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## Neonatal transfusion practices

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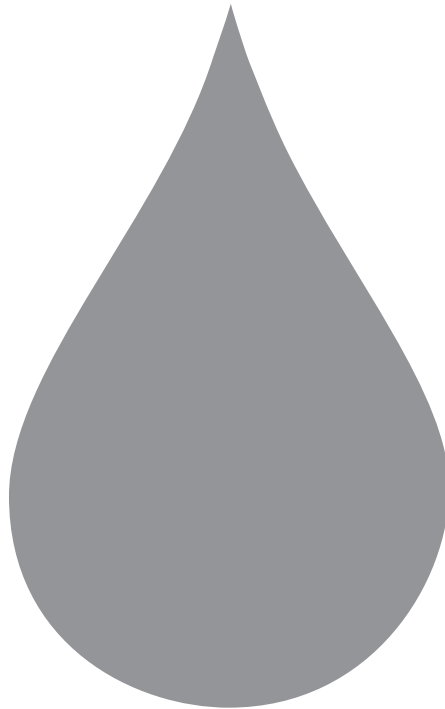
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# Chapter 2

# 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 17<sup>th</sup> week of gestation. In experienced centres, morbidity and mortality is 1–2 % per procedure,<sup>1</sup> although the complication rate is higher if the gestational age is <20 weeks or the fetus has thrombocytopenia.<sup>1,2</sup> 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  $\alpha$ -thalassaemia. 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.<sup>1</sup>

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.<sup>1</sup> As deduced from small series, IUT is also beneficial for hydrops arising from parvovirus B19 infection.<sup>3</sup>

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:<sup>4-6</sup>

- 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.<sup>5</sup> 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.<sup>7,8</sup> Leucocytes must be removed by filtration to prevent CMV transmission, although many centres additionally select CMV-seronegative donors,<sup>5</sup> and some also use parvovirus-B19-safe blood.<sup>4</sup> The red cells used for IUT are generally stored for less than 3–5 days to offer a long erythrocyte survival.<sup>4-6</sup> To prevent transfusion-associated graft versus host disease (TA-GVHD), the red cells are irradiated with 25 Gy.<sup>4-6</sup> 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.<sup>9,10</sup> 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.<sup>10</sup> 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.<sup>5</sup>

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.<sup>11</sup> A theoretical advantage is an estimated 50% reduction of maternal new antibody formation.<sup>8</sup> 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.<sup>12</sup>

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.<sup>13,14</sup> 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.<sup>15</sup> Iron overload is a long-term effect of both haemolysis and transfusion. It can play a role in neonatal cholestasis and hepatitis.<sup>16</sup> 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.<sup>17,18</sup>

## 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.<sup>19</sup> In 15–20% of FNAITP cases, non-HPA-1a/non-HPA-5b antibodies are present or antibodies cannot be detected.<sup>20</sup> 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.<sup>21</sup>

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.<sup>22</sup>

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.<sup>23</sup> 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%.<sup>24</sup> 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.<sup>25</sup>

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.<sup>21,25</sup> 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.<sup>24,26</sup>

In maternal autoimmune thrombocytopenia there is virtually no indication for IUPT because in-utero ICH has not been reported.<sup>27</sup> In hydropic foetuses 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.<sup>3,15</sup>



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.<sup>28</sup>

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.<sup>29</sup>

## 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.<sup>30</sup> 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.<sup>31,32</sup> 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.<sup>33</sup> 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.<sup>34</sup>

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.<sup>35,36</sup>

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.<sup>37</sup> 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-

ite 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;<sup>38</sup> 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 ( $3.3 \pm 2.9$  versus  $5.2 \pm 4.5$ ;  $p=0.025$ ).

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.<sup>39</sup> 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.<sup>40</sup>

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%.<sup>41</sup> 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.<sup>35,42</sup> 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,<sup>41</sup> could be used safely. Because a small volume is given, there is hardly any rise in serum potassium or decrease in RBC 2,3-biphosphoglycerate, and the potential negative influence of

**Table 1.** Transfusion thresholds for red blood cells

Study/centre	Endotracheal ventilation	NCPAP/IF	Breathing spontaneously
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 Term without SoA: Hb <8.0 g/dL
Franz et al. <sup>74</sup>	FiO <sub>2</sub> >25% Ht <sub>v</sub> <0.40 FiO <sub>2</sub> ≤25% Ht <sub>v</sub> <0.30		FiO <sub>2</sub> >25% or SoA present Ht <sub>v</sub> <0.30 Ht <sub>v</sub> <0.21
Kiriplani et al. <sup>37</sup>	<1 week of age Hb <sub>c</sub> <13.5/11.5 g/dL 1-2 weeks of age Hb <sub>c</sub> <12.0/10.0 g/dL >2 weeks of age Hb <sub>c</sub> <10.0/8.5 g/dL		<1 week of age Hb <sub>c</sub> <12.0/10.0 g/dL 1-2 weeks of age Hb <sub>c</sub> <10.0/8.5 g/dL >2 weeks of age Hb <sub>c</sub> <8.5/7.5 g/dL
Miyashiro et al. <sup>75</sup>	MAP ≥8 cm H <sub>2</sub> O Ht <0.40 MAP 6-8 cm H <sub>2</sub> O Ht <0.35 MAP <6 cm H <sub>2</sub> O Ht <0.30	FiO <sub>2</sub> > 35% Ht <0.35 FiO <sub>2</sub> < 35% Ht <0.30	reticulocyte count <2% Ht <0.20
Fabres et al. <sup>76</sup>	Acute phase of RDS Hb <12 g/dL		Supplemental oxygen Hb <10g/dL
Maier et al. <sup>77</sup>	Ht <sub>v</sub> <0.40		Hb <7 g/dL Ht <sