transfusion and/or other policies (e.g. phlebotomy, fluid management, clinical opinion) in the hospitals.

Observational studies like this have many limitations. All data were collected and analysed in retrospect. Because we expected that the indication for transfusion would be similar, we anticipated on investigating the effect of a different transfusion volume using the same transfusion product. This clinical research question was initiated by observations in critically ill adult patients after cardiac surgery revealing that post-operative complications were associated with transfusions of 4 units or more, representing more than 15 mL/kg body weight.¹⁸

Overall, we observed no differences in clinical composite complications and cannot conclude that a smaller or larger transfusion dose is to be preferred. Transfusing with 20 mL/kg may reduce donor exposure in infants born between gestational ages of 28 0/7 weeks and 31 6/7 weeks if a single-donor program is not used. Despite the use of the same national guideline, the clinical motivation for the decision to transfuse appeared to differ between both hospitals

in our study, especially in case of more stable infants. Audits among neonatal intensive care units, could be used to better understand why such differences exist when the same guidelines are supposedly being used.

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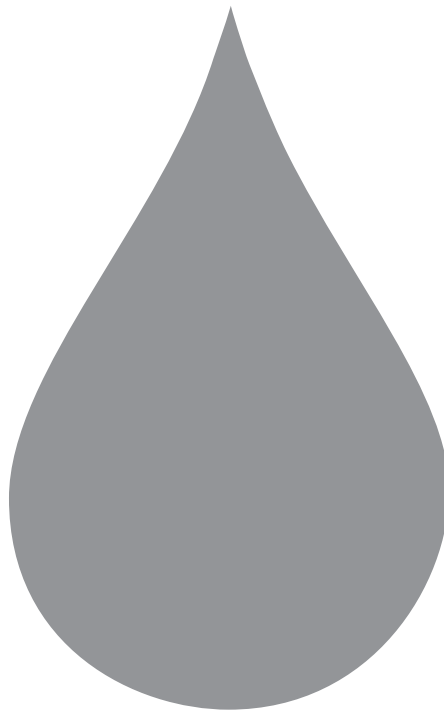
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Chapter 6

Long-term outcome in relationship to neonatal transfusion volume in extremely premature infants: a comparative cohort study



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Abstract

Background In premature born infants red blood cell (RBC) transfusions have been associated with both beneficial and detrimental sequels. Upon RBC transfusion, improvement in cerebral blood flow and oxygenation have been observed, while a more liberal transfusion policy may be associated with a better developmental outcome. The effect of the transfusion volume on long-term outcome is not known.

Methods Observational follow-up study of a cohort of extremely premature born infants, treated in 2 neonatal intensive care units using a different transfusion volume (15 mL/kg in Unit A and 20 mL/kg in Unit B). The primary outcome was a composite of post discharge mortality, neuromotor developmental delay, blindness or deafness, evaluated at a mean corrected age (CA) of 24 months related to the transfusion volume/kg bodyweight administered during the postnatal hospital stay.

Results Despite the difference in transfusion volume in clinically comparable groups of infants, they received a similar number of transfusions (5.5 ± 3.2 versus 5.5 ± 2.3 respectively in Unit A and B). The total transfused volume in unit A was 79 ± 47 mL/kg and 108 ± 47 mL/kg in unit B ($p=0.02$). Total transfused RBC volume per kg bodyweight was not an independent predictor of the composite outcome ($p=0.96$, OR 1.0, CI 0.9–1.1).

Conclusion There was no relationship between the composite outcome at 24 months CA and transfusion volume received during the post natal hospital stay. As there was no clinical advantage of the higher transfusion volume, a more restrictive volume will reduce total transfusion volume and donor exposure. Future research on the optimal transfusion volume per event to extremely preterm infants should include larger, prospective studies with a longer follow-up period through to childhood or even adolescence.

Background

There is ongoing uncertainty whether transfusion of red blood cells (RBC) in the neonatal period influences the clinical outcome and development of premature infants. In the international literature there is discussion about the optimal transfusion volume and trigger.¹

We previously published a study comparing two transfusion dosages of the same RBC product in two Dutch tertiary care neonatal units (NICUs) using the same transfusion trigger protocols.² No difference in short-term outcome and mortality was observed in extremely and very preterm infants when a dose of 15 mL/kg had been administered compared to a volume of 20 mL/kg bodyweight.

Little is known about the long-term follow-up of extremely premature infants after RBC transfusion. Only a few randomized trials have been published on long-term outcome in premature infants with difference in transfusion practice.^{3,4} However, these were studies (the PINT trial and the Iowa trial) comparing liberal and restrictive transfusion triggers, exposing the infants in the restrictive group to the possible risks of a low hemoglobin level.^{5,6} In sequel to our study on short-term outcome we also wanted to compare long-term outcome in the same group of extremely premature infants treated with different transfusion volumes but with otherwise comparable transfusion triggers and transfusion products.

In our follow-up study we evaluated post discharge mortality, neuromotor developmental outcome and disabilities in a cohort of extremely premature born infants born before 28 gestational weeks in two Dutch tertiary NICUs.

Methods

Ethics

In the Netherlands no ethical approval is required for this type of research as no new intervention or treatment is studied. All collected data were anonymous.

Study population

This is a follow-up study of a cohort of extremely premature infants born before 28 gestational weeks that participated in a previous study on transfusion practice and short-term outcome in two Dutch NICUs. All infants were transfused using the same transfusion triggers and with a similar RBC blood product with only a different volume per event, i.e. 15 and 20 mL/kg bodyweight. The study design was previously described.² The primary outcome measure was a composite of post discharge mortality, neuromotor developmental outcome and disabilities.

Transfusion guideline and product

All infants were transfused according to the Dutch consensus for blood transfusion 2004.⁷

The recommended transfusion triggers vary with postnatal age, degree of illness and need for respiratory support:

- Hb <8 mmol/L (13g/dL) (hematocrit (Hct) range 0.38–0.40 L/L) capillary (or <7 mmol/L arterial (11 g/dL) (Hct 0.32–0.35 L/L)): the first 24 hours after birth in all infants with clinical symptoms of anemia (tachycardia, supplemental oxygen need, apnoea, bradycardia); in all infants on mechanical ventilation or severely ill.
- Hb <7 mmol/L (12g/dL) (Hct 0.32–0.35 L/L) capillary: reasonably stable infants with cardio-respiratory problems (patent ductus arteriosus, apnoea, bronchopulmonary dysplasia, need for supplemental oxygen).
- Hb <6 mmol/L (10g/dL) (Hct 0.27–0.30 L/L) capillary: stable premature infants with a postnatal age <4 weeks.
- Hb <4.5 mmol/L (7 g/dL) (Hct 0.2–0.23 L/L) capillary: stable infants with a postnatal age >4 weeks if there are no signs of anemia (apnoea, tachycardia, poor weight gain, poor feeding). In case of symptomatic anemia, transfusion is recommended at a higher threshold.

Transfusion volume per kg bodyweight was different between the two hospitals; 15 mL/kg in Unit A and 20 mL/kg in Unit B. This transfusion volume was part of the standard practice in the hospitals and was not chosen for study purposes. The same transfusion product was used in both hospitals. All products consisted of pre-storage filtered RBC stored in additive solution Saline Adenine Glucose Mannitol (SAG-M) (maximum storage time 35 days), with a hematocrit of 0.58 ± 0.05 L/L. The products were irradiated with 25 Gy less than 24 hours before transfusion.

Preventative measures for anemia of prematurity were not standardised. Erythropoietin was not used in the study units. Iron supplementation was started 6 to 8 weeks after birth if there had been no previous RBC transfusion. After RBC transfusion iron supplementation was postponed for four weeks because of the assumed iron load given with the transfusion.

Data collection and outcome parameters

Infants with a syndrome or congenital/hereditary anomaly known to cause a neuromotor developmental delay were excluded from analysis. Data on neuromotor developmental outcome, major disabilities (deafness, blindness) and survival at a mean ($\pm$ SD) corrected age (CA) of 24 ± 3.4 months were obtained from each child's outpatient follow-up physician, child-psychologist and/or pediatric physiotherapist, who were all trained in neonatal follow-up. Developmental examination was done in different hospitals. The children were assessed with

various instruments depending on the hospital of follow-up. Instruments used were the Dutch 2nd version of the Bayley Scales of Infant Development-II (BSID-II-NL),⁸ the Griffiths Mental Development Scales,⁹ Alberta Infant Motor Scale (AIMS),¹⁰ and the Hempel,¹¹ Touwen,¹² and van Wiechen¹³ assessments of neuromotor development. The children were classified as normal, mildly delayed or severely delayed, using the cut-off values of each test, classifying severe neuromotor developmental delay as a score of more than 2 SD below average and mild neuromotor developmental delay as a score 1 to 2 SD below the mean. Non-cooperative children received a general assessment by the physician, pediatric physiotherapist and/or child-psychologist. A parental questionnaire was sent to the parents of the children if follow-up at age 2 years was unknown by any of the previously mentioned professionals. Our primary outcome was the composite of post discharge mortality, severe hearing or visual impairment, or neuromotor developmental delay at 24 months CA. Visual impairment was defined as <20/200 of best eye, hearing impairment was defined as the need for a hearing aid or cochlear implant. Neuromotor developmental delay was defined as a score more than 1 SD below the mean. The definition for bronchopulmonary dysplasia (National Institutes of health Consensus, USA) was used as described by Ehrenkranz et al.¹⁴ For retinopathy of prematurity the revised international classification was used.¹⁵ Intraventricular hemorrhage was graded according to Volpe.¹⁶

Statistical Analysis

All variables were analyzed by univariate analysis for continuous variables and chi-square or Fishers exact probability test for nominal variables. Backward step wise logistic regression analysis was used for the independent effect of the following factors: hospital (representing transfusion dose and unknown factors), number of RBC transfusions, total transfused RBC volume per kg bodyweight, gender, gestational age, birth weight, CRIB II score and Apgar-score at 5 minutes. (SPSS 17 Chicago, United States of America). A p-value of less than 0.05 was considered significant.

Results

Eighty-seven extremely premature infants were included in our earlier cohort study (44 in Unit A, 43 in Unit B). Four infants died the first day of life and were excluded from this follow-up study (1 lung hypoplasia, 1 massive pulmonary hemorrhage, 1 severe perinatal asphyxia and 1 cause unknown). None of these children had received an RBC transfusion. Twelve other patients died in the neonatal period for various reasons; they had all received RBC transfusions. They were also excluded from follow-up. Seventy-one of the 87 infants (82%) left the

hospital alive (in total 9 in unit A and 7 in unit B died ($p=0.78$). There were no deaths after discharge from the units. One other patient was excluded from follow-up analysis because of a severe myopathy. Three children were lost to follow-up in the first year of life; one due to emigration, of the other two (twins) the reason is unknown, leaving 67/70 (96%) of the eligible infants surviving the post-natal period for neuromotor developmental follow-up. There were no statistically significant differences when comparing the baseline characteristics of the patients from both units (Table 1). The number of transfusions administered was similar, 5.5 ± 3.2 (Unit A) versus 5.5 ± 2.3 (Unit B) respectively. As the volume per transfusion event per center differed, the mean total volume transfused in unit A and B was significantly different (79 ± 47 mL/kg versus 108 ± 47 mL/kg ($p=0.02$) respectively). Neuromotor development was evaluated at a mean 24 ± 3.4 months CA. One infant had a severe visual impairment and one child had severe hearing loss. Forty-seven out of 67 (70.1%) infants showed normal neuromotor development for corrected age. Seventeen infants (25.4%) had a mild developmental delay and three infants were severely impaired (4.5%).

Table 1. Characteristics of patients per unit included in follow-up study

	Total (n=67)	Unit A (n=31)	Unit B (n=36)	p-value
Male, n	61% (41)	74% (23)	50% (18)	0.05
Gestational age, weeks mean SD	26 6/7 $\pm$ 4/7	26 6/7 $\pm$ 4/7	26 6/7 $\pm$ 4/7	0.65
Birth weight, grams mean SD	900 $\pm$ 187	906 $\pm$ 178	895 $\pm$ 197	0.59
AS 5 minutes, median (IQR)	8 (7–9)	8 (7–9)	8 (7–9)	0.88
IVH $\geq$ grade 3	9% (6)	13% (4)	6% (2)	0.40
BPD $\geq$ moderate	35% (23)	29% (9)	40% (14)	0.35
ROP $\geq$ grade 2	12% (8)	13% (4)	11% (4)	1.0
Total number of transfusions, mean SD	5.5 $\pm$ 2.7	5.5 $\pm$ 3.2	5.5 $\pm$ 2.3	0.98
ML/per kg RBC administered, mean SD	95 $\pm$ 49	79 $\pm$ 47	108 $\pm$ 47	0.02
Composite outcome	31% (21)	29% (9)	33% (12)	0.71

AS=Apgar score; IQR=interquartile range; IVH=intraventricular hemorrhage; BPD=bronchopulmonary dysplasia; ROP=retinopathy of prematurity; RBC=red blood cells; composite outcome=post discharge mortality, impaired neuromotor development, deafness or blindness

There was no statistically significant difference in the transfused volume for the primary outcome (composite of post discharge mortality, neuromotor developmental delay, blindness or deafness) compared to children with a normal outcome (105 ± 52 mL versus 90 ± 47 mL respectively) ($p=0.96$, OR 1.0 (0.9–1.1)). In multivariate analysis none of the tested variables reached statistical significance for an independent association with the composite outcome (Table 2).

Table 2. Predictors of the composite outcome of neuromotor developmental delay, post-discharge mortality, blindness or deafness

	p-value	OR	CI of OR (95%)
Hospital	0.8	1.6	0.1–24.7
Number of RBC transfusions	1.0	1.0	0.2–4.8
ML/kg RBC transfused	1.0	1.0	0.9–1.1
Gender	0.2	0.4	0.0–1.6
Gestational age	0.8	1.0	0.9–1.1
Birth weight	0.4	1.0	1.0–1.0
CRIB II score	0.6	1.0	1.0–1.0
Apgar score 5 min	0.3	1.2	0.8–1.9

RBC=red blood cells

Discussion

Transfusion of RBC has been associated with negative and positive effects on clinical outcome. Our study showed no significant differences between a smaller and a larger transfusion volume regarding a composite outcome of post discharge mortality, neuromotor developmental outcome and disabilities at 2 years CA. This would imply that a smaller transfusion volume rather has advantages (generally limiting donor exposure and costs), than negative effects on neuromotor developmental outcome. It is possible that no effects were seen because the difference in transfusion volume per transfusion event was not large enough. On the other hand, the total transfusion volume/kg during the post natal hospital stay was significantly different in the two NICUs. Several studies associated RBC transfusion with the development of retinopathy of prematurity^{17–20} and chronic lung disease.^{21–23} It is hypothesized that iron overload, caused by multiple RBC transfusions, increases oxidative stress leading to free radical-induced injury to the premature retina and developing lungs.^{24,25} However, anemia has been associated with negative effects as well and in particular the brain may be susceptible to low hemoglobin levels. Bell et al, in a randomized study, found a statistically significant higher incidence in the number of infants with intracranial hemorrhage grade IV or periventricular leukomalacia when a restrictive transfusion threshold was applied as compared to a more liberal threshold.⁵ On the other hand, neither Chen²⁴ nor Kirpalani (PINT trial)⁶ found a statistically significant difference in the occurrence of (severe) intracranial pathology. However, the follow-up study by Whyte et al. of infants included in the PINT trial, suggested that preterm infants treated with a more liberal transfusion regime may have a better developmental outcome.⁴ The premature infants included in this multicenter PINT trial were analyzed at 18–21 months CA with regard to mortality, cerebral palsy, severe visual impairment, hearing-loss, a Bayley-Mental Developmental Index (MDI) score <70, and a composite of these variables. Although

there was no statistically significant difference in composite outcome (45% in the restrictive and 38% in the liberal group), the difference in cognitive delay (MDI score <70) approached statistical significance in favor of the liberal group. A post-hoc analysis with cognitive delay redefined (MDI score more than 1SD below the age-standardized mean) showed a significant difference favoring the liberal threshold group. This suggests that premature infants may benefit from a higher hemoglobin threshold for transfusion.⁴ In the study by Nopoulos et al. on the brain structure of 12 year old children previously enrolled in the Iowa trial by Bell and colleagues, only 44 of the 100 children participated.³ The children who were exposed to the liberal transfusion threshold appeared to have a substantially smaller intracranial volume compared to the children transfused according to the restrictive guideline. Nopoulos mentions unpublished data on long-term cognitive follow-up showing (non-significant but) overall poorer outcome in the group of liberal transfused children.

Another recent study has shown that RBC transfusions increase cerebral oxygenation, thereby decreasing the risk of tissue hypoxia.²⁶ Mercer et al. showed that premature male infants after delayed cord clamping, had a better developmental outcome at seven months CA compared to infants after immediate cord clamping.²⁷ It is conceivable that upon transfusion of a larger volume, an increase in cerebral flow and better oxygenation can be obtained with a similar effect as described by Mercer.

In transfusion guidelines, the range of recommended RBC volume per kilogram bodyweight is rather wide, 10–20 mL/kg. Given the variation in hematocrit between used RBC products, the guidelines result in a huge variation in actually transfused RBC.

We observed no influence of transfused volume RBC per kg bodyweight on a composite of impaired neuromotor development, post discharge mortality and deafness or blindness in extremely preterm born infants evaluated at 24 months CA. This finding remained unchanged after logistic regression analysis correcting for other factors relevant for outcome.

The two Dutch NICUs participating in this study used the same transfusion guideline, transfusion trigger and transfusion product, which virtually excludes important differences in degree of anemia between infants. Our study has its limits being a retrospective, non-randomized trial. The neuromotor development was assessed using various different tests. In Unit B all children were assessed by the same special educator who is also a pediatric physiotherapist (ICvH) using the same test (BSID-II-NL) in 95% of the children. The children from Unit A were assessed using various validated tests, performed in different hospitals by different professionals. It may be an incentive to perform a larger, prospective randomized controlled trial, focusing not only on the transfusion trigger but also on the total transfused RBC volume to recommend an optimal transfusion trigger and dose.

Conclusion

A high total transfused volume of RBC per kg was not correlated with the composite outcome of impaired neuromotor developmental outcome, post discharge mortality, blindness or deafness when analyzed at 24 ± 3.4 months CA, hereby questioning the previously presumed possible detrimental effects of RBC transfusions.

Recommendations

Due to differences in neonatal transfusion practice, conclusions on the effect of maintaining a higher Hb value by the use of liberal transfusion triggers or a larger volume of RBC per transfusion are not based on strong evidence. A larger transfusion volume may not be associated with deleterious effects, whereas a smaller transfusion volume may limit donor exposure. Through randomized clinical studies, the effect of more liberal or restrictive triggers on long-term outcome can be studied, as well as the combination with a low (10 mL/kg) or high (20 mL/kg) transfusion volume. A longitudinal study to school age or even adolescence looking for instance at academic competencies might give further insight in the effects of different transfusion volumes.

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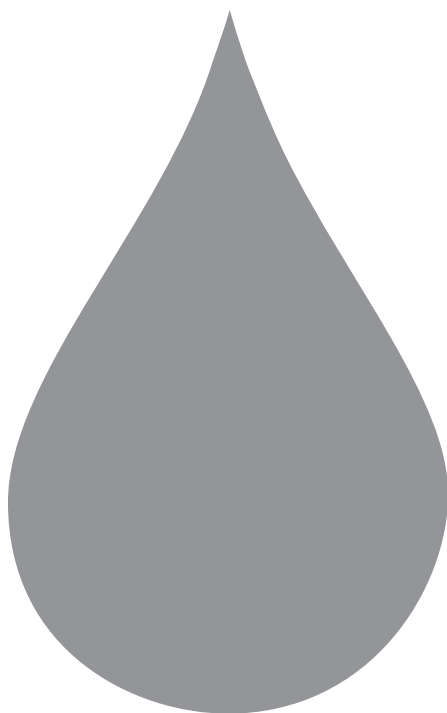
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Chapter 7

Erytrocyten transfusie bij neonaten: huidige praktijk en gereviseerde landelijk richtlijn



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namens de werkgroep CBO-richtlijn bloedtransfusie 2011

Submitted

Samenvatting

Doel 1. Inventariseren van de huidige protocollen met betrekking tot erytrocyten transfusies bij pasgeborenen in de 10 Nederlandse neonatale intensive care units (NICUs) en deze vergelijken met de huidige CBO (Centraal Begeleidings Orgaan/Kwaliteitsinstituut voor de gezondheidszorg) richtlijn bloedtransfusie 2004.

2. Samenvatten van de nieuwe gereviseerde CBO richtlijn 2011 en vergelijken met de internationale literatuur.

Opzet Inventariserend.

Methoden De lokale erytrocyten transfusie protocollen van de 10 NICUs werden opgevraagd en vergeleken met de huidige CBO 2004 richtlijn. Internationale richtlijnen en literatuur werden bestudeerd en vergeleken met de gereviseerde CBO richtlijn 2011.

Resultaten Slechts twee NICUs houden zich aan de CBO richtlijn 2004, al kan niet altijd aan de bestralingsindicatie worden voldaan. Alle 10 protocollen houden rekening met de zwangerschapsduur en geboortegewicht naast de mate van ziek zijn en (cardio-) respiratoire ondersteuning voor het vaststellen van de transfusiedrempel. De transfusievolumina variëren tussen de 10 en 20 ml/kg. Ook de internationale richtlijnen lopen uiteen zowel qua transfusiedrempel als volumina.

Conclusie Er is geen consensus over erytrocyten transfusies bij pasgeborenen. Een heldere gereviseerde richtlijn gebaseerd op (inter-)nationale studies zou uniformiteit kunnen geven. Tot die tijd kan een landelijke consensus zorgen voor uniformiteit in Nederland met de mogelijkheid voor deelname aan (inter-)nationale studies.

Summary

This article describes the red blood cell (RBC) transfusion practices in the ten Dutch neonatal tertiary care intensive care units (NICUs) and compares them to the current Dutch consensus guideline 2004 (Centraal Begeleidings Orgaan/Kwaliteitsinstituut voor de gezondheidszorg (CBO)). In 2011 a revised CBO guideline will be published. This guideline has been summarized and compared to the international literature.

Only two NICUs comply with the national guideline 2004, although they cannot always adhere to the irradiation advice. All 10 NICUs take gestational age, birth weight and disease severity, including cardio-respiratory support, into account to establish the transfusion threshold. RBC volume per transfusion ranges from 10 to 20 mL/kg. International guidelines vary in transfusion threshold as well as transfusion volume, irradiation indication and Parvovirus B19 and cytomegalovirus safety of products.

In conclusion, there is no consensus in RBC transfusion practices in neonates. A clear, revised guideline based on (inter-)national evidence based studies could lead to uniformity. Until then a national consensus can lead to uniformity within the Netherlands and thus to the possibility of participating in international studies.

Introductie

In Nederland worden jaarlijks 180.000 kinderen geboren waarvan 5000 prematuur (<37 weken zwangerschapsduur) en ruim 2000 zeer prematuur (<32 weken) en/of met een extreem laag geboorte gewicht (<1000 gram). De meeste a terme geboren kinderen hebben geen rode bloed cel (RBC) transfusie nodig. Fors (perinataal) bloedverlies, ernstige hemolyse door bijvoorbeeld bloed groep of Rhesus antagonisme, ernstige infecties of intra-uterien ontstane anemie kan reden zijn om deze groep kinderen wel een bloedtransfusie te geven in de neonatale periode. De zeer prematuur geboren kinderen ontvangen daarentegen frequent bloedtransfusies voor anemie van de prematuur (AOP). Van de kinderen met een geboorte gewicht onder de 1000 gram krijgt meer dan 90% minimaal één transfusie.^{1,2}

De literatuur geeft echter weinig houvast voor consensus met betrekking tot transfusieprotocollen voor de pasgeborene. Wel houdt nagenoeg ieder protocol rekening met de klinische conditie van het kind, zoals zuurstofbehoefte of ademhalings-ondersteuning, groei en de leeftijd.

Recent is vanuit het Centraal Begeleidings Orgaan/Kwaliteitsinstituut voor de Gezondheidszorg (CBO) de landelijke richtlijn bloedtransfusies uit 2004 door een werkgroep herzien. Met dit artikel willen we de huidige transfusie praktijk in de tien Nederlandse neonatale intensive care units (NICUs) weergeven en vergelijken met de CBO richtlijn bloedtransfusie 2004.³

Daarnaast werd de internationale literatuur geïnventariseerd. Dit artikel beoogt een breed draagvlak te creëren voor de gereviseerde richtlijn.

Data en methoden

In 2008 zijn de 10 NICUs gevraagd hun lokale erytrocyten transfusie protocol op te sturen. Deze werden met elkaar en de CBO richtlijn bloedtransfusie 2004 vergeleken. Daarnaast maakten we door te zoeken op Pubmed en internet een inventarisatie van internationale richtlijnen en studies met betrekking tot erytrocyten transfusies bij neonaten.

Resultaten

Van alle centra werd het protocol ontvangen. Nagenoeg elk centrum heeft een eigen protocol, deels aansluitend bij de landelijke richtlijn, deels op basis van de literatuur en op de lokale gewoonten.

Transfusiegrenzen

Hemoglobine (Hb) of hematocriet waarde (Ht) worden beide gehanteerd als RBC transfusie grens. De transfusie grens wordt in alle centra bepaald aan de hand van de amenorroeduur, het geboortegewicht, de postnatale leeftijd en/of klinische parameters. Deze grens wisselt tussen de verschillende centra met als hoogste waarde een Hb van 9.0 mmol/l (Ht 0.47 l/l) voor een zieke neonat aan de beademing met zuurstof behoefte. De laagst gehanteerde grens voor een niet ziek kind, ouder dan 4 weken zonder enige symptomen is 4.5 mmol/l (Ht 0.23 l/l) (tabel 1). De uiterste grenzen die de CBO richtlijn aan geeft, zowel in 2004 als 2011, zijn 8.0 mmol/l voor een kind met respiratoire insufficiëntie waarvoor beademing en 4.5 mmol/l voor een stabiel kind zonder symptomen, ouder dan 4 weken. De CBO transfusie grenzen worden door slechts 2 NICUs volledig aangehouden.

In de gereviseerde CBO richtlijn is de drempel van 8 mmol/l die gold voor de eerste postnatale 24 uur losgelaten. Een laag Hb op dag 1 blijkt vrijwel altijd het gevolg van acuut bloedverlies, al of niet tengevolge van foetomaternal bloeding, of door reeds intra-uterien bestaande anemie (zoals bij Rhesus antagonisme of Parvovirus B19 infectie). De indicatie tot RBC transfusie is klinisch bepaald en niet zozeer aan de hand van het gemeten Hb op dag 1. Tabel 2 laat verschillende internationale richtlijnen zien. Er zijn enkele gerandomiseerde onderzoeken verricht naar hogere versus lagere RBC transfusie grenzen. Hierbij varieert de hogere drempel van 7.5 tot 8.4 mmol/l (Ht 0.30–0.46 l/l) afhankelijk van de klinische situatie waarbij vooral respiratoire insufficiëntie en zuurstof behoefte genoemd worden.⁴⁻⁶ De ondergrens varieert tussen een Hb van 4.3 mmol/l of een Ht van 0.20 l/l.⁶⁻¹⁰ Twee gerandomiseerde studies, de studie van Brooks e.a.⁵ en de PINT studie (Premature Infants in Need of Transfusion) door Kirpalani e.a.⁶, lieten zien dat een lagere transfusie drempel op de korte termijn als veilig kan worden beschouwd. Een observationele studie vond zelfs dat een restrictievere RBC transfusiedrempel gepaard ging met een afname van het aantal intracraniele bloedingen.¹¹ Echter, een gerandomiseerd onderzoek door Bell en collega's (Iowa trial) laat een verhoogd aantal ernstige intracraniele bloedingen zien indien een meer restrictieve RBC transfusiegrens wordt gehanteerd.⁴ De lange termijn uitkomst van de verschillende RBC transfusiegrenzen is tot nu toe alleen van de bovengenoemde PINT studie en van de Iowa trial bestudeerd. Hoewel er in de PINT trial geen significant verschil werd gevonden tussen beide groepen ten aanzien van de lange termijn psychomotorie ontwikkeling, was bij post hoc analyse de mentale ontwikkeling in de groep met een hogere Hb trigger beter.¹² Bij de kinderen uit de Iowa trial bleek op de leeftijd van 12 jaar juist de cognitieve uitkomst van de kinderen met de liberale transfusie grens lager dan die van de kinderen uit de restrictieve transfusie grens.¹³

Tabel 1. Transfusie gegevens erytrocyten NICUs en CBO

ziekenhuis	Hoogste grens Hb	Hb <7	Hb <6	laagste grens Hb	Dosis ml/kg	Tx snelheid (ml/kg/u)	bestralingsindicatie
1	Hb<8 (Hb<7): - 1e 24 uur indien verschijnselen ¹ - ernstig zieke kinderen - beademing	- redelijk stabiele prematuren met cardio-respiratoire problemen: ODB, apneu's, BPD, extra zuurstof	- stabiele prematuren jonger dan 4 weken	Hb 4.5: - stabiele prematuren >4 weken mits afwezigheid van symptomen ²	10-20	2,5-5	- >80ml/kg/24u én <1500 gram - na IUT - verdenking - immuundeficiënte - product van familielid 1e-3e graad
2	Hb 8: - beademing, ECMO	- afhankelijk van kliniek ²	- nno	nno	?	?	- ?
3	Hb 8: - beademing	- <4 weken oud, niet beademd	- >4 weken oud mits geen problemen		10-20	2,5-10	- bij verdenking - immuundeficiënte
4	Hb 8: - beademing, - 1e 24 uur en klachten	- stabiele pasgeborene met cardio-pulmonale problematiek en O ₂ -behoefte	- stabiele prematuur <4 weken oud	Hb 4.5: - stabiele prematuur >4 weken	15	2,5-7,5	- <1500 gram
5	CBO 2004	CBO 2004	CBO 2004	CBO 2004	CBO 2004	CBO 2004	- <32 weken of <1500 gram
6	CBO 2004	CBO 2004	CBO 2004	CBO 2004	CBO 2004	CBO 2004	- CBO 2004 (niet altijd haalbaar)
7	Hb 9: - cyanotisch cor vitium of ECMO Hb 8: - 1e 24 uur bij circulatie of ademhalingsstoornissen - PPHN - dag 2-28 bij beademing met FIO ₂ >30%	- <4 weken oud beademing met FIO ₂ < 30% - nCPAP (MAP >6cm H2O) - spontaan ademend met FIO ₂ >35% - dag 2-28 bij beademing met FIO ₂ >30%	- >4 weken oud en beademd - nCPAP < 6 cm H2O - spontane ademhaling en FIO ₂ 22-35% - klinische symptomen ² - preoperatief iom anesthesist	Hb 5: - spontaan ademend met FIO ₂ 21%	10-15	5	- IUT en na IUT tot 6 maanden na à terme datum - product van familielid (1e of 2e graad) - ECMO - wisseltransfusie bij <1500 gram
8	Hb 8: - prematuur <36 weken met beademing en O ₂ behoefte - instabiele pasgeborene met cardiopulmonale pathologie en O ₂ behoefte	- stabiel met cardio-pulmonale pathologie - stabiel aan beademing met gering of geen extra O ₂ - stabiel aan nasale IMV - prematuur <32 weken met symptomen ²	- stabiele prematuur geboren voor 32 weken, <4 weken oud, geen symptomen - à terme neonaat met symptomen ²	Hb 5: - a terme zonder symptomen - prematuur (geboren <32 weken) >4 weken oud zonder symptomen ²	15-20	5-6,6 (max 10)	- en/of <32 weken - >80ml/kg/24u én <1500 gram - verdenking - immuundeficiënte - product van familielid 1e-3e graad

ziekenhuis	Hoogste grens Hb	Hb <7	Hb <6	laagste grens Hb	Dosis ml/kg	Tx snelheid (ml/kg/u)	bestraalingsindicatie
9	-direct postnataal indien Ht_c <0.47 of H_s <0.43	Ht_c <0.37 of H_s <0.33: - CPAP of beademing met MAP >6-8 cm H_2O - >40% O_2	Ht_c <0.32 of H_s <0.28: - CPAP of beademing met MAP <6cm H_2O - klinische symptomen ² - preoperatief	Ht_c <0.27, H_s <0.23	?	?	- ?
10	Hb 8: - respiratoire ondersteuning met extra O_2	- respiratoire ondersteuning zonder extra O_2	- prematuur <4 weken, a terme met symptomen ⁴	Hb 5: - prematuur >4 weken, a terme zonder symptomen	15	5	- IUT en na IUT - <32 weken en/of <1500 gram - <1500 gram - wisselbloed voor prematuren <1500 gram en/of <32 weken - product van 1e-3e graad verwanten - IUT en na IUT tot 6 maanden na à terme
CBO 2004	Hb 8: - 1e 24 uur bij symptomen ³ , beademing	- stabiel met cardiopulmonale afwijkingen en zuurstofbehoefte	- stabiele prematuur <4 weken	Hb 4.5: - stabiele prematuur >4 weken	10-15	2,5-5	- < 1500 gram en/of <32 weken tot 6 maanden na a terme - product van 1e-3e graad verwanten - IUT en na IUT tot 6 maanden na à terme - immuundeficentie
CBO 2011	Hb8: - respiratoire insufficiëntie met beademing	- stabiel met cardiopulmonale afwijkingen en zuurstofbehoefte	- <4 weken	Hb 4.5: - >4 weken tenzij symptomen nno	15	max 5	- IUT en na IUT tot 6 maanden na à terme - immuundeficentie

¹ tachycardie, extra zuurstof behoefte, apneu's, bradycardia, ² tachycardie, apneu, tachypnoe, slecht groeien, slecht drinken, ³ tachycardie, bradycardie, apneu, tachypnoe, slecht groeien, slecht drinken en/of O_2 behoefte, Hb in mmol/l, Ht in l/l, nno=niet nader omschreven, Ht_a =arterieel hematocriet, Ht_c =capillair hematocriet, Hb_c =capillair hemoglobine, Hb_s =veneus hemoglobine, PPHN=persistierende pulmonale hypertensie van de neonat, ECMO=extra corporele membraan oxygenatie, IMV=intermitterende mechanische ventilatie, MAP= mean airway pressure, (n)CPAP=(nasale) continuous positive airway pressure, IUT=intra uterine transfusie

Tabel 2. Internationale erythrocyten transfusie grenzen

Studie	Endotracheale beademing	nCAP/IF	Spontane ademhaling	Overig
Kiriplani (PINT trial VS) 2006 ⁶	<1 week oud, Hb 8.4/7.1 1-2 weken oud, Hb 7.5/6.2 >2 weken oud, Hb 6.2/5.2		<1 week oud, Hb 7.5/6.2 1-2 weken oud, Hb 6.2/5.3 >2 weken oud, Hb 5.3/4.3	
Miyashiro (Brazilië) 2005 ⁵	MAP >8 cm H ₂ O, Ht 0.40 MAP 6-8 cm H ₂ O, Ht 0.35 MAP <6 cm H ₂ O, Ht 0.30 Ht 0.40	FI _O ₂ >35%, Ht 0.35 FI _O ₂ <35%, Ht 0.30	FI _O ₂ <35% met zuurstofkoepel, Ht 0.30 Geen extra O ₂ en reticulocyten <2%, Ht 0.20	
Maier (Europa, epo studie) 2002 ²²			FI _O ₂ >40%, Ht 0.40 FI _O ₂ <40% en <2 weken oud, Ht 0.35 3-4 weken oud, Ht 0.30 >4 weken oud, Ht 0.25	Levensbedreigende anemie Hypovolemie Chirurgie
Juul (Washington, VS) 2003 ³	MAP >6 cm H ₂ O en FI _O ₂ >5%, Ht 0.35 MAP <6 cm H ₂ O en FI _O ₂ <35%, Ht 0.30	FI _O ₂ <35%, Ht <0.30	FI _O ₂ <35% of SoA/sepsis, Ht 0.30 Geen extra O ₂ , Ht 0.20	
Bell (Iowa, VS) 2005(4)	Ht 0.46/0.34	Ht 0.38/0.28	Extra O ₂ , Ht 0.38/0.28 Geen extra O ₂ , Ht 0.30/0.22	
Harding (Nieuw Zeeland) 2003 ³⁴	Ziek beademend, <1000 gram, problemen) Ht <0.40 Lage frequentie ventilator en O ₂ <30%, Ht 0.30	O ₂ <30%, Ht 0.30	O ₂ >30%, matige longziekte, apneus en bradycardieën, Ht 0.35	Shock agv bloedverlies <6 weken, stabiel maar SoA, Ht 0.30 Chirurgie >6 weken en SoA, Ht 0.25
American Red Cross, naar Strauss (VS), 2010 ¹⁰	Eerste cardiopulmonale ziekte Ht0. 40-0.45	Ht 0.30-0.35	Extra O ₂ , Ht 0.30-0.35 Stabiel, Ht 0.20-0.30, tenzij SoA Chronisch O ₂ gebruik Hb 6.8	Voor grote chirurgie, Ht 0.30-0.35
Gibson (Groot Brittannië) 2004 ¹⁸				1 ^o 24 uur, Ht 0.36 Intensive care, Hb 7.5 Cumulatief bloed verlies in 1e week 10% + intensive care indicatie Acuut bloedverlies ≥10% Late anemie, stabiele patiënt, Hb 4.3
Canadian Paediatric Society (Canada) 2002 ⁷	Zeer ernstig ziek waarbij transfusie zuurstof transport naar vitale organen kan verbeteren, Ht 0.30-0.35 Ernstig ziek en/of beademing waarbij zuurstof afgifte in het gedrang is, Ht 0.20-0.30		Ht <0.20 met reticulocyten <100-150 x 10 ⁹ /l en SoA	Hypovolemische shock geassocieerd met acuut bloedverlies
CBO 2011 (Nederland)	Hb 8.0		Stabiel maar cardiopulmonale afwijkingen en extra O ₂ , Hb 7 Stabiel <4 weken oud, Hb 6 Stabiel >4 weken oud, Hb 4.5	

Hb = hemoglobine in mmol/l; Ht = hematocriet in l/l; SoA = tekenen van anemie (tachycardie, bradycardie, tachypneu, apneu, groei <10 g/dag); NCPAP = nasal continuous positive airway pressure; IF = infant flow; MAP = mean airway pressure; RDS = respiratoir distress syndroom

Transfusie volume en snelheid

De gebruikte RBC transfusie volumina in de Nederlandse NICUs variëren van 10 tot 20 ml/kg (tabel 1). In de CBO consensus 2004 wordt geadviseerd 10–15 ml/kg te transfunderen. De nieuwste richtlijn adviseert 15 ml/kg naar aanleiding van onderstaande studies.

In de literatuur worden volumina tussen de 5 en 20 ml/kg genoemd.^{14–16} In een gerandomiseerd onderzoek door Paul e.a. werd 10 met 20 ml/kg vergeleken, waarbij een groter volume resulteerde in een significant hoger posttransfusie Hb, zonder nadelige effecten op de ademhaling.¹⁵ Wong e.a. toonde aan dat door het geven van een groter RBC transfusie volume het aantal transfusies kon worden teruggebracht zonder nadelige effecten als gevolg van het grotere volume.¹⁶ In een Nederlands vergelijkend onderzoek tussen 2 NICUs bleek het totaal getransfundeerd erythrocyten volume bij prematuren geboren voor 28 weken zwangerschapsduur bijna 50% hoger bij een volume per transfusie van 20 ml/kg dan van 15 ml/kg.¹⁷

De RBC transfusie snelheid in de Nederlandse NICUs varieert van 2,5 tot 10 ml/kg/uur. De CBO richtlijn 2004 en 2011 adviseren een transfusie snelheid van maximaal 5 ml/kg/uur. Internationale richtlijnen noemen een transfusiesnelheid van 5–10 ml/kg/uur.^{14,18} Er is geen onderzoek verricht naar de gevolgen van hogere of lagere snelheid van transfusie.

Product specificaties

Het door de nationale bloedvoorziening, Sanquin, geleverde standaard erythrocyten product, bedoeld voor zogenoemde top-up transfusies bij neonatale anemie heeft een Ht tussen 0.55 en 0.65 l/l. Eén volwassen RBC donoreenheid wordt verdeeld in 4 of 5 eenheden van ca 50 ml. Deze pedipacks kunnen worden gereserveerd voor 1 of 2 kinderen, het zogenaamde single donor programma. Dit houdt in dat wanneer meerdere RBC transfusies bij één patiënt in de neonatale periode te verwachten zijn, er pedipacks van dezelfde donor voor het kind gereserveerd worden om daarmee de donorexpositie te verlagen. Er zijn vier centra die dit single donor programma voor geselecteerde patiënten proberen toe te passen. Het single donor programma wordt voor premature neonaten door het CBO (2004 en 2011) geadviseerd. In de Westerse landen bestaat nagenoeg overal de mogelijkheid van single donor programma's. Ze worden echter niet overal gebruikt.¹⁹

Alle erythrocytenproducten geleverd door Sanquin zijn leukocyten gedepleteerd door filtratie en bevatten <10⁶ leukocyten per (volwassen) donor eenheid.³ Hierdoor is de transmissie van latente intracellulaire virussen zoals Cytomegalovirus (CMV) geminimaliseerd en worden deze producten als CMV veilig beschouwd.^{20–24} In Nederland geeft één NICU aan prematuren <32 weken en/of <1500 gram bij voorkeur bloed wat tevens afkomstig van donoren die geen CMV hebben doorgemaakt. De CBO richtlijnen 2004 en 2011 adviseren anti-CMV serologisch negatief bloed alleen voor intra-uteriene transfusies. In Westerse landen wordt er zowel

leukogedepleteerd als CMV-antistof negatief bloed aan pasgeborenen gegeven.¹⁹ Voor intra uterine transfusies (IUTs) worden in buitenlandse richtlijnen, vaak bloedproducten geadviseerd die zowel serologisch anti-CMV negatief als leukogedepleteerd zijn. In sommige westerse landen worden extreme prematuren ook gezien als ernstig immuun gecompromitteerd en krijgen om deze reden ook CMV seronegatieve producten.

Parvovirus B19 (Parvo-B19) geeft risico op ernstige anemie indien intra-uterien opgelopen en kan hydrops foetalis veroorzaken.²⁵ Postnataal opgelopen, kan Parvo-B19 infectie met name bij ontvangers met een aangeboren of verworven hemolytische anemie ernstige en langdurige beenmerg depressie en aplasie geven.²⁶ In Nederland is het mogelijk Parvo-B19 veilig bloed te geven afkomstig van donoren met neutraliserende antistoffen. Twee van de tien klinieken geven aan bij prematuren jonger dan 32 weken en/of <1500 gram een Parvo-B19 veilig product te geven. Eén NICU zegt zo mogelijk aan alle kinderen Parvo-B19 veilig bloed te geven. In de richtlijn 2004 staan geen indicaties voor Parvo-B19 veilig bloed behalve voor vrouwen in de eerste 2 trimesters van de zwangerschap. De richtlijn 2011 geeft meer indicaties (tabel 3) waarbij de notitie van de Gezondheidsraad wordt gevolgd.²⁷ In de internationale richtlijnen is het beleid met betrekking tot Parvo-B19 nauwelijks beschreven. Een recente internationale “survey” suggereert dat alleen in Nederland rekening gehouden wordt met het Parvo-B19.¹⁹ De bewaarduur van erythrocyten voor top-up transfusies is 35 dagen.

Bestralingscriteria

Bestraling van cellulaire bloedproducten dient ter voorkoming van graft versus host-ziekte door immuun competente donorlymfocyten bij immuun gecompromitteerde ontvangers. De verschillende centra houden allemaal andere bestralingindicaties aan (tabel 1). Niet één kliniek handhaaft protocollair alle CBO 2004 criteria of kan aan alle criteria voldoen. De bestralingscriteria in de nieuwste richtlijn zijn uitgebreid ten opzichte van 2004 met kinderen geboren na een zwangerschapsduur van minder dan 32 weken, tot 6 maanden na de a terme datum. De reden is dat deze kinderen gezien worden als immuun gecompromitteerd. Bloed voor postnatale transfusies bij kinderen die een IUT hebben ondergaan moeten bestraald worden tot 6 maanden na de a terme datum. Andere indicaties genoemd in de CBO 2011 zijn naast (extreme) prematuriteit, producten van familiendonoren of bij (vermoeden op) immuun deficiëntie. De bestralingsindicaties volgens de CBO richtlijn 2011 staan in tabel 3 vermeld. Internationale richtlijnen benoemen wisselende bestralingscriteria.^{18,19,28} Bloed voor intra-uterine transfusie wordt overal bestraald. De Verenigde Staten hanteren de meest ruime criteria, al verschilt dit per staat of ziekenhuis. Daar variëren de bestralingscriteria van alle kinderen <1250 gram tot alle kinderen in het eerste jaar, ongeacht een onderliggende aandoening.¹⁹

Speciale indicaties

Voor massale transfusies (≥ 80 ml/kg/24 uur) zoals bij wisseltransfusies, cardiochirurgie of extracorporele membraan oxygenatie worden in alle centra erytrocytenproducten gegeven die maximaal 5 dagen oud zijn in verband met de hyperkaliëmie die ontstaat na een langere bewaarduur. Dit is conform beide CBO richtlijnen.

Indicaties voor IUT zijn ernstige anemie met (dreigende) hydrops foetalis, meestal als gevolg van hemolyse door bloedgroep antagonisme of intra-uteriene infecties met Parvo-B19.²⁹

Hiervoor mag het bloed niet ouder dan 3 dagen zijn en dient te worden bestraald. Deze transfusies worden in Nederland alleen in het Leids Universitair Medisch Centrum verricht. Bij acuut massaal bloedverlies bij de geboorte door bijvoorbeeld een ernstige foetomaternale transfusie is er de mogelijkheid een partiële wisseltransfusie uit te voeren met ongekruste O-Rhesus negatieve RBC om zo het Hb te verhogen zonder volume belasting.³⁰

Beschouwing

De meeste NICUs hebben hun eigen beleid met betrekking tot het geven van RBC transfusies. De literatuur geeft ook weinig houvast voor consensus. Gevolg is dat de klinische beoordeling een belangrijke rol speelt bij RBC transfusie-indicaties en dat dit leidt tot aanzienlijke verschillen in transfusiegebruik in de Nederlandse NICUs.

Hemoglobine of hematocriet waarde worden beide gehanteerd als transfusie grens; echter het Ht is mede bepaald door het hoge foetale MCV, wat afneemt met de postnatale leeftijd en als gevolg van transfusie. Een ander aspect, zelden bij de transfusiedrempel betrokken, is de betere zuurstofafgifte van foetaal HbF in vergelijking tot volwassen HbA.

Prematuur geboren kinderen hebben een relatief insufficiënte (hepatische) erytropoïetine productie, een korte levensduur van de rode bloed cellen, die voornamelijk foetaal hemoglobine (HbF) bevatten, en ondergaan veelvuldige bloedafnames voor diagnostiek. Nog altijd is deze iatrogene component het meest belangrijk in het ontstaan van transfusiebehoefte van de prematuur.

Het risico op overdracht van infecties door een bloedtransfusie is tegenwoordig zeer laag, echter het risico van RBC transfusies is niet volledig verwaarloosbaar. Elektrolyt stoornissen, volume belasting, (passieve) immunologische reacties en eventuele immunosuppressie met toegenomen gevoeligheid voor nosocomiale infecties behoren tot de mogelijke risico's.^{31,32} Een voordeel van een groter volume per transfusie kan zijn een afname in het aantal donorexposities, zeker indien een centrum geen single donor programma toepast. Een aantal preventieve maatregelen verdienen nader onderzoek.

Preventieve maatregelen

Studies hebben laten zien dat het hebben van een duidelijke richtlijn leidt tot afname in het aantal bloedtransfusies.^{33,34} Alternatieven om het aantal RBC transfusies en de donor expositie terug te dringen zijn bijvoorbeeld single donor programma's, laat afnavelen, autologe navelstrengbloedtransfusies en erythropoïetine. De meeste consensus bestaat over terugdringen van de iatrogene component. Microanalyses hebben het benodigde volume voor laboratorium onderzoek al voor een deel gereduceerd, zo ook intravasale katheters met meetinstrumenten en transcutane methodes om bijvoorbeeld bilirubine, O₂ en CO₂ te meten. Kritisch prikken, beoordelen of een bloedafname wel strikt noodzakelijk is, naast afnemen van het minimaal benodigde volume, blijft zeer belangrijk in de reductie van het afnamevolume en hiermee de noodzaak tot bloedtransfusie.

Laat afnavelen (minimaal 30 seconden tot maximaal 3 minuten wachten na de geboorte) of het 'melken' van de navelstreng is, ook voor prematuren, een veilige methode om de kans op een bloedtransfusie te verminderen zonder toename van het aantal kinderen met klinisch relevante polycythemie of hyperbilirubinemie.³⁵⁻⁴⁰

Het oogsten van navelstrengbloed voor autologe RBC transfusies bij ernstig prematuur geboren kinderen met een zwangerschapsduur van minder dan 30 weken is technisch mogelijk. Helaas leidt dit voor een onvoldoende aantal kinderen tot een significante reductie in het gebruik van donor bloed.^{1,41,42} Bij a term geboren kinderen waar antenataal de noodzaak tot chirurgisch ingrijpen kort na de geboorte was vastgesteld, is in een aantal gevallen met succes autoloog navelstrengbloed geoogst en getransfundeerd.⁴³⁻⁴⁵

Alle kinderen laten in de weken na de geboorte een dalend Hb zien waarbij de afbraak van HbF niet volledig gecompenseerd wordt door de aanmaak van HbA. Bij a terme kinderen daalt het Hb tot gemiddeld 6.8 mmol/l. Prematuur geboren kinderen hebben bij de geboorte, naast een lager Hb, een geringer ijzer voorraad dan a terme kinderen omdat zij minder hebben kunnen profiteren van de transplacentaire ijzerverdracht in het derde trimester door de kortere zwangerschapsduur. Ook verliezen zij extra ijzer en erythrocyten door laboratorium diagnostiek. Ijzer suppletie kan al vroeg na de geboorte gegeven worden aan kinderen met een verhoogd risico op ijzergebreks anemie. Bij kinderen met een geboorte gewicht <1301 gram, resulteerde vroege ijzer suppletie (2–6 mg/kg/dag, gestart zodra enterale voeding minimaal 100 ml/kg/dag is) vergeleken met late ijzersuppletie (start na 2 maanden of bij ijzerdeficiëntie) in een reductie in het aantal bloedtransfusies na de tweede week.⁴⁶

Het gebruik van erythropoëetine zowel kort na de geboorte als later geeft nauwelijks afname van het aantal bloedtransfusies bij prematuren kinderen, bij wie de grootste transfusiebelasting in de eerste weken na de geboorte ligt.^{8,47-50} Daarnaast is er zorg over een verhoogd risico op retinopathie.⁵¹ Er lijkt in de neonatologie geen routinematige plaats voor erythropoëetine, met uitzondering, misschien, voor de kinderen van Jehova getuigen ter voorkoming van de late anemie.

Nieuwe richtlijn

De adviezen van de CBO richtlijn 2011 met betrekking tot de pasgeborenen zijn samengevat in tabel 3.

Tabel 3. CBO richtlijn erythrocyten transfusies bij neonaten

Transfusie grenzen	Hb (mmol/l)
Beademing	8
Stabiele pasgeborenen met cardiopulmonale afwijkingen en extra O ₂ behoefte	7
Stabiele (premature) neonaat leeftijd < 4 weken	6
Stabiel (prematuur) kind leeftijd > 4 weken	4,5

Indicaties bestraling

Prematuren jonger dan 32 weken en/of kleiner dan 1500 gram tot 6 maanden na a terme
Intra-uteriene transfusies, hierna tot 6 maanden na de a terme datum
Kinderen met een aangeboren gecombineerde immuun-deficiëntie (SCID)
Transfusie tussen eerste tot en met 3e graads verwanten
Wisseltransfusie bij kinderen < 32 weken en/of < 1500 gram

Indicaties Parvovirus B 19 veilig

Intrauteriene transfusie (IUT)
Na IUT tot 6 maanden na de a terme datum
Alle prematuren jonger dan 32 weken en/of kleiner dan 1500 gram
Aangeboren of verworven hemolytische anemie zonder Parvovirus B19 antistoffen
Cellulaire immuundeficiëntie zonder Parvovirus B 19 antistoffen
Altijd leukocyten gedepleteerd
CMV-vrij alleen voor intrauteriene transfusies
Transfundeer 15 ml/kg, transfusiesnelheid maximaal 5 ml/kg/uur

De werkgroep concludeert dat:

1. Er meer bewustwording moet komen voor het voorkómen van erythrocyten transfusies door kritisch gebruik van diagnostische testen, micro analyse technieken en laat afnavelen (30 seconden tot maximaal 3 minuten na de geboorte).
2. Het vroeg starten met ijzer een gunstig effect heeft op de noodzaak voor erythrocyten transfusies. Er wordt dan ook geadviseerd met ijzer suppletie te starten (6 mg/kg/dag) in die kinderen die at risk zijn voor het ontwikkelen van anemie, zodra enterale voeding (>100 ml/kg) goed verdragen wordt.
3. Er nog steeds onbeantwoorde vragen zijn met betrekking tot de veiligheid op korte en lange termijn van restrictieve en liberale transfusie grenzen. Dit geldt eveneens voor het toedienen van grotere transfusie volumina per RBC transfusie.

4. Al bestaande opties zoals het single donor programma meer routine matig gebruikt moeten worden.

Het hebben van een landelijke richtlijn, die gedragen wordt door alle behandelaars van pasgeborenen, kan leiden tot een uniform transfusie beleid en een reductie in het aantal RBC transfusies. Met name het vergelijken van de indicaties en de resultaten van RBC transfusie ten aanzien van klinische verbetering, geeft een mogelijkheid tot het genereren van gegevens om vragen te kunnen beantwoorden over veiligheid en lange termijn gevolgen van RBC transfusie grenzen en transfusie dosis.

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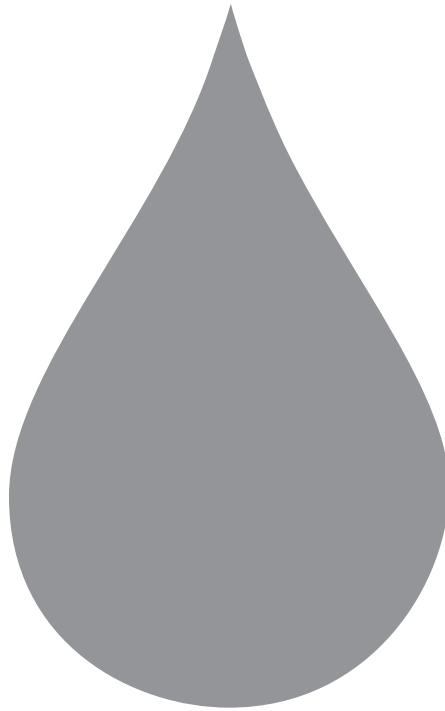
Part III

Platelets



Chapter 8

Thrombocytopenia in neonates and the risk of intraventricular hemorrhage: a retrospective cohort study



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Abstract

Background The overall prevalence of thrombocytopenia in neonates admitted to neonatal intensive care units ranges from 22 to 35%. There are only a few small studies that outline the relationship between the severity of thrombocytopenia and the risk of bleeding. This makes it difficult to form an evidence-based threshold for platelet transfusions in neonatal patients. The aim of this study was to determine the prevalence of thrombocytopenia in a tertiary neonatal intensive care unit and to study the relation between thrombocytopenia and the risk of intraventricular hemorrhage (IVH).

Methods We performed a retrospective cohort study of all patients with thrombocytopenia admitted to our neonatal tertiary care nursery between January 2006 and December 2008. Patients were divided into 4 groups according to the severity of thrombocytopenia: mild ($100\text{--}149 \times 10^9/\text{L}$), moderate ($50\text{--}99 \times 10^9/\text{L}$), severe ($30\text{--}49 \times 10^9/\text{L}$) or very severe ($<30 \times 10^9/\text{L}$). The primary outcome was IVH $\geq$ grade 2. Pearson's chi-square and Fischer's exact tests were used for categorical data. ANOVA, logistic regression analysis and multivariate linear regression were used for comparisons between groups and for confounding factors.

Results The prevalence of thrombocytopenia was 27% (422/1569). Risk of IVH $\geq$ grade 2 was 12% (48/411) in neonates with versus 5% (40/844) in neonates without thrombocytopenia ($p<0.01$). After multivariate linear regression analysis, risk of IVH $\geq$ grade 2 in the subgroups of thrombocytopenic infants was not significantly different ($p=0.3$). After logistic regression analysis the difference in mortality rate in neonates with and without thrombocytopenia was not significant ($p=0.4$). Similarly, we found no difference in mortality rate in the subgroups of neonates with thrombocytopenia ($p=0.7$).

Conclusion Although IVH $\geq$ grade 2 occurs more often in neonates with thrombocytopenia, this relation is independent of the severity of thrombocytopenia. Prospective studies should be conducted to assess the true risk of hemorrhage depending on underlying conditions. Randomized controlled trials are urgently needed to determine a safe lower threshold for platelet transfusions.

Background

The overall prevalence of thrombocytopenia in neonates ranges from 1 to 5% and is reported to be much higher in neonates admitted to neonatal intensive care units, ranging from 22 to 35%.¹⁻⁶ From 22 weeks' gestation onwards, the platelet count reaches and maintains a level above $150 \times 10^9/L$, thereby defining thrombocytopenia in the newborn as a platelet count below $150 \times 10^9/L$.^{1,2} Many neonatal and maternal conditions are associated with thrombocytopenia, of which septicemia and prematurity are the most common.^{2,6} In thrombocytopenia the major concern is an increased risk of bleeding.

In 1882 Bizzozzero was the first to describe the role of platelets in coagulation and thrombosis.⁷ Since then, only a few small studies investigating the relationship between the severity of thrombocytopenia and the risk of bleeding in newborns have been reported.^{4,8,9} Likewise, the number of clinical trials examining thrombocytopenia and the effects on bleeding in adults is limited.¹⁰⁻¹³ The lack of studies makes it difficult to form an evidence-based threshold for platelet transfusions in neonatal patients.

The aim of this study was to analyze and describe all cases with thrombocytopenia admitted to our neonatal nursery during a 3-year period and study a possible relationship between the risk of intraventricular hemorrhage (IVH) and the severity of thrombocytopenia. We studied the prevalence and risk factors of thrombocytopenia in relation to the risk of IVH and mortality.

Methods

Patients

We retrospectively collected data from all neonates admitted between January 2006 and December 2008 to the neonatal department of the Leiden University Medical Center, a tertiary neonatal care center in The Netherlands. In the Netherlands no ethical approval is required for this type of research as no new intervention or treatment is studied. Nor is any randomization needed. All collected data was anonymized. We identified all thrombocytopenic newborns by extracting data from our dedicated patient-database, medical files, laboratory system and electronic blood banking records. We excluded neonates with only one platelet count measurement below $150 \times 10^9/L$. We considered these isolated counts as clotted samples, platelet clumping, laboratory error or one-time only measurements with immediate normalization. Thrombocytopenia was defined as a platelet count below $150 \times 10^9/L$. The included neonates with thrombocytopenia were divided into 4 groups, based on their lowest platelet count during their stay in our unit, and classified as mild (platelet count

100–149 x 10⁹/L), moderate (platelet count 50–99 x 10⁹/L), severe (platelet count 30–49 x 10⁹/L) or very severe (platelet count <30 x 10⁹/L), according to standard classification.^{1,2,6,14-17}

We recorded the presence of IVH detected by cranial ultrasound and classified according to Volpe.¹⁸ IVH grade 2, grade 3 or grade 4 (i.e. periventricular hemorrhagic infarction (PVHI)) were recorded.

Cranial ultrasounds were performed according to local protocol depending on gestational age and degree of illness.

Data for demographic as well as clinical conditions of all infants were collected, including gender, gestational age at birth, birth weight, small for gestational age, chromosomal disorders, perinatal asphyxia, necrotizing enterocolitis, sepsis/meningitis, hemorrhage, thrombosis, central catheters, polycythemia, rhesus hemolytic disease, exchange transfusion, neonatal allo-immune thrombocytopenia and the number of blood product transfusions (platelets, erythrocytes, fresh frozen plasma). Small for gestational age was defined as a birth weight <3rd percentile for the corresponding gestational age.¹⁹ Chromosomal disorders were defined as congenital anomalies related to thrombocytopenia, such as trisomy 18 and 21. Perinatal asphyxia was defined as a five minute Apgar score <5, a decelerative heart rate on a cardiotocogram and/or an arterial umbilical cord pH below 7.0. Hypotension was defined as a mean blood pressure below the 3rd percentile for gestational age and requiring inotropic support. Sepsis was defined as a positive blood culture in a neonate with clinical signs of infection. Necrotizing enterocolitis was scored based on Bell staging criteria.²⁰ Polycythemia was defined as a venous hematocrit ≥65% in symptomatic infants or ≥70% with or without symptoms. A thrombus could be catheter related, in a major blood vessel or intracardial, detected with ultrasound.

The primary outcome measure was IVH ≥ grade 2. The secondary outcomes were total number of platelet transfusions and mortality.

In our hospital a platelet transfusion for neonates is a concentrated single donor product in plasma and is leukocyte depleted. The dose is a median of 20 x 10⁹ platelets per kg. The product is irradiated with 25 Gy for all infants with a gestational age below 32 weeks and/or a birth weight below 1500 grams and/or for neonates that previously underwent an intra-uterine transfusion. Guidelines for platelet transfusions in our department were as follows: 1) platelet count <30 x 10⁹/L and stable, 2) platelet count <50 x 10⁹/L and unstable, and/or birth weight <1000 g, and/or previous major bleeding, and/or after exchange transfusion, and/or before planned surgery and/or rapid decrease of platelets, or 3) platelet count <100 x 10⁹/L in neonates with active bleeding and/or at start of exchange transfusion.¹⁷

Statistics

Data analyses were performed using Statistical Package for Social Sciences (SPSS), version 16.0 (SPSS, Inc., Chicago, Illinois, USA). For every separate variable the Pearson's chi-square test was used. If the chi-square test could not be used (frequency of an event was <5) the Fisher's exact test was used. Comparisons between group means were analyzed using the one way ANOVA test (with a 95% confidence-interval). Logistic regression was performed to evaluate the confounders between the infants with and without thrombocytopenia. Factors considered potential confounders were variables with a significant difference in thrombocytopenia. Multivariate linear regression was used to compare for confounders in the subgroups of thrombocytopenic neonates, because of the small number in some of the subgroups of thrombocytopenic neonates. A p-value smaller than 0.05 was considered to be significant.

Results

Total patient population

A total of 1727 neonates were admitted to our neonatal nursery during the 3-year study period. Thrombocytopenia was detected in 580 neonates, of which 158 were excluded because of only one platelet count below $150 \times 10^9/L$. The prevalence of thrombocytopenia was 27% (422/1569). Neonates with thrombocytopenia were divided into four groups according to their lowest platelet count; 122 (29%) mild, 164 (39%) moderate, 67 (16%) severe

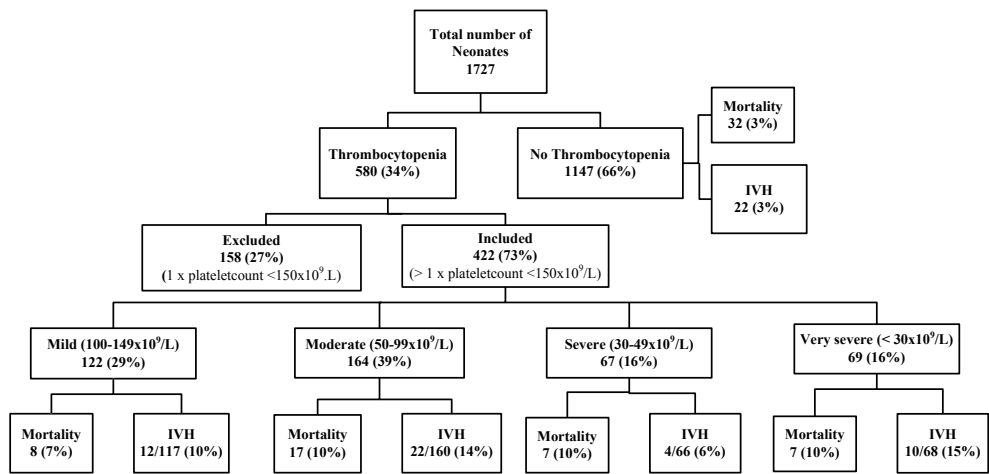


Figure 1. Flow-chart of all in- and exclusions of the cohort

and 69 (16%) with very severe thrombocytopenia. The distribution of included and excluded neonates is shown in Figure 1.

An overview of the baseline characteristics of all included neonates with (n=422) and without (n=1147) thrombocytopenia is presented in Table 1. Except for gender, single or multiple births and chromosomal disorders, every characteristic was significantly different.

Table 1. Patient characteristics of neonates with and without thrombocytopenia

	Neonates without thrombocytopenia (N=1147)	Neonates with thrombocytopenia (N=422)	p-value
Female gender, n (%)	513 (45)	179 (42)	0.41
GA at birth (weeks), mean $\pm$ SD	35 $\pm$ 4.2	32.5 $\pm$ 4.8	<0.01
Birth weight (gram), mean $\pm$ SD	2537 $\pm$ 968	1864 $\pm$ 1067	<0.01
SGA, n (%)	44 (4)	62 (15)	<0.01
Multiple births (single), n (%)	270 (24)	100 (24)	0.96
Postnatal condition			
Asphyxia, n (%)	42 (4)	46 (11)	<0.01
Hypotension, n (%)	68 (6)	123 (29)	<0.01
Sepsis, n (%)	77 (7)	129 (30)	<0.01
NEC, n (%)	9 (1)	10 (2)	0.01
Central catheter, n (%)	403 (35)	346 (82)	<0.01
Thrombus, n (%)	6 (1)	14 (3)	<0.01
NAITP, n (%)	1 (0)	14 (3)	<0.01
Polycythemia, n (%)	32 (3)	26 (6)	<0.01
RHD, n (%)	61 (5)	40 (10)	<0.01
Exchange transfusion, n (%)	1 (0)	14 (3)	<0.01
Chromosomal disorders, n (%)	9 (1)	7 (2)	0.15

GA=gestational age; SGA=small for gestational age; NEC=necrotizing enterocolitis; NAITP=Neonatal alloimmune thrombocytopenia; RHD=Rhesus hemolytic disease

Primary and secondary outcome in total patient population

Cranial ultrasound was performed in 97% (411/422) of neonates with thrombocytopenia and in 74% (844/1147) of infants without thrombocytopenia. The rate of IVH $\geq$ grade 2 in neonates with and without thrombocytopenia was 12% (48/411) and 5% (40/844), respectively ($p<0.01$). After multiple regression analysis, with all significantly different variables, the correlation between IVH and thrombocytopenia was still statistically significant ($p=0.045$); gestational age remained an independent significant risk factor for IVH ($p<0.01$).

Mortality rate in neonates with and without thrombocytopenia, respectively 9% (39/422) vs. 3% (32/1147), was not significantly different after multiple regression analysis ($p=0.4$).

Thrombocytopenic patient population

Thrombocytopenia was detected at a mean of 2 days after birth (range 0–56 days). In the group of thrombocytopenic neonates (n=422), 27 died before thrombocytopenia had resolved and in 32 neonates laboratory testing was discontinued before a platelet count above $150 \times 10^9/\text{L}$ was recorded during follow-up. In these 32 infants platelet counts were not measured because of already increasing platelet counts with a value above $120 \times 10^9/\text{L}$. In the remaining 363 neonates, the mean duration of thrombocytopenia was 9 days (range 0–112 days). We found a significant positive correlation between severity of thrombocytopenia and the time to recovery. Duration of thrombocytopenia in the mild, moderate, severe and very severe group was 5, 8, 10 and 16 days, respectively ($p < 0.01$).

Mean gestational age at birth was 32.5 (range 24 to 42) weeks. Of all thrombocytopenic neonates, 75% (316/422) were preterm (< 37 weeks). Patient characteristics divided into subgroups according to severity of thrombocytopenia are shown in Table 2. After linear

Table 2. Patient characteristics of thrombocytopenic neonates, divided into subgroups by severity

	Mild ($100\text{--}149 \times 10^9/\text{L}$) (N=122)	Moderate ($50\text{--}99 \times 10^9/\text{L}$) (N=164)	Severe ($30\text{--}49 \times 10^9/\text{L}$) (N=67)	Very severe ($< 30 \times 10^9/\text{L}$) (N=69)	p-value
Gender (female), n (%)	51 (42)	66 (40)	32 (48)	30 (43)	0.77
GA (weeks), mean $\pm$ SD	32.8 ± 4.7	32.1 ± 4.7	32.5 ± 4.7	32.9 ± 5.0	0.56
Weight (gram), mean $\pm$ SD	2004 ± 1172	1715 ± 984	1854 ± 1058	1979 ± 1046	0.11
SGA, n (%)	13 (11)	29 (18)	12 (18)	8 (12)	0.28
Postnatal condition					
Asphyxia, n (%)	11 (9)	14 (9)	9 (13)	12 (17)	0.19
Hypotension, n (%)	27 (22)	46 (28)	28 (42)	22 (32)	0.04
Sepsis, n (%)	26 (21)	43 (26)	25 (37)	35 (51)	< 0.01
NEC, n (%)	1 (1)	4 (2)	3 (4)	2 (3)	†
Central catheter, n (%)	90 (74)	138 (84)	56 (84)	62 (90)	0.03
Thrombus, n (%)	2 (2)	3 (2)	3 (4)	6 (9)	†
NAITP, n (%)	1 (1)	5 (3)	5 (8)	3 (4)	†
Polycythemia, n (%)	6 (5)	12 (7)	3 (5)	5 (7)	†
RHD, n (%)	7 (6)	13 (8)	8 (12)	12 (17)	0.05
Exchange transfusion, n (%)	1 (1)	4 (2)	2 (3)	7 (10)	†
Chromosomal disorders, n (%)	0 (0)	2 (1)	1 (1)	4 (6)	†

†=No analysis possible, because some of the subgroups were too small.

GA=gestational age; SGA=small for gestational age; NEC=necrotizing enterocolitis; FFP=fresh frozen plasma; NAITP=Neonatal alloimmune thrombocytopenia; RHD=Rhesus hemolytic disease

regression analysis, severity of thrombocytopenia remained associated with sepsis ($p < 0.01$) and thrombi ($p < 0.01$).

In 66% of the 105 thrombocytopenic neonates with sepsis, the low platelet count was already present before the child became ill, whereas in 15% thrombocytopenia developed after the onset of sepsis.

Primary and secondary outcome in thrombocytopenic patient population

In the majority (87%) of neonates with an IVH $\geq$ grade 2 the hemorrhage occurred within the first 3 days of life. In 23% of the 48 neonates (11/48) with an IVH, the hemorrhage was discovered on the same day as the thrombocytopenia. In 20 (42%) neonates the hemorrhage was discovered after and in 16 (33%) before the thrombocytopenia even existed. In 1 neonate we could not trace the timing of the hemorrhage.

Among the 122 neonates who received a platelet transfusion 17 had an IVH $\geq$ grade 2. Two (13%) of these infants developed an IVH during thrombocytopenia despite platelet transfusions (one IVH grade 2, one IVH grade 3).

The primary and secondary outcome in the 4 subgroups of neonates with thrombocytopenia is presented in Table 3. Risk of hemorrhage was 10% (12/117), 14% (22/160), 6% (4/66) and 15% (10/68). We found no significant association between bleeding and severity of thrombocytopenia ($p = 0.3$). After logistic regression analysis, the severity of thrombocytopenia was not a significant risk factor for IVH.

Logistic regression analysis showed no significant relation between mortality and the severity of thrombocytopenia ($p = 0.7$). In 5 patients treatment was withdrawn because of poor

Table 3. Primary and secondary outcome in the 4 subgroups of thrombocytopenic infants

	Mild (100–149x10 ⁹ /L) (n=122)	Moderate (50–99x10 ⁹ /L) (n=164)	Severe (30–49x10 ⁹ /L) (n=67)	Very severe (<30x10 ⁹ /L) (n=69)	p-value
Primary Outcome	(n=117)		(N=66)	(N=68)	
Number of neonates with IVH $\geq$ grade 2, n (%)	12 (10)	22 (14)	4 (6)	10 (15)	0.32
IVH, grade 2	3 (3)	7 (4)	3 (5)	6 (9)	0.26
IVH, grade 3	4 (3)	6 (4)	1 (2)	2 (3)	0.86
PVHI (i.e., IVH grade 4)	5 (4)	9 (6)	0 (0)	2 (3)	0.25
Secondary Outcome	(n=122)	(n=164)	(n=67)	(n=69)	
Number of platelet Tx per transfused patient, median (range), N	0 (n=0)	1 (1–1) (n=9)	1 (1–5) (n=48)	3 (1–28) (n=65)	< 0.01
Mortality, n (%)	8 (7)	17 (10)	7 (10)	7 (10)	0.69

neurological prognosis due to major hemorrhage (IVH grade 3 or PVHI) in combination with respiratory and/or cardiac insufficiency.

Of all included neonates with thrombocytopenia, 29% (122/422) received a platelet transfusion (Table 3). The median number of platelet transfusions in the mild, moderate, severe and very severe groups was 0, 1, 1 and 3, respectively. The 9 neonates in the moderate thrombocytopenia group ($50\text{--}99 \times 10^9/\text{L}$) were transfused because of a rapid drop in platelet count in combination with sepsis ($n=4$), IVH $\geq$ grade 2 ($n=3$) or adrenal hemorrhage ($n=1$). In most cases the infants with severe thrombocytopenia ($30\text{--}49 \times 10^9/\text{L}$) received a platelet transfusion before or during an intervention (such as a lumbar puncture or exchange transfusion), because of active bleeding or if they were clinically unstable. The rest of the newborns were transfused when the platelet count was below $30 \times 10^9/\text{L}$. Only 4 neonates with very severe thrombocytopenia did not receive a platelet transfusion. Three of them had a gestational age of 37 and the fourth one of 31 weeks. All four were clinically stable, 2 had polycythemia and 2 had Rhesus hemolytic disease. None of them had IVH.

Discussion

This study shows that although thrombocytopenic neonates are a high risk group (more unstable, and sicker than non-thrombocytopenic neonates), the severity of thrombocytopenia is not related to IVH or mortality. In 33% of the thrombocytopenic neonates with IVH grade 2 or more, IVH occurred before the thrombocytopenia even existed. Our data confirm that risk of IVH in neonates is a complex mechanism related to a wide variety of factors, of which low platelet counts is only one.

Prevalence of thrombocytopenia

The overall prevalence of thrombocytopenia found in this study (27%) is in accordance with the rates reported in the literature for tertiary care centers (22–35%).^{1–6} The prevalence of severe ($<50 \times 10^9/\text{L}$) thrombocytopenia (8%) was also similar to other studies (2–25%).^{1,3} Our findings confirm that thrombocytopenia in neonates is associated with a wide variety of factors, including prematurity and low birth weight, small for gestational age, sepsis, hypotension, necrotizing enterocolitis, asphyxia, thrombi and exchange transfusions.^{1,4,14,21}

Platelet transfusion

We found a positive correlation between the severity of thrombocytopenia, the duration of thrombocytopenia and with the total number of platelet transfusions. In the subgroup of infants with severe thrombocytopenia, platelet transfusion resulted in a good, but less

sustained rise in platelet count (data not shown). A few studies have suggested that a fast drop in platelet count after transfusion is caused by ongoing platelet consumption instead of platelet underproduction.²¹

The reason for platelet transfusions in more than half of the cases was an existing thrombocytopenia. Despite the platelet transfusions, 13% (2/17) developed an IVH ($\geq$ grade 2), independent of the severity of thrombocytopenia. In newborns that required more transfusions (data not shown) no increased risk for IVH was seen, comparable to other studies.^{9,16,21} Therefore the jury is still out on the protective value of platelet transfusions.^{1,4,6,9,22}

Risk of hemorrhage

We found no significant relationship between hemorrhage and severity of thrombocytopenia. This suggests that bleeding in neonates depends on more variables than a platelet count alone. In approximately one-third of the thrombocytopenic neonates the IVH was discovered before the thrombocytopenia existed and this raises the question whether IVH can be explained as a cause or an effect of thrombocytopenia.²²⁻²⁴ Hemorrhage is probably due to pre-existing fragility in vessel wall structure (especially in premature neonates) and damaged blood vessels, amongst others by cytokines and/or a co-existing coagulopathy.^{9,22}

Our results are important in the discussion whether thrombocytopenia is one of the major causes of IVH in neonates. Major IVH has a large impact on neurological development and mortality. However, the risk for an IVH cannot be predicted based on a platelet count alone. We also looked at other bleedings of importance in the thrombocytopenic neonates, such as pulmonary- or gastrointestinal bleedings, but the number of these hemorrhages was too small to analyze. Other variables such as gestational age, birth weight and underlying illness are of equal importance and should be taken into account. Several studies have searched for other factors that may influence the development of hemorrhage in thrombocytopenic neonates. Deficiencies, immaturity or increased consumption of other blood products, such as thrombopoietin, coagulation factors, megakaryocyte progenitor cells, cytokines and mean platelet volume, have been reported.^{3,25}

One of the current major issues in transfusion medicine is the appropriate trigger for platelet transfusion. Different triggers are being used for platelet transfusion. While some centers transfuse all neonates with platelet counts below $50 \times 10^9/L$, other centers use lower thresholds such as <30 or $<20 \times 10^9/L$.^{1,2,14,17,26} There is an apparent evolvement amongst neonatologists towards more liberal platelet transfusion practices, even in the absence of evidence based data.²⁷ This study does not demonstrate a difference in IVH between neonates with a platelet count below $50 \times 10^9/L$ or below $30 \times 10^9/L$ (nor between <150 , <100 or lower for that

matter), questioning the different transfusion thresholds. Whether platelet transfusions have a protective value in neonates with a platelet count below $20 \times 10^9/L$ is still not known.

Conclusion

In this study, we found no relationship between the severity of thrombocytopenia and IVH, suggesting that the etiology of IVH in neonates is a complex multifactorial process. However, our findings should be interpreted with care due to limitations associated with the retrospective nature of the study and the relatively small sample sizes of some variables in the subgroups.

Prospective studies should be conducted to assess the true risk of hemorrhage depending on underlying conditions. Randomized controlled trials are urgently needed to determine a safe lower threshold for platelet transfusions.

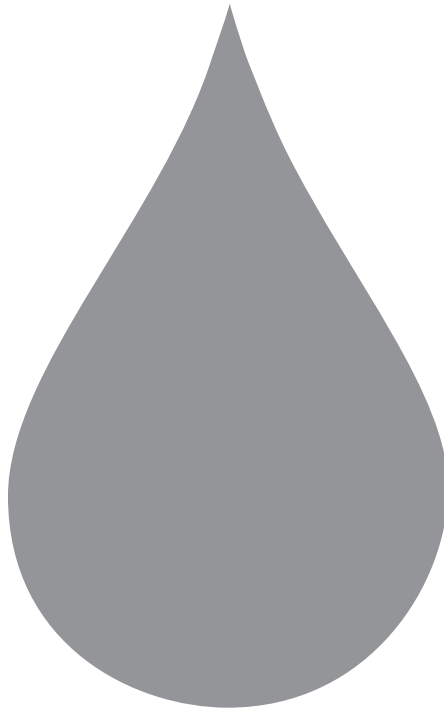
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Chapter 9

Thrombocytopenia and intraventricular hemorrhage in very premature infants: a tale of two cities



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Submitted

Abstract

Objective To study whether the incidence of intraventricular hemorrhage (IVH) in very premature infants with thrombocytopenia is lower when using a liberal platelet transfusion guideline compared to a restrictive guideline.

Study design A retrospective cohort study comparing the incidence of IVH in very premature infants with thrombocytopenia (platelet count $<150 \times 10^9/L$) admitted between 2007 and 2008 to two neonatal intensive care unit in The Netherlands. The restrictive platelet transfusion unit (N=353) transfused only in case of active hemorrhage and a platelet count $<50 \times 10^9/L$. The liberal-transfusion unit (N=326) transfused according to predefined platelet count thresholds. Primary outcome was the incidence and severity of IVH in thrombocytopenic infants in both units.

Results The incidence of IVH in thrombocytopenic infants in the restrictive-transfusion and liberal-transfusion unit was 30% (44/145) and 29% (41/141), respectively ($p=0.81$). The incidence of severe IVH (grade 3 or 4) in the restrictive-transfusion and liberal-transfusion unit was 8% (12/145) and 11% (16/141), respectively ($p=0.38$).

Conclusion In the restrictive-transfusion unit, the rate of platelet transfusions was significantly lower, but the incidence and severity of IVH was similar to the liberal-transfusion unit.

Introduction

Thrombocytopenia, defined as a platelet count below $150 \times 10^9/\text{L}$, occurs in approximately 21–33% of all neonates admitted to a neonatal intensive care unit (NICU).^{1–4} The incidence of thrombocytopenia in extremely low birth weight infants (≤ 1000 grams) is even higher and may reach 73%.⁵ Various studies in premature infants have reported co-occurrence of thrombocytopenia and intraventricular hemorrhage (IVH).^{2,6,7} However, the etiology of IVH in premature infants is multicausal and related to various risk factors including extreme prematurity, patent ductus arteriosus (PDA), sepsis and mechanical ventilation.⁸ Whether (severe) thrombocytopenia is independently associated with an increased risk of IVH is not known. Whether platelet transfusions in premature infants with thrombocytopenia reduce the risk of IVH is not known either. The only randomized controlled trial (RCT) in this field reported similar incidences and grades of IVH when comparing prophylactic platelet transfusions with no platelet transfusion for newborn infants with moderate thrombocytopenia.⁹ The paucity of data on the effects of platelet transfusions has led to multiple consensus-based platelet transfusion guidelines, some of them being liberal, whereas others are restrictive. We analyzed the incidence and grade of IVH in very premature infants in two comparable Dutch NICUs using different platelet transfusion guidelines.

Materials and methods

Subjects studied

We included all premature infants with a gestational age < 32 completed weeks born between January 1st 2007 and December 31st 2008 and admitted to the NICUs of the Leiden University Medical Center and the University Medical Center Groningen. These centers are two of the 10 neonatal tertiary care centers in the Netherlands.

Platelet transfusion guidelines

The platelet transfusion guidelines used during the study period in the two units differed significantly. One unit used a liberal transfusion guideline based primarily on platelet count thresholds, the other unit used a more restrictive guideline based primarily on signs of clinical bleeding.

Platelet transfusions in the liberal-transfusion unit were given when platelet counts fell below the following thresholds: 1) $< 30 \times 10^9/\text{L}$ in clinically stable infants, 2) $< 50 \times 10^9/\text{L}$ in unstable infants and/or before planned surgery, 3) $< 100 \times 10^9/\text{L}$ in neonates with active bleeding and/or at the start of an exchange transfusion.

Platelet transfusions in the restrictive-transfusion unit were given if the platelet count fell below $50 \times 10^9/\text{L}$ in the presence of any of the following: major bleeding, massive petechiae, bruising, need for surgery, need for invasive procedures or the need for indomethacin treatment for a patent ductus arteriosus. Premature infants with (very) severe thrombocytopenia but without clinical signs of bleeding did not routinely receive prophylactic platelet transfusions.

Cranial ultrasound guidelines

During the study period both units used a similar cranial ultrasound protocol: scans were performed on day 1, 3 and 7 of life. Ultrasound scans were repeated within 1 week if abnormalities were present. Follow-up ultrasound scans were performed until normalization or long-standing stabilization of the IVH, periventricular leukomalacia (PVL) or (post-hemorrhagic) dilatation of the ventricles. Additional ultrasound scans were performed in case of illness known to increase the risk for hemorrhage such as sepsis, necrotizing enterocolitis (NEC) or thrombocytopenia.

We recorded the platelet count and platelet transfusions in both units. Thrombocytopenia was defined as a platelet count $<150 \times 10^9/\text{L}$. The severity of thrombocytopenia was graded as mild ($100\text{--}150 \times 10^9/\text{L}$), moderate ($50\text{--}100 \times 10^9/\text{L}$), severe ($30\text{--}50 \times 10^9/\text{L}$) and very severe ($<30 \times 10^9/\text{L}$).

We recorded the rate and severity of IVH in both units. IVH was graded according to Papile.¹⁰ In short, grade 1 is a subependymal hemorrhage, grade 2 an IVH without ventricular dilatation, grade 3 an IVH with ventricular dilatation and grade 4 an IVH with parenchymal hemorrhage.

Additional data were collected from patient files, local databases, and laboratory and transfusion records. The data included gestational age at birth, birth weight, gender, Apgar score, days on respiratory support, sepsis, NEC ($\geq$ grade 2 according to Bell, i.e. clinically ill children with abdominal distention, gastric residuals, signs of ileus, occult or gross hemorrhage from the gastrointestinal tract, and on abdominal radiography pneumatosis intestinalis, portal vein gas or (in grade 3) presence of pneumoperitoneum)¹¹, mortality, and major hemorrhage defined as a pulmonary or intestinal hemorrhage or massive petechiae and bruising. Sepsis was defined as clinical symptoms of infection and a positive blood culture.

Outcome

The primary outcome was the incidence and severity of IVH in thrombocytopenic infants in both units.

Statistical analysis

SPSS Statistics 17.0 (SPSS Inc., Chicago, IL, USA) was used. All variables were analyzed using the t-test for continuous variables and chi-square or Fisher's exact test for nominal variables. Logistic regression analysis was performed for potential confounding factors. A p-value of <0.05 was considered significant. Odds ratios were calculated.

Approval from the medical ethical committee was not requested for this study as anonymous data collection is part of standard care in the Netherlands.

Results

Total study population

A total of 689 infants were admitted during the study period. In 28 infants no cranial ultrasound scans were performed. Of these 28 infants, 11 died of whom 10 shortly after birth before a cranial ultrasound scan could be made. These 10 infants were not included in the analysis because several other variables (for example platelet counts) were also missing. The characteristics of the study population are presented in Table 1. There were no significant differences in demographic and clinical characteristics between the two populations in both units. There were no premature infants with fetal/neonatal autoimmune thrombocytopenia. Only one infant (in the liberal-transfusion unit) received an exchange transfusion but had a normal platelet count. For the analysis of (intraventricular) hemorrhage only those infants were included of whom a cranial ultrasound scan was made and a platelet count was known (323 infants in the liberal-transfusion unit and 330 in the restrictive-transfusion unit).

Table 1. Characteristics of all patients per unit

	Liberal transfusion unit (N=326)	Restrictive transfusion unit (N=353)	p-value
Gestational age at birth, wk ^a	28.9 (±1.9)	28.9 (±1.9)	0.91
Birth weight, g ^a	1259 (±326)	1270 (±353)	0.67
IUGR, n (%)	19 (6)	10 (3)	0.05
Male, n (%)	172 (53)	195 (55)	0.52
Apgar score 5 min ^a	7.7 (±2.0)	7.6 (±1.5)	0.54
RBC Tx, n (%)	163 (50)	159 (45)	0.20
Patent ductus arteriosus, n (%)	60 (18)	81 (23)	0.14
Sepsis, n (%)	98 (30)	89 (25)	0.16
NEC, n (%)	9 (3)	14 (4)	0.39
Mortality, n (%)	22 (7)	25 (7)	0.86
IVH, n/N (%) ^b	63/323 (20)	75/330 (23)	0.31

^aValue given as mean ± SD; ^bN=number of infants with a cranial ultrasound scan

IUGR=intra-uterine growth retardation; RBC Tx=red blood cell transfusion; NEC=necrotizing enterocolitis

Platelet transfusions

Data on the incidence and severity of thrombocytopenia and the incidence of platelet transfusions in the two units are shown in Table 2. The overall rate and severity of thrombocytopenia was similar in both units. The percentage of thrombocytopenic patients who received one or more platelet transfusions was significantly higher in the liberal-transfusion unit than in the restrictive-transfusion unit, 31% (44/141) versus 15% (21/145), respectively ($p<0.001$). The mean ($\pm$ SD) number of platelet transfusions per thrombocytopenic infant was significantly higher in the liberal-transfusion unit than in the restrictive-transfusion unit, 1.1 ± 3.0 SD versus 0.2 ± 0.7 SD, respectively ($p=0.001$).

Table 2. Rate and severity of thrombocytopenia in the 2 units and IVH and platelet transfusion practices in the thrombocytopenic infants per unit

Variable	Liberal transfusion unit	Restrictive transfusion unit	p-value
Thrombocytopenia all, n/N (%)	141/325 (43)	147/334 (44)	0.87
Mild ($100-150 \times 10^9/L$), n/N (%)	41/141 (29)	58/147 (40)	0.06
Moderate ($50-100 \times 10^9/L$), n/N (%)	55/141 (39)	50/147 (34)	0.38
Severe ($30-50 \times 10^9/L$), n/N (%)	21/141 (15)	19/147 (13)	0.63
Very severe ($<30 \times 10^9/L$), n/N (%)	24/141 (17)	20/147 (14)	0.42
Platelet Tx in thrombocytopenic infants, n/N (%)	44/141 (31)	21/145 (15)	<0.001
Platelet Tx per thrombocytopenic infant, n ^a	1.1 (± 3.0)	0.2 (± 0.7)	0.001
Platelet Tx per transfused infant, n ^a	3.6 (± 4.6)	1.6 (± 0.9)	0.05
IVH grade 1, n/N (%)	15/141 (11)	30/145 (21)	0.02
IVH grade 2, n/N (%)	10/141 (7)	2/145 (1)	0.02
IVH grade 1 or 2, n/N (%)	25/141 (18)	32/145 (22)	0.36
IVH grade 3, n/N (%)	8/141 (6)	2/145 (1)	0.06
IVH grade 4, n/N (%)	8/141 (6)	10/145 (7)	0.67
IVH grade 3 or 4, n/N (%)	16/141 (11)	12/145 (8)	0.38

^aValue given as mean $\pm$ SD

Tx=transfusion; IVH=intraventricular hemorrhage

Intraventricular hemorrhage

The overall incidence of IVH was similar in the liberal- and restrictive-transfusion unit, 20% (63/323) versus 23% (75/330), respectively ($p=0.31$). The rate of IVH in thrombocytopenic infants in the liberal-transfusion and restrictive-transfusion unit is shown in Table 2. The rate of clinically significant, severe IVH (grade 3 or 4) was similar, 11% (16/141) versus 8% (12/145), in the liberal- and restrictive-transfusion unit respectively ($p=0.38$). We found no differences in the incidence of IVH after stratifying for severity of thrombocytopenia (data shown in Table 3). Logistic regression analysis with gestational age at birth (<28 weeks or 28–32

Table 3. Rate of intraventricular hemorrhage per unit versus severity of thrombocytopenia

Platelet count x 10 ⁹ /L	No IVH			IVH grades 1+2			IVH grades 3+4		
	Lib	Restr	p-value	Lib	Restr	p-value	Lib	Restr	p-value
>150	160/182	157/185	0.40	12/182	24/185	0.04	10/182	4/185	0.10
	(88%)	(85%)		(7%)	(13%)		(6%)	(2%)	
100–149	30/41	44/58	0.76	6/41	12/58	0.44	5/41	2/58	0.12
	(73%)	(76%)		(15%)	(21%)		(12%)	(3%)	
50–99	38/55	32/49	0.68	9/55	13/49	0.21	8/55	4/49	0.31
	(69%)	(65%)		(16%)	(27%)		(15%)	(8%)	
30–49	16/21	12/19	0.37	5/21	4/19	1.00	0/21	3/19	0.10
	(76%)	(63%)		(24%)	(21%)		(0%)	(16%)	
<30	16/24	13/19	0.90	5/24	3/19	1.00	3/24	3/19	1.00
	(67%)	(68%)		(21%)	(16%)		(13%)	(16%)	

Numbers are n/N (%)

IVH=intraventricular hemorrhage; Lib=liberal platelet transfusion unit; Restr= restrictive platelet transfusion unit

weeks), thrombocytopenia and severity of thrombocytopenia, sepsis, intra-uterine growth retardation (IUGR), NEC, platelet transfusion, restrictive- or liberal-transfusion unit and patent ductus arteriosus as confounders for IVH was performed. Thrombocytopenia, irrespective of the severity, was significantly related to the incidence of IVH (all grades combined ($p=0.000$), grade 1+2 ($p=0.002$) or grade 3+4 ($p=0.009$)). Gestational age <28 weeks was a significant causal factor for all grades of IVH ($p=0.003$) and IVH grade 3+4 combined ($p=0.000$).

Other major hemorrhages

In the liberal-transfusion unit 2 infants had a pulmonary hemorrhage versus 1 in the restrictive-transfusion unit. The two infants in the liberal-transfusion unit received one or more platelet transfusions. The pulmonary hemorrhage in the infant in the restrictive-transfusion unit ceased after instituting mechanical ventilation with PEEP and endotracheal xylomethazoline. In the liberal-transfusion unit 5 infants were born with extensive bruising; an expectative approach was used in these infants. In the restrictive- transfusion unit 3 infants received one or more platelet transfusions for a major hemorrhage other than IVH (gastro-intestinal ($n=1$), adrenal ($n=1$), at site of surgery after abdominal surgery ($n=1$)).

Discussion

This study shows that the overall incidence and severity of IVH in the NICU unit using a liberal platelet transfusion guideline was similar to the unit using a restrictive transfusion guideline. Although the number and rate (per infant) of platelet transfusions was significantly higher in

the liberal-transfusion unit, platelet transfusions did not reduce the incidence or grades of IVH in premature infants with thrombocytopenia.

The etiology of IVH is multi-factorial and related to several other factors besides a low platelet count, including gestational age, birth weight, sepsis, NEC, acidosis, patent ductus arteriosus and the fragility of the vessel wall in (extremely) premature infants.¹² In our study gestational age was the only confounding factor. Thrombocytopenia is a frequent finding in premature infants and may be due to impaired platelet production by intra-uterine factors such as placental insufficiency or postnatal factors increasing platelet consumption such as sepsis.^{3,4,13} Several studies in premature infants have shown that the rate of IVH is increased in thrombocytopenic infants.^{6,14,15} These data contrast with other studies, which have shown that the risk for IVH in thrombocytopenic premature infants is not related to the severity of thrombocytopenia.^{2,7} Our study also shows no correlation between the severity of thrombocytopenia and IVH. The causal relationship between (severe) thrombocytopenia and IVH remains thus unclear.^{16,17}

Whether restrictive platelet transfusion guidelines based on lower thresholds and/or on clinical bleeding signs are safe in premature infants is not known and international consensus is lacking. In 2008 an international forum published an article on transfusion policies and practices in various countries. Platelet transfusion thresholds varied between 20 and 100 x 10⁹/L depending on disease severity and birth weight or gestational age of the infant.¹⁸ In the previously mentioned RCT by Andrew et al. no benefit was found in terms of IVH reduction by maintaining platelet counts >150 x 10⁹/L with platelet transfusions in infants with moderate thrombocytopenia (50 to 150 x 10⁹/L).⁹ However, in this RCT, all infants with platelet counts <50 x 10⁹/L received prophylactic platelet transfusions and were excluded from analysis. The risk of IVH in premature infants with platelet counts <50 x 10⁹/L remains unclear. Despite the lack of high-quality evidence, it is intuitive to think that prophylactic platelet transfusion may prevent IVH. However, recent studies provide evidence that platelet transfusion may have potential harmful effects and even increase mortality rates.^{7,19,20} In our study there was no difference in mortality rate, NEC or septicemia between the children treated with a liberal or a restrictive transfusion regimen. Moreover there was no difference in mortality rate in infants with and without a platelet transfusion in this study (data not shown). The possible causes of the detrimental effect of platelet transfusions in premature infants are not known and may be related to activation of inflammatory and coagulative necrosis, particularly in patients with sepsis or NEC.³

The data in our study should be interpreted with caution since bias may have been introduced by the retrospective nature of the study. Despite the large cohorts of comparable infants in both units, the incidence and grading of IVH may be center-dependent due to possible

differences in interpretation of cranial ultrasound scans. A study design using an independent reviewer evaluating all ultrasound scans would have been preferable.

Due to the multi-factorial cause of IVH more research needs to be performed to determine which factors are independently related to the risk of bleeding. Our study shows that a restrictive platelet transfusion guideline is not associated with a higher incidence of IVH or other major hemorrhage. Therefore randomized controlled trials are warranted and urgently needed to determine the safety of restrictive platelet transfusions guidelines in premature infants.

Conclusions

Liberal-transfusion guidelines in premature infants suffering from thrombocytopenia result in more platelet transfusions but do not reduce the incidence and rate of IVH compared to restrictive-transfusion guidelines.

Acknowledgements

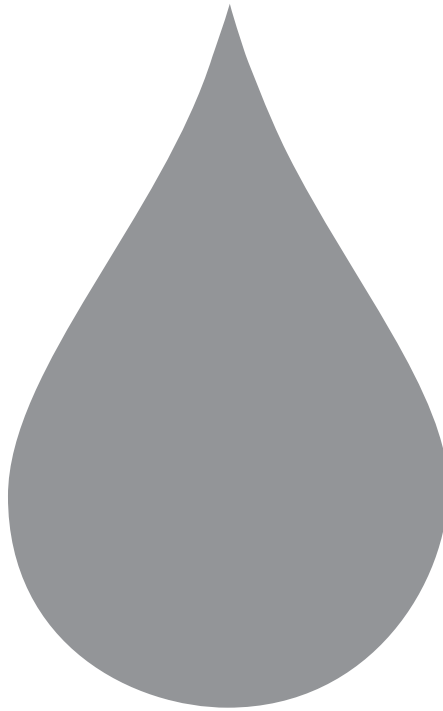
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Chapter 10

Trombocyten transfusie bij neonaten: huidige praktijk en gereviseerde landelijke richtlijn



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Submitted

Samenvatting

Doel 1. Inventariseren van de huidige protocollen met betrekking tot trombocyten transfusies bij neonaten in de 10 Nederlandse neonatale intensive care units (NICUs) en deze vergelijken met de CBO (Centraal Begeleidings Orgaan/Kwaliteitsinstituut voor de gezondheidszorg) richtlijn bloedtransfusie 2004.

2. Samenvatten van de nieuwe gereviseerde richtlijn 2011 en vergelijken met de internationale literatuur.

Opzet Inventariserend.

Methoden De lokale trombocyten transfusie protocollen van de 10 Nederlandse NICUs werden opgevraagd en vergeleken met de huidige CBO richtlijn (2004). Internationale richtlijnen en de literatuur werden geanalyseerd en vergeleken met de nieuwe gereviseerde CBO richtlijn (2011).

Resultaten Alle NICUs hebben een trombocyten transfusie protocol. Slechts 1 NICU volgt volledig de CBO richtlijn 2004. De transfusie protocollen houden allen rekening met de mate van ziek zijn, zwangerschapsduur bij geboorte en/of geboorte gewicht. Er worden 3 verschillende transfusieproducten gebruikt. Het aantal trombocyten per transfusie varieert van 5 tot $20 \times 10^9/\text{kg}$. De transfusiegrenzen in de internationale richtlijnen lopen eveneens wijd uiteen.

Conclusie Er werd zelden volledig aan de CBO richtlijn 2004 voldaan. Aandacht voor de nieuwe richtlijn kan meer uniformiteit in de transfusie protocollen geven.

Summary

The current platelet transfusion guidelines of the ten Dutch tertiary care neonatal intensive care units (NICUs) were studied and compared to the national consensus guideline blood-transfusion 2004 (Centraal Begeleidings Orgaan/Kwaliteitsinstituut voor de gezondheidszorg (CBO)). A revised CBO guideline blood transfusion 2011 will be published this year. This CBO guideline 2011 has been summarized and compared with the international literature.

All 10 NICUs have their own platelet transfusion protocol. Only 1 NICU adheres completely to the national consensus 2004. All protocols take gestational age, birth weight and disease severity into account in defining the transfusion threshold, but these differ nationwide. In the Netherlands 3 different platelet transfusion products are used. The number of transfused platelets varies between 5 and 20 x 10⁹/kg.

Platelet transfusion thresholds in the international guidelines also vary widely.

In conclusion, as in the international literature, platelet transfusion guidelines differ between the different NICUs. The national CBO consensus 2004 was almost never followed completely. Awareness of the new CBO transfusion guideline 2011 will hopefully give more uniformity in the platelet transfusion protocols.

In Nederland worden jaarlijks ongeveer 180.000 kinderen geboren. Studies hebben aangetoond dat vanaf een zwangerschapsduur van 22 weken het trombocytenaantal bij de foetus meer dan $150 \times 10^9/l$ is.^{1,2} Trombocytopenie bij neonaten wordt dan ook gedefinieerd als een trombocytenaantal $<150 \times 10^9/l$. Eén tot 5% van alle pasgeborenen heeft een trombocytopenie.²⁻⁴ Bij pasgeborenen die op een neonatale intensive care unit (NICU) verblijven loopt dit op tot 30%.^{1,2,4-6} De belangrijkste oorzaken van trombocytopenie bij de pasgeborenen zijn sepsis, dysmaturiteit, verbruik door bloeding, prematuriteit en asfyxie.

Een te laag trombocytenaantal hoeft niet altijd met een transfusie te worden behandeld. Bij neonaten met een te laag aantal bloedplaatjes zijn er twee indicaties voor behandeling met trombocyten transfusies: 1. Wanneer er sprake is van een (acute) bloeding en 2. Wanneer er een risico bestaat op een ernstige bloeding. Het risico op een (ernstige) bloeding dient afgewogen te worden tegen het risico van een trombocyten transfusie, zoals overdracht van infecties of transfusiereacties. Vaak is er spontane normalisering van het trombocytenaantal of wordt deze bereikt na behandeling van de onderliggende oorzaak van de trombocytopenie. In de internationale literatuur is geen consensus over de transfusie grenzen, het product of het aantal te transfunderen trombocyten.

Recent is vanuit het Centraal Begeleiding Orgaan/Kwaliteitsinstituut voor de gezondheidszorg (CBO) de landelijke richtlijn bloedtransfusies uit 2004 door een werkgroep herzien. Dit artikel beoogt, naast het weergeven van de huidige transfusie praktijk in de Nederlandse NICUs, een breed draagvlak te creëren voor de nieuwe CBO richtlijn, en hiermee een uniform beleid in Nederland.

Data en Methoden

In 2008 zijn de 10 neonatale intensive care units (NICUs) van Nederland gevraagd hun protocol op te sturen, en als deze er niet was een vragenlijst in te vullen over het gebruik van trombocyten (bijlage 1). Deze werden met elkaar, met de CBO richtlijn 2004⁷ en de nieuwe richtlijn 2011 (in press) vergeleken. Op Pubmed en internet werd gezocht naar internationale richtlijnen met betrekking tot trombocyten transfusies.

Resultaten

Van alle NICUs werden de protocollen ontvangen. Nagenoeg elk centrum heeft een eigen protocol, deels aansluitend bij de landelijke richtlijn, deels op basis van de literatuur en deels op de lokale gewoonten. Slechts twee klinieken geven aan de CBO richtlijn te volgen. Eén hiervan voldoet ook aan de bestralings adviezen van de richtlijn. Een tweetal klinieken

Tabel 1. Landelijke gebruiken en CBO richtlijn 2004

Unit	Indicatie en grenzen	Bestralingsindicaties (Na) IUT	Product en dosering trombocyten Hyperconcentraat van 1 donor in PAS-II 60% plasma 40% 20 x 10 ⁹ /kg
1	-20 <1500 gram en stabiel, >1500 gram ziek of niet ziek -50 <1500 gram en ziek, alle neonaten met manifeste bloeding of noodzaak tot ingreep/lumbaal punctie, na wisseltransfusie -100 bij aanvang wisseltransfusie, dan halverwege trombocyten geven -Trombocytopenie met evidente bloedingsneiging -Massaal bloedverlies -Trombopathie met actieve bloeding of geplande operatie -20 profylaxe stabiele neonaat of ziek en >1500 gram -50 zieke neonaat <1500gr -50 actieve bloeding of operatie, na wisseltransfusie -100 voortgaand aan wisseltransfusie -manifeste bloeding bij familiair bekende trombopathie -10 in de eerste levensweek -30 bij sepsis, DIS, NEC, verse ICB > graad II, bloedige ingrepen -50 manifeste bloeding -100 bij start wisseltransfusie -Manifeste bloeding in combinatie met trombopenie/-pathie -20 overwegen als profylaxe -50 bij herhaalde wisseltransfusie -20 altijd -50 en slijmvlies bloeding, massale petechiën en/of echymosen, of <1000g én <1 week -100, manifeste bloeding, ICB, of noodzaak tot operatie, wisseltransfusie -20, zonder bloedingsneiging -40 en bloedingsneiging -40 wisseltransfusie -80 ECMO Conform CBO 2004	(Na) IUT <32 weken <1500 gram HIA identiek <1000 gram	Hyperconcentraat van 1 donor in PAS-II 60% plasma 40% 20 x 10 ⁹ /kg
2	-100 bij aanvang wisseltransfusie, dan halverwege trombocyten geven -Trombocytopenie met evidente bloedingsneiging -Massaal bloedverlies -Trombopathie met actieve bloeding of geplande operatie -20 profylaxe stabiele neonaat of ziek en >1500 gram -50 zieke neonaat <1500gr -50 actieve bloeding of operatie, na wisseltransfusie -100 voortgaand aan wisseltransfusie -manifeste bloeding bij familiair bekende trombopathie -10 in de eerste levensweek -30 bij sepsis, DIS, NEC, verse ICB > graad II, bloedige ingrepen -50 manifeste bloeding -100 bij start wisseltransfusie -Manifeste bloeding in combinatie met trombopenie/-pathie -20 overwegen als profylaxe -50 bij herhaalde wisseltransfusie -20 altijd -50 en slijmvlies bloeding, massale petechiën en/of echymosen, of <1000g én <1 week -100, manifeste bloeding, ICB, of noodzaak tot operatie, wisseltransfusie -20, zonder bloedingsneiging -40 en bloedingsneiging -40 wisseltransfusie -80 ECMO Conform CBO 2004	<1500 gram	Plaatjesrijk plasma concentraat (50 x 10 ⁹ in 50 ml) van 1 donor 10 ml/kg
3	-100 bij start wisseltransfusie -Manifeste bloeding in combinatie met trombopenie/-pathie -20 overwegen als profylaxe -50 bij herhaalde wisseltransfusie -20 altijd -50 en slijmvlies bloeding, massale petechiën en/of echymosen, of <1000g én <1 week -100, manifeste bloeding, ICB, of noodzaak tot operatie, wisseltransfusie -20, zonder bloedingsneiging -40 en bloedingsneiging -40 wisseltransfusie -80 ECMO Conform CBO 2004	nee	Plaatjesrijk plasma concentraat 50ml van 5 donoren (speciale reden 1 donor) 10–15 ml/kg
4	-100 bij start wisseltransfusie -Manifeste bloeding in combinatie met trombopenie/-pathie -20 overwegen als profylaxe -50 bij herhaalde wisseltransfusie -20 altijd -50 en slijmvlies bloeding, massale petechiën en/of echymosen, of <1000g én <1 week -100, manifeste bloeding, ICB, of noodzaak tot operatie, wisseltransfusie -20, zonder bloedingsneiging -40 en bloedingsneiging -40 wisseltransfusie -80 ECMO Conform CBO 2004	Na IUT cellulaire immuundeficiëntie familie donor <1500gram <32 weken <1500 gram immuundeficiëntie	Plaatjesrijk plasma concentraat van 1 donor (50 x 10 ⁹ trombocyten in 40–70 ml) 10 ml/kg
5	-100 bij start wisseltransfusie -Manifeste bloeding in combinatie met trombopenie/-pathie -20 overwegen als profylaxe -50 bij herhaalde wisseltransfusie -20 altijd -50 en slijmvlies bloeding, massale petechiën en/of echymosen, of <1000g én <1 week -100, manifeste bloeding, ICB, of noodzaak tot operatie, wisseltransfusie -20, zonder bloedingsneiging -40 en bloedingsneiging -40 wisseltransfusie -80 ECMO Conform CBO 2004	Plaatjesrijk plasma concentraat van 1 donor (50 x 10 ⁹ trombocyten in 40–70 ml) 10 ml/kg	Plaatjesrijk plasma concentraat van 1 donor (50 x 10 ⁹ trombocyten in 40–70 ml) 10 ml/kg
6	-100 bij start wisseltransfusie -Manifeste bloeding in combinatie met trombopenie/-pathie -20 overwegen als profylaxe -50 bij herhaalde wisseltransfusie -20 altijd -50 en slijmvlies bloeding, massale petechiën en/of echymosen, of <1000g én <1 week -100, manifeste bloeding, ICB, of noodzaak tot operatie, wisseltransfusie -20, zonder bloedingsneiging -40 en bloedingsneiging -40 wisseltransfusie -80 ECMO Conform CBO 2004	Plaatjesrijk plasma concentraat van 1 donor (50 x 10 ⁹ trombocyten in 40–70 ml) 10 ml/kg	Plaatjesrijk plasma concentraat van 1 donor (50 x 10 ⁹ trombocyten in 40–70 ml) 10 ml/kg
7	-100 bij start wisseltransfusie -Manifeste bloeding in combinatie met trombopenie/-pathie -20 overwegen als profylaxe -50 bij herhaalde wisseltransfusie -20 altijd -50 en slijmvlies bloeding, massale petechiën en/of echymosen, of <1000g én <1 week -100, manifeste bloeding, ICB, of noodzaak tot operatie, wisseltransfusie -20, zonder bloedingsneiging -40 en bloedingsneiging -40 wisseltransfusie -80 ECMO Conform CBO 2004	Plaatjesrijk plasma concentraat van 1 donor (50 x 10 ⁹ trombocyten in 40–70 ml) 10 ml/kg	Plaatjesrijk plasma concentraat van 1 donor (50 x 10 ⁹ trombocyten in 40–70 ml) 10 ml/kg
8	-100 bij start wisseltransfusie -Manifeste bloeding in combinatie met trombopenie/-pathie -20 overwegen als profylaxe -50 bij herhaalde wisseltransfusie -20 altijd -50 en slijmvlies bloeding, massale petechiën en/of echymosen, of <1000g én <1 week -100, manifeste bloeding, ICB, of noodzaak tot operatie, wisseltransfusie -20, zonder bloedingsneiging -40 en bloedingsneiging -40 wisseltransfusie -80 ECMO Conform CBO 2004	Plaatjesrijk plasma concentraat van 1 donor (50 x 10 ⁹ trombocyten in 40–70 ml) 10 ml/kg	Plaatjesrijk plasma concentraat van 1 donor (50 x 10 ⁹ trombocyten in 40–70 ml) 10 ml/kg
9	-100 bij start wisseltransfusie -Manifeste bloeding in combinatie met trombopenie/-pathie -20 overwegen als profylaxe -50 bij herhaalde wisseltransfusie -20 altijd -50 en slijmvlies bloeding, massale petechiën en/of echymosen, of <1000g én <1 week -100, manifeste bloeding, ICB, of noodzaak tot operatie, wisseltransfusie -20, zonder bloedingsneiging -40 en bloedingsneiging -40 wisseltransfusie -80 ECMO Conform CBO 2004	Plaatjesrijk plasma concentraat van 1 donor (50 x 10 ⁹ trombocyten in 40–70 ml) 10 ml/kg	Plaatjesrijk plasma concentraat van 1 donor (50 x 10 ⁹ trombocyten in 40–70 ml) 10 ml/kg
10	-100 bij start wisseltransfusie -Manifeste bloeding in combinatie met trombopenie/-pathie -20 overwegen als profylaxe -50 bij herhaalde wisseltransfusie -20 altijd -50 en slijmvlies bloeding, massale petechiën en/of echymosen, of <1000g én <1 week -100, manifeste bloeding, ICB, of noodzaak tot operatie, wisseltransfusie -20, zonder bloedingsneiging -40 en bloedingsneiging -40 wisseltransfusie -80 ECMO Conform CBO 2004	Plaatjesrijk plasma concentraat van 1 donor (50 x 10 ⁹ trombocyten in 40–70 ml) 10 ml/kg	Plaatjesrijk plasma concentraat van 1 donor (50 x 10 ⁹ trombocyten in 40–70 ml) 10 ml/kg
CBO 2004	-20 <1500 gram en stabiel >1500 gram ziek/niet ziek -50 <1500 gram en ziek, manifeste bloeding, ingreep, na wisseltransfusie -80 ECMO ongecompliceerd -100 ECMO en bloeding/ingreep, bij start wisseltransfusie	PAS-II 70% / plasma 30% 10 x 10 ⁹ /kg Alerse concentraat van 1 donor Na IUT HUA-getypeerd immuundeficiëntie	PAS-II 70% / plasma 30% 10 x 10 ⁹ /kg Alerse concentraat van 1 donor 10 x 10 ⁹ /kg

NAITP=neonatale alloimmun trombocytopenie; IUT=intrauteriene transfusie; ICB=intracranieële bloeding; ECMO=extracorporele membraan oxygenatie

hebben literatuur referenties bij het protocol staan. Van Sanquin, de nationale producent van bloedproducten, ontvingen we de gegevens van de 4 Nederlandse Sanquin vestigingen met betrekking tot de specificaties van de trombocyten producten die voor de neonatologie afdelingen worden geleverd. De resultaten zijn weergegeven in tabel 1.

Transfusiegrenzen

De grens die in de verschillende Nederlandse NICUs voor bloedplaatjes transfusie wordt aangehouden varieert van 10 tot $100 \times 10^9/l$ trombocyten en is afhankelijk van meerdere factoren, waarbij bij prematuriteit of ernstig ziek zijn een hogere drempel wordt aangehouden. De CBO richtlijn 2004 en 2011 houden afhankelijk van de klinische situatie 20 tot $100 \times 10^9/l$ aan.

In de internationale richtlijnen worden eveneens wisselende transfusie drempels genoemd waarbij $20 \times 10^9/l$ trombocyten eigenlijk overal als absolute ondergrens wordt gehouden voor pasgeborenen. Afhankelijk van de mate van ziek zijn, zwangerschapsduur bij geboorte en geboorte gewicht wordt een hogere grens aangehouden (tabel 2).

Transfusie dosering

De gebruikte transfusie hoeveelheid wisselt. Twee NICUs geven $10 \times 10^9/kg$ trombocyten en één $20 \times 10^9/kg$. Eén NICU geeft 0.1–0.2 van een volwassen eenheid/kg, bij voorkeur van 1 donor. Drie klinieken geven 10 ml/kg en 3 klinieken geven 10–15ml/kg trombocyten van een pediatrische eenheid. Volgens Sanquin wordt er altijd een aferese product van 1 donor geleverd. Het aferese product wat geleverd wordt voor pediatrische doeleinden is een deel hiervan in een zakje van 40–70 ml met minimaal 50×10^9 trombocyten.⁷ Dit betekent dat de dosering trombocyten wisselt van 5 tot $20 \times 10^9/kg$. De CBO richtlijnen 2004 en 2011 adviseren 10×10^9 trombocyten/kg lichaamsgewicht.

In een Canadese studie van Andrew wordt gesproken van 10 ml/kg van één donor.⁸ Het aantal trombocyten wordt niet genoemd. De Britse richtlijn spreekt over 10–20 ml/kg van een volwassen (1 donor aferese) trombocyten transfusie.⁹ Het volwassen product moet $>240 \times 10^9$ trombocyten bevatten (voor intra-uteriene transfusie zelfs $>120 \times 10^9$ in 60 ml plasma).¹⁰ Calhoun adviseert een dosering van 10–15 $\times 10^9/kg$ CMV-negatieve bloedplaatjes.¹¹

Product specificaties

Eén Sanquin vestiging levert aan 1 NICU trombocyten hyperconcentraat in 60% PAS-II/40% plasma, aan de andere NICU wordt plaatjesconcentraat in 70% PAS-II/30% plasma geleverd. De overige 8 NICUs krijgen plaatjes-rijk plasma.

Tabel 2. Internationale trombocyten transfusie grenzen

	Transfusie drempel	Transfusie indicatie
Calhoun, Florida ¹¹	<25	Altijd
	<50	Chirurgie of binnen 5 dagen na chirurgie, cardiovasculaire instabiliteit, respiratoire instabiliteit, <72 uur na convulsie, DIS, <1500 gram en <7 dagen
Strauss, Iowa, 2008 ³⁴	<20	Altijd
	<50	Invasieve procedures
	<50–100	Klinisch niet stabiel, significante bloeding
NHS, Groot Britannie, 2007 ³⁹	<30	Altijd
	< 50	Gewicht <1000 gram en <1 week, stollingsstoornis, eerdere grote bloeding, actuele kleine bloeding (petechiën, siepelen uit prik gaatjes), geplande operatie of wisseltransfusie, dalend trombocyten getal naar waarschijnlijk <30, NAITP indien eerder kind met ICB
Murray, Groot Britannie, 2003 ⁴⁰	<30	Altijd
	30–49	Overweeg indien <1000 gram en <7 dagen, actieve bloeding, klinisch niet-stabiel, eerdere ernstige bloeding (IVH 3-4 of long bloeding), actuele kleine bloeding (petechiën, siepelen uit prik gaatjes, bloederige endotracheale secreties), stollingsstoornis, noodzaak voor chirurgie of wisseltransfusie
	50–99	Bij bloeding
	<20–30	Altijd
Melbourne, 2008 ⁴¹	<30–50	Geen verschil tussen a terme of prematuur
	<50	Kleine bloeding, stollingsstoornis. Overweeg bij chirurgische ingreep of wisseltransfusie
	<100	Grote orgaan bloeding, ECMO
American Red Cross, 2010 ⁴²	<20	Stabiel a terme
	<30	Stabiele prematuur, hoog risico neonaten a terme (perinatale asfyxie, sepsis, beademing met >40% O ₂ , klinisch instabiel)
	<50	Hoog risico neonaten prematuur (extreem laag geboorte gewicht, perinatale asfyxie, sepsis, beademing met >40% O ₂ , klinisch instabiel)
		Invasieve ingreep of operatie, klinisch significante bloeding
	<100	ECMO

Getallen zijn aantal trombocyten x 10⁹/l, tenzij anders benoemd

DIS = diffuse intravasale stolling; NAITP = neonatale allo immuun trombocytopenie; IVH = intraventriculaire bloeding; ICB = intracraniele bloeding; ECMO = extracorporele membraan oxygenatie

Sinds 1985 worden in Nederland alle trombocyten producten leukocyten gedepleteerd door filtratie en bevatten <10⁶ leukocyten per volwassen donor eenheid. Leukodepletie wordt gezien als een methode om intracellulaire virussen, zoals cytomegalovirus (CMV), te minimaliseren en zo CMV veilige producten te produceren.¹²⁻¹⁵ De CBO richtlijn 2004 en 2011 adviseert serologisch CMV-negatieve trombocyten alleen voor intra-uteriene transfusies (die plaats vinden in het Leids Universitair Medisch Centrum). Voor alle andere indicaties wordt leukodepletie als afdoende gezien. In sommige westerse landen worden echter ook aan extreem prematuur geboren kinderen (<32 weken zwangerschapsduur) serologisch CMV-negatieve producten gegeven omdat zij als immuun gecompromitteerd worden beschouwd.

Voor zover te vinden in de literatuur, is Nederland het enige land waar donoren gescreend worden op Parvovirus B19. Het Parvovirus B19, indien intra-uterien opgelopen, kan ernstige anemie en hydrops foetalis tot gevolg hebben.¹⁶ Post nataal opgelopen Parvovirus B19 infectie kan langdurige beenmerg depressie met aplasie geven. Om deze reden zijn de indicaties voor het geven van Parvo veilige producten (tabel 3) ten opzichte van 2004 uitgebreid naar aanleiding van het advies van de Gezondheidsraad.¹⁷

Bestralingscriteria

Bestraling van bloedproducten dient ter voorkoming van graft versus host ziekte bij immuun gecompromitteerde patiënten. Geen enkele NICU geeft op zich aan alle CBO bestralingscriteria te (kunnen) houden (tabel 1). De bestralingsindicaties in de nieuwste CBO richtlijn 2011 zijn uitgebreid ten opzichte van 2004 met alle prematuren geboren <32 weken tot 6 maanden na de a terme datum omdat deze kinderen als immuun gecompromitteerd worden beschouwd. Ook producten van familieleden dienen te worden bestraald. Bij (vermoeden op) een immuundeficiëntie zijn bestraalde producten eveneens geïndiceerd (tabel 3). Internationaal worden verschillende criteria aangehouden. Alle richtlijnen adviseren bloedproducten voor intra-uteriene transfusies te bestralen. De post natale indicaties wisselen sterk.

(Foetale) Neonatale alloimmuun trombocytopenie ((F)NAITP)

Hierbij is sprake van maternale allo-antistoffen tegen een trombocyten antigeen bij de foetus. De, door afbraak van de foetale bloedplaatjes, ontstane trombocytopenie vormt een risico op (intracraniele) bloedingen, waarvan tot 80% van deze hersenbloedingen in utero optreden.¹⁸⁻²⁰ Het gaat in de meeste gevallen om antilichamen tegen het humane plaatjes antigeen (HPA)-1a of HPA-5b.²¹ Er zijn geen gerandomiseerde studies verricht bij neonaten naar de optimale behandelstrategie. Experts suggereren dat postnatale transfusie van HPA-1a en HPA-5b negatieve trombocyten in het grootste deel van de gevallen het meest effectief is.²² Bij onvoldoende effect kunnen gewassen trombocyten van moeder uitkomst bieden. Bij spoed is er geen contra-indicatie voor het gebruik van trombocyten transfusies van random donoren, al zal dit een korter durende verhoging van het trombocytenaantal geven.²³ Een aantal studies hebben aangetoond dat postnataal intraveneuze immuunglobuline (IVIG) toediening eveneens effectief kan zijn, maar stijging van het trombocytenaantal is trager dan met trombocyten transfusies.^{24,25} Een studie door te Pas e.a. laat echter geen effect van IVIG zien.²² Het nieuwe CBO advies is daarom om neonaten met NAITP en een bloeding(sneiging) primair te behandelen met compatibele trombocyten transfusies, en IVIG alleen te gebruiken bij persisterende trombocytopenie (ondanks ≥ 2 compatibele transfusies).

NAITP studies laten zien dat antenatale toediening van hoge doses IVIG aan zwangere vrouwen met een kind met FNAITP leidt tot stijging van het trombocytenaantal en een vermindering van het aantal kinderen met een intracranieële bloeding. Intra-uteriene trombocyten transfusies, met de risico's hiervan, lijken geen meerwaarde te hebben boven IVIG aan de zwangere vrouw.²⁶⁻²⁸

Beschouwing

De verschillende NICUs houden verschillende transfusiegrenzen aan, deels gebaseerd op de literatuur, deels aan de hand van de CBO richtlijn en deels historisch gegroeid. Ook in de internationale literatuur is er geen consensus (tabel 2).

Er zijn nauwelijks gerandomiseerde studies verricht naar een veilige ondergrens voor het trombocytenaantal. Er is één gerandomiseerde studie verricht bij premature neonaten naar het effect van profylactische trombocyten transfusies op de incidentie van intracranieële bloedingen.⁸ In deze studie van Andrew e.a. uit 1993 werd een trombocyten transfusie grens van $50 \times 10^9/l$ (restrictief beleid) vergeleken met $150 \times 10^9/l$ (liberaal beleid). Er was geen significant verschil tussen beide groepen met betrekking tot het ontstaan van een intracranieële bloeding of uitbreiding van een reeds bestaande intracranieële bloeding. In verschillende retrospectieve studies wordt geen statistisch significant verschil in mortaliteit of ernstige bloeding gezien bij kinderen met een trombocytenaantal $<50 \times 10^9/l$ ten op zichten van $>50 \times 10^9/l$.^{29,30} Een prospectieve observationele studie door Stanworth en collega's laat zien dat 91% van de neonaten met een trombocytenaantal $<20 \times 10^9/l$ geen ernstige bloeding ontwikkelde.³¹ Dit roept de vraag op of een profylactische transfusiedrempel van $20 \times 10^9/l$ (of hoger) voor de meeste kinderen dan ook niet te hoog is. Bij volwassenen bestaan overigens vergelijkbare dilemma's omtrent de adequate transfusiedrempel.³² Volwassen studies zijn niet zonder meer naar pasgeborenen te vertalen maar kunnen wel richting geven aan verdere studies bij neonaten.

Er zijn geen studies gedaan bij pasgeborenen waar het effect van verschillende hoeveelheden getransfundeerde bloedplaatjes wordt bekeken. De meeste studies noemen het aantal milliliters getransfundeerd product, soms met een schatting van het aantal bloedplaatjes. Waar de dosis wel genoemd is variëren de transfusie adviezen van 10 tot $20 \times 10^9/kg$.^{33,34} Een grote studie bij volwassen hemato-oncologische patiënten (PLADO studie) met ernstige trombocytopenie ($<10 \times 10^9/l$), vergeleek een lage, versus standaard, versus hoge dosering trombocyten per profylactische transfusie en liet geen verschil zien in de incidentie van bloedingen.³⁵ Een kleinere studie (SToP) bij volwassenen met een soortgelijke opzet is echter

voortijdig afgebroken in verband met een verschil van >5% in ernstige bloedingen ten nadele van de laag gedoseerde groep.³⁶

Met betrekking tot het soort transfusie product is er geen consensus in de internationale literatuur. De meeste protocollen zijn eminence based. Het voordeel van PAS-II (Platelet Additive Solution type II) als bewaarvloeistof ten opzichte van plasma is dat er minder plasma gegeven wordt met eventuele anti-A en/of B antistoffen. Echter de opbrengst van de transfusie is mogelijk iets lager dan met plasma als bewaarvloeistof.^{37,38} In de praktijk is de opbrengst voldoende met beide soorten bewaarvloeistof. Momenteel loopt er een retrospectief onderzoek door Sanquin naar de opbrengst van de drie verschillende trombocyten producten (70% PAS-II/30% plasma, hyperconcentraat in 60% PAS-II/40% plasma en plaatjes-rijk plasma) die bij neonaten worden gebruikt.

Verder onderzoek naar trombocyten transfusiebeleid (met name bij prematuren) is noodzakelijk om een veilige lage transfusie drempel vast te stellen.

Nieuwe richtlijn

Er zijn slechts geringe verschillen voor trombocyten transfusies bij pasgeborenen tussen de CBO richtlijn Bloedtransfusie 2004 en 2011. De gereviseerde richtlijn (2011) is samengevat in tabel 3.

De werkgroep heeft, samengevat, het volgende trombocyten transfusie advies opgesteld:

1. Bij stabiele neonaten, ongeacht het geboorte gewicht of bij zieke pasgeborenen ≥ 1500 gram of ≥ 32 weken bij $< 20 \times 10^9/l$ trombocyten.
2. Bij manifeste bloeding of chirurgische ingreep of bij zieke pasgeborenen < 1500 gram en < 32 weken bij een trombocytenaantal $< 50 \times 10^9/l$.
3. Bij pasgeborenen aan de ECMO indien $< 100 \times 10^9/l$ trombocyten.
4. Halverwege een wisseltransfusie indien het trombocytenaantal voor aanvang van de wissel $< 100 \times 10^9/l$ is en indien na de wisseltransfusie het trombocytenaantal $< 50 \times 10^9/l$ is.
5. Bij een trombocyten transfusie aan pasgeborenen wordt een dosis gegeven van minimaal 10×10^9 trombocyten/kg lichaamsgewicht.
6. De voorkeur gaat uit naar een aferese product afkomstig van één donor.
7. Voor prematuren < 32 weken en/of < 1500 gram dienen Parvovirus B19 veilige en bestraalde bloedproducten te worden gegeven.
8. Bloedproducten voor intra-uteriene transfusies dienen Parvovirus B19 veilig, en afkomstig van een CMV seronegatieve donor te zijn en te worden bestraald.
9. Na een eerdere bestralingsindicatie worden de bloedproducten tot 6 maanden na de a terme datum bestraald.

Tabel 3. Samenvatting CBO richtlijn 2011

Trombocyten transfusie grens	Transfusie indicatie
<20 x 10 ⁹ /l	- altijd
<50 x 10 ⁹ /l	- <1500 gram en <32 weken, ziek - manifeste bloeding - ingreep - na wisseltransfusie
<100 x 10 ⁹ /l	- indien bij aanvang wisseltransfusie, dan halverwege transfusie geven - extra corporele membraan oxygenatie

Indicaties bestraling

- Prematuren <32 weken en/of <1500 gram tot 6 maanden na a terme
- Intra-uteriene transfusies, hierna tot 6 maanden na de a terme datum
- Kinderen met een aangeboren gecombineerde immuun deficiëntie (SCID)
- Transfusie van eerste tot en met 3e graad verwanten
- Wisseltransfusie bij kinderen <32 weken en/of <1500 gram

Indicaties Parvovirus B 19 veilig

- Intra uteriene transfusie (IUT)
- Na IUT tot 6 maanden na de a terme datum
- Alle prematuren <32 weken en/of <1500 gram
- Aangeboren of verworven hemolytische anemie zonder Parvovirus B19 antistoffen
- Cellulaire immuundeficiëntie zonder Parvovirus B 19 antistoffen

Altijd leukocyten gedepleteerd, bij voorkeur aferese concentraat van 1 donor

CMV-vrij alleen voor intra-uteriene transfusies

Transfundeer 10 x 10⁹/kg trombocyten

Bijlage 1 Vragenlijst NICUs

Trombocyten transfusies in de neonatologie

1. Wat is de indicatie voor het geven van een trombocyten transfusie bij prematuren?
(Welke transfusie grenzen houden jullie aan?) Indien het afhangt van klinische parameters, graag specificeren.
2. Wat is de indicatie voor het geven van een trombocyten transfusie bij a terme neonaten?
(Welke transfusie grenzen houden jullie aan?) Indien het afhangt van klinische parameters, graag specificeren.
3. Worden er standaard trombocyten gegeven bij een (partiële) wissel transfusie? Zo nee, welke grenzen worden er gehanteerd?
4. Hoeveel trombocyten worden per transfusie gegeven?
5. Zijn de gegeven trombocyten per transfusie van een of meerdere donoren?
6. Worden de trombocyten als concentraat gegeven of in oplossing?
7. Welk oplosmiddel/bewaarvloeistof wordt gebruikt?
8. Worden trombocyten bestraald? Zo ja voor welke kinderen/indicaties?
9. Zijn de trombocyten producten leukogedepleteerd?
10. Wat is de maximale bewaartijd van de trombocyten na oogsten en voor transfusie?
11. Gebruikt u trombopoietine? Zo ja, wat is de indicatie?
12. Op welk protocol of artikel is het trombocyten transfusiebeleid op uw afdeling gebaseerd?

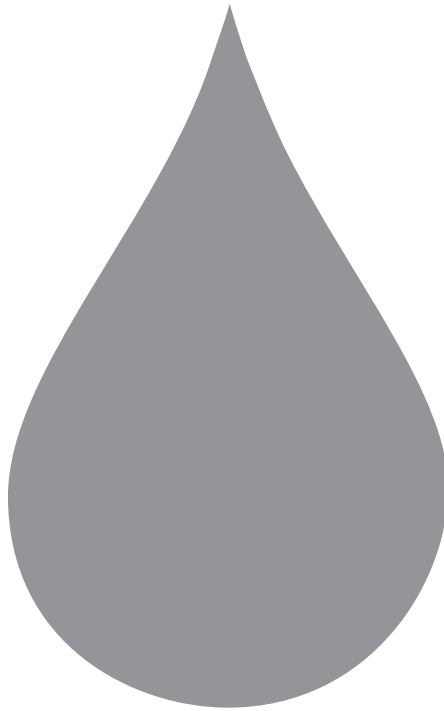
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Chapter 11

Discussion and future perspectives



Anemia and thrombocytopenia are common hematologic disorders in (preterm) infants admitted to neonatal intensive care units (NICUs). In the first months of life all newborn infants experience a decrease in hemoglobin (Hb) level due to normal physiologic processes such as body growth, shorter life span of neonatal red blood cells (RBCs) compared to adult RBCs, and relative insufficient erythropoiesis. Very preterm infants, however, also experience iatrogenic blood loss due to routine laboratory investigations increasing the decline in RBCs. For healthy term infants a decrease in Hb to 10 g/dL at 10–12 weeks of age is considered normal. In preterm infants the nadir is reached at a younger age (4–6 weeks after birth) and can be as low as 7 g/dL in infants with a birth weight <1 kg.¹ Nearly all of very preterm infants (<32 weeks gestation) develop anemia and 25–35% may encounter thrombocytopenia (a platelet count <150 × 10⁹/L). Severe thrombocytopenia (a platelet count <50 × 10⁹/L) is seen in approximately 5% of extremely preterm infants.² Blood products, especially RBCs, are therefore frequently used in neonatal medicine. RBC transfusion might well be the single most prescribed ‘drug’ for extremely preterm infants (born before 28 completed weeks’ gestation) as up to 95% of these infants receive at least one RBC transfusion during their postnatal hospital stay. In newborn infants with severe thrombocytopenia (platelet count <50 × 10⁹/L) up to 86% receive one or more platelet transfusions.³ Despite the frequent use of RBC and/or platelet-transfusions, there is no international consensus on the transfusion threshold, the correct dose, the optimal product and no adequate information available on the long-term effects of transfusions.

Red blood cells

Due to lack of large randomized controlled trials (RCT), guidelines for RBC transfusions differ all over the world. The uncertainty of the effects of RBC transfusions, both positive and detrimental, is probably the cause of the wide variety in transfusion guidelines. Iron deficiency and relative hypoxia due to a lower Hb level may damage the developing brain.⁴ Prolonged anemia has a negative effect on growth, although a threshold is not known.⁵ On the other hand, a low Hb level stimulates the production of erythropoietin, a growth factor with neuroprotective properties.^{6,7} RBC transfusion can increase oxidative stress and injure the immature lung, resulting in prolonged oxygen dependency and chronic lung disease.⁸ The increase in free iron in preterm infants after RBC transfusion increases the presence of oxygen radicals which may induce the development of retinopathy of prematurity.⁹ Several recent studies have reported an increase in the incidence of necrotizing enterocolitis (NEC) within 24–48 hours after a RBC transfusion.^{10,11} Krimmel et al. showed that the usual postprandial increase in blood flow to the gut is not present immediately after a RBC transfusion, which may explain the increased

risk for NEC.¹² A low level of nitric-oxide in stored RBCs may reduce their vasodilatory effects and ability to enhance oxygen delivery and increase the risk of relative hypoxia in the gut.¹³ El-Dib showed that withholding feeds during RBC transfusion decreased the previously found higher incidence of NEC significantly from 5.3% to 1.4% ($p=0.047$).¹⁴ Due to the increased risk of NEC after RBC transfusions, especially in preterm infants and in infants receiving larger volumes of (cow's milk based formula) feeds, Christensen also advises to stop enteral feeding prior to and during RBC transfusions.¹³

Another aspect that may be related to blood flow reduction affected by RBC transfusions is the observation of Baer et al., who even showed that RBC transfusions given before the development of an intraventricular hemorrhage (IVH) are an independent risk factor for the development of a severe IVH.¹⁵

The pro's and con's for giving a RBC transfusion and at which moment should therefore be taken into consideration before actually giving the transfusion.

Product and volume

Transfusion products worldwide differ greatly. In the United States most RBC products have a high hematocrit (Hct) up to 0.80 L/L compared to, for example, a Hct of 0.55–0.65 L/L in the Netherlands. Even though the transfusion volume may be equal when comparing studies, the number of erythrocytes given may differ greatly making comparisons difficult, if not impossible. Studies comparing transfusions of similar volumes need therefore take the Hct into account. The volume per transfusion given varies (inter)nationally between 5 and 20 mL/kg with possible effects on the hemodynamics of the infant and as a result a change in cerebral blood flow or pressure which may influence neuromotor and cognitive development.

We performed a two-center study in very preterm infants (<32 weeks gestation) comparing two different transfusion volumes of an identical transfusion product using the same transfusion protocol except for a different volume per transfusion (15mL/kg vs. 20 mL/kg). We found no difference in the number of transfusions given, short-term and long-term outcome in the infants born before 28 weeks' gestation as is shown in chapter 5 and 6.^{16,17} One explanation could be that the difference in transfusion volume per transfusion event is too small to detect a difference in outcome or that the volume per transfusion does not influence outcome. The total transfusion volume, however, was significantly lower in the unit transfusing the lower volume per transfusion event and reduced the number of donors to which the infants were exposed. In preterm infants born between 28 and 31 6/7 weeks' gestation, the lower transfusion volume per event led to an increase in the number of RBC transfusions needed and thus a higher donor exposure if a single-donor program was not used. A study by Paul et al., comparing RBC transfusions of 10 to 20 mL/kg, showed a significantly higher post-transfusion

Hct, but no negative effects on pulmonary function when giving the higher volume.¹⁸ A study by Wong et al. comparing 15 to 20 mL/kg also showed higher post transfusion Hcts, but no impact on the number of RBC transfusions given.¹⁹

The only study focusing on the relation between long-term outcome and transfused RBC volume is our observational follow-up study comparing transfusion volumes of 15 and 20 mL/kg. This study did not show a significant difference in post-discharge mortality, deafness, blindness and neuromotor developmental delay.¹⁷

More research is clearly needed, preferably RCT, focusing on long-term outcome comparing identical transfusion products with different transfusion volumes per transfusion given as well as long-term outcome taking total transfusion volume into account. Our results suggest, in particular with respect to donor exposure, that it may be important to have a different policy in extremely preterm infants (born <28 weeks' gestation) and less preterm infants.

Triggers

In analogy with optimal volume, guidelines on optimal thresholds for RBC transfusion also differ greatly between (and within) countries. Some studies suggest that restrictive transfusion guidelines lead to similar results compared to liberal guidelines and reduce donor exposure and total transfusions. However, which of the two strategies (restrictive versus liberal) is superior remains controversial.

In the Premature Infants in Need of Transfusion (PINT) trial, comparing restrictive to liberal RBC transfusion thresholds, there was no difference in short-term mortality or major disability (retinopathy of prematurity, chronic lung disease or ultrasound findings of white matter injury).²⁰ A study by Chen et al. also did not show a difference in short-term neurological outcome when comparing liberal to restrictive guidelines.⁸ However, they did find a significantly higher rate of chronic lung disease among infants with a total transfused red blood cell volume >30 mL in 30 days. In another recent RCT (Iowa trial), a group of very low birth weight infants (500–1300 gram) was treated with either a restrictive or liberal transfusion guideline; the infants in the restrictive transfusion group more frequently had an IVH grade 4 and cystic periventricular leukomalacia, while other clinical parameters were equal.²¹

Long-term follow-up of the PINT study at a corrected age (CA) of 18–21 months showed no difference in the primary composite outcome defined as severe cognitive delay (Mental Developmental Index (MDI) <70 (<–2 SD), death, blindness or deafness.²² However, in a post-hoc analysis with developmental delay redefined as a MDI < 85 (<–1 SD), the authors found a significant difference in favor of the liberal RBC transfusion threshold. Long-term developmental follow-up in the Iowa trial has not been published yet. However, the authors recently published a study on cerebral imaging performed in infants at 12 years included in the initial

Iowa trial and reported that intracranial volume was significantly smaller in the liberal group compared with term controls.²³ These finding may seem to contradict the conclusions from their short-term follow up. However, the imaging study was hampered by several important methodological limitations: A) a high loss to follow-up rate (only 44% of the initial cohort was available for analysis), and B) intracranial volume (which includes cerebrospinal fluid) is not the best predictor of neurodevelopmental outcome.

The relatively small number of infants in these studies (the PINT study is the only larger study on this topic) makes it difficult to use them as principal guidance for a transfusion guideline and is reflected in the prevailing variety of guidelines shown in a recent international practice survey.²⁴

Alternatives

Micro-blood sampling, transcutaneous measurements and increased awareness on anemia of prematurity (AOP) have diminished the amount of blood drawn for laboratory sampling, one of the most important causes for AOP. Baer et al. showed that implementation of a transfusion ordering and monitoring instrument improved compliance to the local transfusion guideline and diminished the number of transfusions given.²⁵

A Cochrane review in 2004 showed that delaying cord clamping (DCC) for 30 seconds to 3 minutes in preterm infants reduces the number of RBC transfusions needed and the number of IVH without detrimental effects.²⁶ In term infants, however, DCC leads to an increased need for phototherapy to treat hyperbilirubinemia, but not to an increase in the indication for partial exchange transfusion because of polycythemia. The Hb level in these infants is better than in infants subjected to immediate cord clamping and their ferritin levels are significantly higher at six months of age.²⁷

Umbilical cord blood (UCB) harvesting is a possibility to obtain red blood cells for autologous transfusion. It is a costly procedure and harvesting for extremely preterm infants (<28 weeks gestation), who would benefit most from these products, is often difficult due to their small placenta and thus a small volume retrieved. Our study described in chapter 4 showed that it is not feasible to routinely harvest UCB in all preterm infants for autologous RBC transfusion due to small volumes harvested or processing difficulties.²⁸ Strauss et al. show similar results in their overview article on autologous RBC transfusion for anemia of prematurity.²⁹ Several studies have shown that UCB, harvested at term deliveries, can be used for (full term) neonatal surgical patients to avoid allogenic RBC transfusions,^{30,31} but this is a very limited group of patients. UCB has been safely used for allogenic red blood cell or whole blood transfusions in pediatric patients and adults in source-restricted areas where availability of blood products is scarce.³²

Cochrane reviews have shown that erythropoietin (EPO) administration (early: started in the first week of life or late: started after day 7) in preterm infants has no clinical meaningful benefit in diminishing the number of RBC transfusions, total transfusion volume and, thus donor exposition, when given the first weeks of life, in particular not in sick children. Together with the increased risk for severe retinopathy of prematurity as a side effect of early EPO administration, indications for EPO are very limited.^{33,34} Selected groups of patients such as infants of Jehovah's Witness might benefit from avoidance of late RBC transfusions.

Enteral iron therapy started when enteral feeds have reached 100 mL/kg/day may reduce late RBC transfusions (>2 weeks) in infants <1301 grams without negative side effects.³⁵ It does not affect the number of RBC transfusions given in the first few weeks of life when the majority of these very preterm infants are transfused.

Measures to diminish donor exposure are possible, but mainly used in the Western countries. Single donor programs, dividing an adult unit of RBCs into four or five pediatric units and assigning these to one or two infants can limit donor exposure.^{16,36,37}

Restrictive use of RBC, either in volume per transfusion event or by a restrictive transfusion threshold, can lead to a lower total transfusion volume, and if single donor programs are not used, less donor exposure in particular for infants born before a gestational age of 28 weeks.³⁸⁻⁴⁰

Platelets

Thrombocytopenia can be divided into fetal onset, early onset (<72 hours after birth) and late onset thrombocytopenia (>72 hours after birth), each with their own causes, albeit partly overlapping. Early thrombocytopenia is often caused by maternal causes (auto- and allo-antibodies against platelets) and due to asphyxia during delivery and can affect both term and preterm born infants, whereas late thrombocytopenia often affects mainly preterms in relation to infection and thrombosis and impaired bone marrow response to compensate low platelet counts.

Platelet transfusions may be administered to prevail over an extremely low platelet count (<30–50 x 10⁹/L) or to help stabilize an acute severe hemorrhage before a sustained rise in platelets is induced by treating the underlying cause. Most feared is IVH.

In a study by Bear et al. in neonatal intensive care patients with a platelet count <50 x 10⁹/L the rate and grade of IVH was independent of the severity of the thrombocytopenia below this level.³ In severe thrombocytopenia (<20 x 10⁹/L) the rate of cutaneous bleeding (defined as extensive bruising or oozing from puncture sites) is also reported to be significantly higher than in infants with higher platelet counts. Our study presented in chapter 8 shows that

thrombocytopenia $<150 \times 10^9/L$ in (preterm) neonates is associated with a higher risk of (intraventricular) hemorrhage than a normal platelet count, but that the severity of thrombocytopenia has no correlation with the severity of IVH. Setzer et al. found a higher prevalence of IVH in infants with a lower platelet count, next to an increased number of hemostatic abnormalities in infants with IVH.⁴¹ In 1987 Andrew et al. published a prospective study and found an abnormal bleeding time in thrombocytopenic infants that was corrected after platelet transfusion.⁴² Thrombocytopenia correlated with more severe IVH and other hemorrhages. There was a significantly higher percentage of infants with severe neurologic sequelae (cerebral palsy, deafness or hydrocephalus) at twelve months follow-up in the infants <1500 grams at birth with thrombocytopenia ($<100 \times 10^9/L$) compared to the infants with a normal platelet count. However, the group of thrombocytopenic infants had also a significantly lower Apgar score at 5 minutes after birth. Whether the neurologic sequelae were caused by thrombocytopenia or other factors remains unclear.

Incidentally, platelet transfusions have been associated with detrimental effects such as the transmission of infectious agents and transfusion reactions.^{43,44} Several studies have shown a significantly higher mortality rate after platelet transfusions in neonates.⁴⁵⁻⁴⁷ Whether this higher mortality rate is causally related to the platelet transfusion or the underlying disease severity indicating platelet transfusions is unknown. The question therefore remains whether and when to transfuse thrombocytes.

Product and volume

As far as the properties of the transfusion products are mentioned most clinicians transfuse between 10 and $20 \times 10^9/kg$ thrombocytes. The volumes of these transfusions differ greatly depending on the type of preservation fluid and technique of processing. Products can be random single donor, acquired through apheresis, in compatible plasma or preservation fluid. Alternatively, products can be part of an adult unit derived from 5 random donors, either whole-blood derived or by buffy-coat technique. In our medical center a single donor plasma reduced platelet product, acquired through apheresis, is used limiting the volume transfused to approximately 5 mL/kg (containing 20×10^9 platelets/kg). Platelets can be suspended in plasma or a PAS (platelet additive) solution (PAS-1: D-mannitol, phosphate and citrate; PAS-2: supplemented with acetate, but low citrate concentration). Each suspension fluid has its advantages and disadvantages. Plasma is known to cause adverse transfusion reactions. PAS-1 and -2 have the advantage of being able to remove the majority of plasma, but during storage in PAS-1 and -2, platelets tend to deteriorate faster because maintenance of the pH above 6.5 is decreased and in adult recipients post-transfusion platelet increment is lower than after storage in plasma.⁴⁸

Infants with thrombocytopenia due to fetal/neonatal allo-immune thrombocytopenia (FNAITP) may need HPA-1a or HPA-5b negative matched platelet products to provide a longer lasting rise in platelet count. In acute situations random platelets can be given awaiting the matched platelets.⁴⁹

Triggers

Various guidelines for platelet transfusions can be found in the literature, but none are evidence based. In the previously mentioned international practice survey by New et al. several transfusion guidelines are mentioned.²⁴ Most guidelines take the platelet count and clinical condition, combined with gestational age, into account when deciding whether to transfuse or not. Most consensus based guidelines have a lower platelet transfusion threshold of $20\text{--}30 \times 10^9/\text{L}$.⁵⁰⁻⁵² For clinically unstable infants or those in need of surgery higher platelet counts are preferred (from 50 to $100 \times 10^9/\text{L}$).

In the only prospective RCT on prophylactic platelet transfusions in non-bleeding preterms by Andrew et al. no reduction in IVH was detected when prophylactic platelet transfusions for platelet counts between 50 and $150 \times 10^9/\text{L}$ were compared with no transfusion.⁵³ Murray et al. performed a retrospective review in a group of 53 preterm infants with severe thrombocytopenia. All infants with a platelet count $<30 \times 10^9/\text{L}$ and all unstable infants or infants with an IVH and a platelet count $30\text{--}50 \times 10^9/\text{L}$ received a platelet transfusion. They found no difference in major hemorrhages whether the infants had received a platelet transfusion or not.⁵⁴ In our study described in chapter 9, we compared the outcome in two neonatal intensive care centers in the Netherlands using different transfusion guidelines (restrictive versus liberal) and found no significant difference in the incidence of any hemorrhage or severe hemorrhages between both units. Bonifacio et al. also reported that the development of IVH was strongly associated with a lower gestational age and not with the severity of thrombocytopenia.⁴⁶

Alternatives

No studies have evaluated the use of thrombopoietin (TPO) mimicking proteins to treat thrombocytopenia in human newborns. Human and animal studies show that these TPO analogues produce a rise in platelet count after 6–7 days of therapy.^{55,56} Because thrombocytopenia in human infants usually resolves within a week, the usefulness of TPO mimicking proteins in neonates is questionable.

To date no studies with substitutes for platelets have been performed in newborn infants. Several (adult) animal studies have shown a possible positive effect of the administration of alternative products such as polymerized albumin particles or activated Factor VII improving hemostasis in patients with bleeding and thrombocytopenia.^{57,58}

High dose intravenous immunoglobulin (IVIG) has a role in immune-mediated (early) thrombocytopenia, such as FNAITP and immune thrombocytopenia (ITP) causing (early) thrombocytopenia irrespective of gestational age. Infusions of high dosages of IVIG given weekly to women pregnant with a fetus with FNAITP decrease the risk of intracranial hemorrhage in all infants and induce an increase in the fetal platelet count in 30–85% of these infants.⁵⁹ How IVIG exactly exerts this effect is unknown but the treatment can replace hazardous intra-uterine platelet transfusions.^{60–62} Postnatal IVIG administration to newborns with NAITP has a delayed effect on their platelet count and HPA-matched transfusions are first line treatment.^{49,63,64} In contrast, in case of neonatal thrombocytopenia due to maternal auto-antibodies in ITP, maternal administration of IVIG is ineffective, but neonatal administration of IVIG can raise the platelet count within 2–3 days and is recommended in a non-bleeding infant.⁶⁵

Due to the pathogenesis of hemorrhage in newborns and especially in preterm infants, focusing on a normal platelet count to diminish bleeding in these infants is not enough. Furthermore, the timing of the hemorrhage does not seem to be related with the onset of a low platelet count.⁶⁶

Concluding remarks and future perspectives

Transfusion guidelines for the treatment of anemia or thrombocytopenia in newborns are not well established nor evidence based. Although various studies have been performed to address this problem, absolute thresholds based on evidence based research have not yet been established. Combining the findings of several studies is not possible due to difference in patient categories, cause of anemia/ thrombocytopenia, transfusion products and outcome measurements. The ten Dutch neonatal intensive care units each use their own guidelines for RBC and platelet transfusions despite the availability of national consensus recommendations. As long as alternatives to donor blood products do not seriously diminish the need for transfusions in neonatal medicine, more sufficiently powered prospective RCTs are needed to establish well founded guidelines. This asks for multicenter cooperation because in a fast evolving field like neonatology, many confounding factors may be introduced due to changes in treatment of this group of patients.

Studies should not only focus on different transfusion regimens but should also take the different products into account. Using RBC products with a different Hct will have impact on the blood viscosity and this in itself may have effect on the blood circulation and oxygen delivery in the body. The effect of transfusion volume per event, total transfused volume and red cell or platelet content of the transfusion product should be studied. The various fluids used for

platelet preservation may also have their effect on the recovery of platelets and their lifespan. Platelet studies should be stratified for the cause of thrombocytopenia, gestational age and have relevant (bleeding) endpoints.

Short-term and especially long-term effects of blood product transfusions need to be investigated as possible later detrimental effects of RBC transfusions have been shown in the few studies that have been conducted.

Ways to improve harvesting and processing of umbilical cord blood should be found to make this a more feasible way to produce autologous RBCs for very preterm infants. Alternatives to donor products should be further studied.

In summary, RBCs are probably the most frequently used drug given to very preterm infants without its long-term effects being well studied and known. Except for reduction of the amount of blood drawn for laboratory tests and use of a single donor program, no measures have been shown to be an irrefutable safe way to reduce donor exposure.

Thrombocytopenia is also a frequently encountered problem in neonatal medicine with an increased risk for hemorrhages. But why, when and what to transfuse remains a topic of clinical research.

To acquire broad support from the clinicians taking care of these vulnerable (preterm) infants, large studies showing safety of the guidelines is of utmost importance. Future research should be directed at identifying a safe lower transfusion trigger for transfusion products without short-term or long-term detrimental effects.

Until that time, the data in this thesis suggest the use of 15 mL/kg body weight of RBCs with a Hct of 60 ($\pm$ 5)%, in particular for extremely preterm infants (gestational age at birth <28 weeks), using a single donor program to reduce donor exposure. RBC transfusion triggers should be based on clinical condition and postnatal age. In stable infants older than 4 weeks Hb should be maintained above 4.5 mmol/L. In younger infants or with signs of anemia or oxygen dependency (O_2 <30%) a Hb of 6.0 mmol/L is recommended. In case of mechanical ventilation, higher oxygen need or cardiopulmonary disease the transfusion threshold has to be adjusted upwards.

Platelet transfusions should contain 10–20 $\times 10^9$ /kg of platelets, derived from a single donor in platelet additive solution or volume-reduced. Platelets should only be given when the platelet count drops below 50 $\times 10^9$ /L in the presence of an active major hemorrhage or the need for invasive procedures. In the absence of a significant bleeding a lower threshold (20 $\times 10^9$ /L) is recommended, unless further decrease of the platelet count is foreseen such as in immune mediated thrombocytopenia's.

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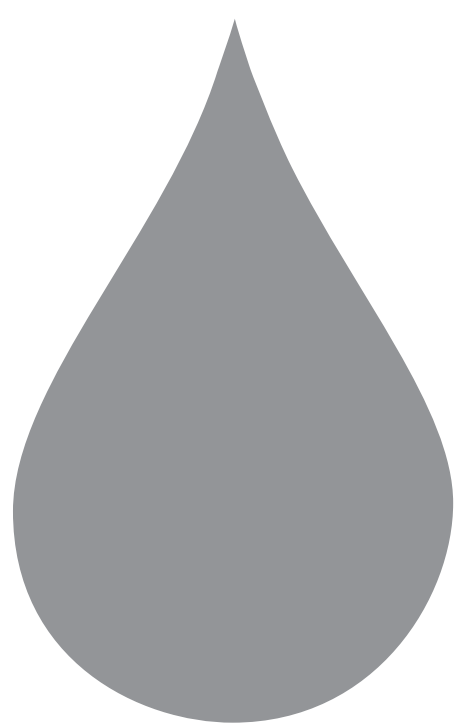
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Chapter 12

Summary



In very premature born infants red blood cells are one of the most commonly used products. Nowadays erythrocytes are deemed fairly safe although not enough is known about the effects of an allogenic red blood cell (RBC) transfusion on the still developing immune system of these young infants. Alternatives to allogenic red blood cell transfusions are sought and ways to reduce the amount of transfusions needed, specifically by premature infants, remain a hot topic.

Thrombocytes are the second most used blood product in perinatal medicine. They are of greater concern in regard to transfer of infectious agents.

There is no consensus on the threshold for transfusion for both erythrocytes and thrombocytes. The effects of liberal or restrictive erythrocyte transfusion strategies are still debated. A safe lower threshold for platelet transfusions has still not been established.

In **chapter 1** the problem concerning blood products in neonatal medicine is outlined. The studies performed for this thesis are introduced.

In **chapter 2** a review of the literature is given concerning the various blood products used in perinatal medicine. The different transfusion thresholds are discussed as well as still standing research questions.

Part I Umbilical cord blood

Chapter 3 describes the possibility at birth to predict the need for a RBC transfusion within the first month of life in a premature born infant. Data on 288 infants born between 24 and 36 weeks of gestation were collected. Gestational age of less than 32 weeks and one minute Apgar score of less than 6 were independently associated with the chance of receiving a RBC transfusion in the first thirty days of life. In the newborns with a gestational age of less than 32 weeks, birth weight was also a factor determining the risk of transfusion.

We also looked at the possibility to harvest umbilical cord blood (UCB) in these infants.

The volume harvested was compared to the total erythrocyte transfusion volume in these children, so as to see whether their transfusion needs could have been covered by autologous RBCs.

Sixty-three% of the collected UCB samples was large enough to process and 61% was suitable as a potential RBC transfusion product. For 41% of the infants with a gestational age less than 32 weeks the autologous UCB product would have been sufficient to meet all transfusion needs in the first 30 days of life.

The feasibility to use red blood cells derived from umbilical cord blood for autologous erythrocyte transfusion is described in **Chapter 4**. The aim of the study was to replace 50% of all donor RBC transfusions by autologous RBC transfusions. In 90% of the 195 cases an attempt to collect UCB was done. In 57% of these cases the volume was large enough (≥ 15 mL) to process, resulting in 64 autologous RBC units (36% of the total population). Due to low collected UCB volumes and subsequent small red blood cell products a transfusion product was available for only 27% of the transfused infants, covering 58% of their transfusion needs within the shelf life time of this autologous RBC product.

Part II Red bloods

In **chapter 5** the results are presented of a prospective study comparing two cohorts of 218 and 241 premature infants born before a gestational age of 32 weeks admitted to two neonatal centers. These centers used the same RBC transfusion trigger but a different transfusion volume (15 vs. 20 mL/kg). The effects of the transfusion volume on the number of RBC transfusion episodes, total transfusion volume and neonatal complications are described. In the lower-transfusion-volume unit we found a significantly lower amount of infants transfused than in the higher-transfusion-volume unit (59% vs. 77%, $p < 0.001$). In the group of infants with a gestational age between 24 6/7 and 27 6/7 weeks both units showed a similar percentage of infants with a RBC transfusion and a similar transfusion frequency per infant leading to a significantly higher total transfusion volume in the higher transfusion volume unit (77 mL/kg in the lower-transfusion-volume unit versus 104 mL/kg in the higher-transfusion-volume unit, $p < 0.01$). In the group of infants with a gestational age between 28 0/7 and 31 6/7 weeks the total transfusion volume was comparable, given in 3 episodes in the lower-transfusion-volume unit and 2 episodes in the higher-transfusion-volume unit. This resulted in a greater donor exposure in the lower-transfusion-volume unit compared to the higher-transfusion-volume unit, as we did not use reserved single-donor pedipacks for these infants. The composite clinical outcome in regard to bronchopulmonary dysplasia, intraventricular hemorrhage (IVH), retinopathy of prematurity and mortality was similar in both units, showing no detrimental effects of a different total transfusion volume on short term outcome.

Chapter 6 shows the neurodevelopmental outcome, post discharge mortality, blindness and deafness at the corrected age (CA) of 24 months in the subgroup infants born before a gestational age of 28 weeks described in chapter 5. Both units transfused an equal percentage of infants in this age group. The infants in both units received a mean number of RBC transfusions of 5.5. Unit A transfused with a volume of 15 mL/kg, Unit B 20 mL/kg. This led to

a significant difference in total transfusion volume of respectively 79 ± 47 mL/kg and 108 ± 47 mL/kg ($p=0.02$). There was no significant relationship between the composite outcome at 24 months CA and the total transfusion volume received during the post natal hospital stay.

Chapter 7 shows that despite a national guideline for the transfusion of RBCs, most neonatal intensive care centers (NICUs) in the Netherlands have their own transfusion protocol taking gestational age and degree of illness into account. The international literature shows a variety of guidelines with no definite thresholds either. The following thresholds are advised: Hb 8.0 mmol/L in case of endotracheal ventilation, Hb 7.0 mmol/L in stable infants with cardiopulmonary problems and extra oxygen need, Hb 6.0 mmol/L in stable infants less than 4 weeks old and Hb 4.5 mmol/L in stable older infants. We recommend 15 mL/kg of RBCs per transfusion. Indications for irradiating blood products and administering Parvovirus B19 safe blood are given.

Part III Platelets

Chapter 8 describes the frequency of thrombocytopenia and the risk of IVH in our tertiary care NICU in Leiden, The Netherlands. We retrospectively studied all infants ($n=1727$) admitted to our ward during a three-year period (January 2006–December 2008). The risk of persistent thrombocytopenia, defined as a platelet count below $150 \times 10^9/L$ found at more than one measurement, was 27% ($n=422$), comparable to the frequency reported in the literature. The difference in the risk of IVH $\geq$ grade 2 in infants with thrombocytopenia compared to a normal platelet count was significant, 12% versus 5% ($p<0.01$) respectively. The severity of thrombocytopenia in relation to the incidence of severe IVH, however, showed no correlation.

In **Chapter 9** the number of very premature thrombocytopenic infants with intracranial hemorrhages and other severe bleedings in two Dutch NICUs with a different platelet transfusion guideline are compared. In the restrictive-platelet-transfusion-guideline unit transfusions were given only in case of a major hemorrhage (IVH $>$ grade 2, mucosal, pulmonary or gastro-intestinal bleeding), severe bruising or massive petechiae, surgery or the need for an invasive procedure *and* a platelet count $<50 \times 10^9/L$. In the liberal-platelet-transfusion-guideline unit the indications for a platelet transfusion were based on platelet counts: 1) platelet count $<30 \times 10^9/L$ in clinically stable infants, 2) platelet count $<50 \times 10^9/L$ in unstable infants and/or before planned surgery, 3) platelet count $<100 \times 10^9/L$ in neonates with active bleeding and/or at start of exchange transfusion. In the restrictive-transfusion

unit the number of thrombocytopenic infants transfused was significantly lower compared to the liberal-transfusion unit (15% (21/145) versus 31% (44/141), $p=0.001$). Despite the more restrictive transfusion strategy the number of infants with a (severe) intracranial hemorrhage was comparable ($p=0.38$). Our conclusion is that more liberal platelet transfusion guidelines do not diminish the number of hemorrhages in very premature thrombocytopenic infants.

Chapter 10 describes the difference in platelet transfusion protocols in the Dutch NICUs despite a national guideline. These guidelines are compared to the national guideline and the revised guideline 2011. International guidelines are presented. Internationally no safe lower threshold has been established. We propose to administer platelets in all infants with a platelet count $<20 \times 10^9/L$. For ill infants younger than 32 weeks of gestation or with a bodyweight below 1500 gram, or for infants with an active major hemorrhage, in need of surgery or an invasive procedure or after an exchange transfusion a minimum platelet count of $50 \times 10^9/L$ is advised. In case of extra corporal membrane oxygenation (ECMO) or before an exchange transfusion platelet counts should be maintained $\geq 100 \times 10^9/L$. A platelet concentrate acquired through apheresis of a single donor containing at least 10×10^9 platelets/kg should be given. Indications for irradiation of products and Parvovirus B19 safety are similar to those for RBCs.

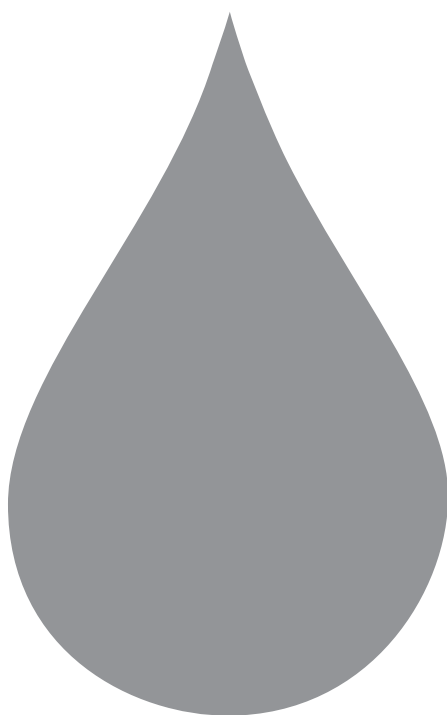
In conclusion, preventative measures for anemia should be used to reduce the number of RBC transfusions needed. Alternatives for allogenic RBC transfusions should be further explored and implemented.

The effect of platelet transfusions on the risk of bleeding is not always evident and needs to be studied further.

We state that both for RBC and platelet transfusions in (premature) newborn infants safe thresholds are still not established. Transfusions may have (late) detrimental effects. Safe thresholds for both erythrocytes and platelets need to be found by large prospective randomized trials focussing not only on the direct effects but also on the long-term effects.

Chapter 13

Samenvatting



In Nederland worden per jaar ongeveer 180.000 kinderen geboren. Circa 7% wordt prematuur (te vroeg) geboren, dat wil zeggen voor 37 voltooide zwangerschapsweken. Er zijn ongeveer 2500 kinderen die zeer prematuur (na minder dan 32 weken zwangerschapsduur) worden geboren. Bij de behandeling van zeer prematuur geborenen worden vaak rode bloed cel (RBC) transfusies gegeven als zij een te laag hemoglobine (Hb), of te wel anemie, hebben. Erytrocyten (=rode bloed cellen) bevatten Hb wat verantwoordelijk is voor het transport van zuurstof en kooldioxide door het lichaam van en naar de longen. Bijna alle kinderen geboren na een zwangerschapduur van 28 weken of minder krijgen minimaal één RBC transfusie. Een van de belangrijkste redenen voor een te laag Hb is iatrogeen. Dat betekent dat de anemie veroorzaakt is doordat er bloed bij de kinderen is afgenomen voor laboratorium onderzoek. Daarnaast groeit een prematuur relatief snel waardoor de aanmaak van rode bloedcellen te kort kan schieten. Ook heeft een zeer prematuur geboren kind minder ijzerreserve bij de geboorte doordat dit pas de laatste 3 maanden van de zwangerschap via de placenta van moeder aan kind wordt doorgegeven. Een pasgeborene heeft ongeveer 80 ml/kg bloed. Tegenwoordig worden RBC transfusies als vrij veilig beschouwd. We weten echter nog onvoldoende over het effect van RBC transfusies van bloed donoren op het afweer systeem van prematuren wat nog volop in ontwikkeling is. Alternatieven voor donor bloed worden onderzocht en manieren om de hoeveelheid bloedtransfusies aan deze kinderen te verminderen blijven een 'hot topic'.

Trombocyten (=bloedplaatjes) zijn het op één na meest gebruikte bloedproduct in de neonatologie. Bij trombocytopenie (= een te laag aantal bloedplaatjes, $<150 \times 10^9/l$) is er een verhoogd risico op het krijgen van een (hersen) bloeding. Trombocytopenie heeft verschillende oorzaken zoals groeivertraging van de foetus of ernstige infecties. Bij trombocyten transfusie is er meer zorg over eventuele besmetting met ziekmakende bacteriën of virussen. Bloedplaatjes zijn nodig voor de stolling (=het stoppen van een bloeding).

Er is, noch voor erytrocyten, noch voor trombocyten, consensus over de transfusiegrenzen voor pasgeborenen. Er is nog steeds discussie over de effecten van een liberaal of meer terughoudend transfusie beleid.

In **hoofdstuk 1** van het proefschrift wordt het vraagstuk van het gebruik van bloedproducten in de neonatologie uiteen gezet. De opzet van het proefschrift wordt beschreven en de uitgevoerde studies worden geïntroduceerd. In **hoofdstuk 2** wordt een literatuur overzicht gegeven van de verschillende bloedproducten die gebruikt worden voor het ongeboren of pasgeboren kind (=de perinatologie). De verschillende transfusie grenzen worden besproken, zo ook de nog uitstaande onderzoeksvragen op dit gebied.

Deel I Navelstrengbloed

In dit deel van het proefschrift wordt het gebruik van navelstrengbloed voor autologe bloedtransfusies beschreven. (Autoloog= afkomstig van de persoon zelf). Hierbij wordt bij de geboorte van een zeer prematuur geboren kind direct na het afnavelen het bloed, wat nog in de navelstreng en placenta zit, verzameld en vervolgens bij de bloedbank bewerkt tot een RBC transfusieproduct. Het kan enkele weken worden bewaard tot het kind een RBC transfusie nodig heeft, waarbij het doel was het kind zijn of haar eigen rode bloed cellen uit navelstrengbloed terug te geven. In **hoofdstuk 3** staat de studie beschreven waarbij gekeken werd in hoeverre bij de geboorte al een inschatting gemaakt kan worden welk kind in de eerste levensmaand een RBC transfusie nodig heeft. Gegevens van 288 kinderen geboren tussen de 24 en 36 weken zwangerschapsduur werden verzameld. Een zwangerschapsduur van minder dan 32 weken en een Apgar score 1 minuut na de geboorte <6 waren onafhankelijk van elkaar geassocieerd met het risico op het nodig hebben van een RBC transfusie in de eerste 30 levensdagen. Bij de kinderen jonger dan 32 weken zwangerschapsduur bij de geboorte was het geboorte gewicht ook een factor die bijdroeg in het risico op de noodzaak voor een RBC transfusie.

We onderzochten ook de mogelijkheid navelstrengbloed te oogsten. De hoeveelheid geoogst bloed werd vergeleken met de totale hoeveelheid toegediend donor bloed bij deze kinderen om te beoordelen of de transfusiebehoefte gedekt had kunnen worden met autologe RBCs. Van de afnames was 63% voldoende groot om te bewerken en was 61% geschikt als potentieel transfusie product. Voor 41% van de kinderen met een zwangerschapsduur van minder dan 32 weken was het autologe navelstrengbloed product voldoende geweest om alle transfusies van de eerste 30 dagen te dekken.

Hoofdstuk 4 beschrijft het onderzoek dat tot doel had de helft van alle donor RBC transfusies te vervangen door autologe navelstrengbloed transfusies. Bij 90% van de 195 geboortes werd een poging gedaan om navelstrengbloed te oogsten. In 57% van de gevallen was het verkregen volume voldoende groot (≥ 15 ml) om tot een autoloog RBC transfusie product te verwerken. Uiteindelijk waren er 64 eenheden autologe RBCs (36% van de totale populatie). Als gevolg van het laag geoogste volume en daardoor kleine transfusie producten waren er uiteindelijk voor slechts 27% van de kinderen met een transfusie noodzaak autologe RBCs beschikbaar. Binnen de houdbaarheid van het autologe RBC product (30 dagen) betekent dit een dekking van 58% van de transfusies in deze groep kinderen.

Deel II Rode bloedcellen

Hoofdstuk 5 beschrijft een prospectieve studie bij 2 groepen van 218 en 241 premature pasgeborenen (<32 weken zwangerschapsduur) opgenomen op twee neonatale intensive care units (NICUs). Deze NICUs hanteerden identieke RBC transfusie grenzen maar gaven een verschillend volume erythrocyten per transfusie (15 versus 20 ml/kg). De effecten van het volume per transfusie op het aantal transfusies, totale transfusie volume en neonatale complicaties worden beschreven. In de NICU met het lagere transfusie volume werd een significant lager percentage kinderen getransfundeerd dan in de hoog-volume NICU (59% versus 77%, $p<0.001$). In de subgroep kinderen met een zwangerschapsduur tussen de 24 6/7 en 27 6/7 week werd een gelijk percentage kinderen getransfundeerd en kregen ze even vaak een transfusie. Dit leidde in de hoog-volume transfusie NICU tot een significant hoger totaal transfusie volume (77 ml/kg in de laag-volume NICU versus 104 ml/kg in de hoog-volume NICU, $p<0.01$). In de groep kinderen geboren met een zwangerschapsduur tussen 28 0/7 en 31 6/7 week was het totale transfusie volume vergelijkbaar, gegeven in 3 transfusies in de laag-volume NICU tegenover 2 in de hoog-volume NICU. Hierdoor was er in de laag-volume NICU een grotere blootstelling aan verschillende bloeddonoren omdat er geen zogenaamd single-donor programma was. Single-donor programma houdt in dat een volwassen donatie eenheid RBCs van één donor verdeeld wordt in 4-5 pediatrische eenheden die dan gereserveerd zijn voor één kind zodat een vervolg transfusie van dezelfde donor eenheid komt. De samengestelde uitkomst van korte termijn effecten, te weten: chronische longziekte, intraventriculaire bloeding (IVH,=hersenbloeding), prematuren retinopathie (stoornis in de uitgroei van de bloedvaten in het netvlies) en overlijden, was vergelijkbaar in de 2 groepen. De conclusie is dat er geen nadelige korte termijn gevolgen gevonden zijn van een verschillend RBC transfusie volume per transfusie.

In **hoofdstuk 6** worden de lange termijn effecten geëvalueerd in een subgroep van de kinderen uit hoofdstuk 5. De kinderen geboren na een zwangerschapsduur van minder dan 28 weken werden op de leeftijd van 24 maanden na de a terme datum (=24 maanden na de uitgerekende datum) beoordeeld op de samengestelde uitkomst van neurologische ontwikkeling, doofheid, blindheid en overlijden na ontslag van de NICU. In beide NICUs werd een gelijk percentage kinderen getransfundeerd. Zij kregen gemiddeld 5,5 transfusies. Door het verschil in RBC volume per transfusie (15 ml/kg in NICU A en 20 ml/kg in NICU B) kregen de kinderen in NICU A significant minder bloed toegediend dan in NICU B (79 ± 47 ml/kg, respectievelijk 108 ± 47 ml/kg, $p=0.02$). Er was geen significante relatie tussen de samengestelde uitkomst op de gecorrigeerde leeftijd van 24 maanden en het ontvangen RBC transfusie volume tijdens de ziekenhuis opname.

Hoofdstuk 7 laat zien dat, ondanks een nationale richtlijn, het merendeel van de 10 NICUs in Nederland een eigen RBC transfusieprotocol heeft waarbij rekening wordt gehouden met de zwangerschapsduur en de mate van ziek zijn. In de internationale literatuur worden ook verschillende richtlijnen gehanteerd zonder eenduidige transfusie grenzen. De nieuwe nationale richtlijn 2011 met transfusie grenzen wordt gepresenteerd. Wij adviseren om 15 ml/kg per RBC transfusie toe te dienen. Indicaties voor bestraling van het bloedproduct (ter vermindering van het risico op afweer reacties) en Parvovirus B19 veilig bloed worden eveneens gegeven.

Deel III Bloedplaatjes

Het voorkomen van trombocytopenie en het risico hierbij op IVH wordt in **hoofdstuk 8** beschreven. Het is een retrospectief onderzoek dat 3 jaar beslaat (2006–2008) waarin 1727 pasgeborenen werden opgenomen op de NICU van het LUMC, Leiden, Nederland. Het risico op trombocytopenie, gedefinieerd als een bloedplaatjes aantal meer dan eenmaal gemeten van minder dan $150 \times 10^9/l$, was 27% (422 pasgeborenen). Dit aantal is vergelijkbaar met de frequentie gerapporteerd in internationale publicaties. Er was een significant verschil in het voorkomen van een IVH graad 2 of meer bij pasgeborenen met trombocytopenie vergeleken met pasgeborenen met een normaal aantal bloedplaatjes, 12% versus 5% ($p < 0.01$). Er was geen correlatie tussen de ernst van de trombocytopenie en de ernst van de hersenbloeding. In **hoofdstuk 9** wordt een vergelijkend onderzoek beschreven tussen 2 groepen zeer prematuur geboren met trombocytopenie die volgens een verschillende trombocyten transfusie richtlijn werden behandeld. In de restrictieve transfusie NICU werd een bloedplaatjes transfusie alleen gegeven bij een bloedplaatjes aantal $< 50 \times 10^9/l$ en een ernstige bloeding (IVH > graad 2, slijmvlies-, long- of maagdarmbloeding), ernstige hematomen (bloeduitstortingen) of massale puntbloedinkjes van de huid, chirurgisch ingrijpen of de noodzaak van een invasieve procedure (bv. ruggenprik). In de liberale transfusie NICU werd de transfusie indicatie gesteld op het trombocytenaantal in combinatie met klinische kenmerken: 1) $< 30 \times 10^9/l$ en stabiel, 2) $< 50 \times 10^9/l$ en niet stabiel en/of geplande chirurgische ingreep, 3) $< 100 \times 10^9/l$ bij pasgeborenen met een actieve bloeding en/of voorafgaand aan een wisseltransfusie. In de restrictieve transfusie NICU was het aantal kinderen dat een trombocyten transfusie kreeg significant lager dan in de liberale transfusie NICU (15% (21/145) versus 31% (44/141), $p = 0.001$). Ondanks het meer restrictieve transfusie beleid was het aantal kinderen met een (ernstige) IVH vergelijkbaar ($p = 0.38$). Onze conclusie is dan ook dat een meer liberaal transfusie beleid het aantal bloedingen bij zeer prematuur geboren niet verlaagd.

Hoofdstuk 10 beschrijft het verschil in de trombocyten transfusie richtlijnen in de 10 Nederlandse NICUs ondanks een nationale richtlijn. Deze richtlijnen werden vergeleken met de huidige nationale richtlijn uit 2004. De internationale richtlijnen worden besproken en de gereviseerde nationale bloedtransfusie richtlijn 2011 wordt gepresenteerd. Internationaal is er geen veilige ondergrens voor bloedplaatjes transfusie vastgesteld. Wij stellen voor alle pasgeborenen met een trombocytenaantal $<20 \times 10^9/l$ te transfunderen. Een transfusie grens van $<50 \times 10^9/l$ wordt geadviseerd voor zieke neonaten jonger dan 32 zwangerschapsweken of met een gewicht <1500 gram, of voor neonaten met een actieve bloeding, met de noodzaak voor chirurgisch ingrijpen of een invasieve procedure of na afloop van een wisseltransfusie. Voorafgaand aan een wisseltransfusie of bij kinderen aan de extracorporele membraan oxygenatie (hart-long machine) wordt een trombocytenaantal geadviseerd van minimaal $100 \times 10^9/l$. Het geconcentreerde transfusie product, verkregen via aferese van één donor moet minimaal $10 \times 10^9/kg$ trombocyten bevatten. Indicaties voor het bestralen van de bloedproducten en Parvovirus B19 veilig bloed zijn vergelijkbaar met de indicaties voor rode bloed cellen.

Samenvattend dienen maatregelen ter voorkoming van bloedarmoede genomen te worden om het aantal RBC transfusies te verminderen. Alternatieven voor RBC transfusies van donoren moeten verder onderzocht worden en worden geïmplementeerd. Het effect van bloedplaatjes transfusies op het risico van bloeding is niet altijd duidelijk en vraagt ook om verder onderzoek.

Zowel voor RBC als voor bloedplaatjes transfusies bij (premature) pasgeborenen zijn veilige transfusie grenzen nog steeds niet duidelijk. Transfusies kunnen (late) nadelige gevolgen hebben. Grote prospectieve gerandomiseerde studies naar veilige transfusie grenzen voor erytrocyten en trombocyten met zowel focus op de korte termijn effecten maar vooral ook op de lange termijn uitkomsten zijn dan ook noodzakelijk.

Aan iedereen die op wat voor een manier dan ook heeft bijgedragen bij de totstandkoming van dit proefschrift:

Bedankt!

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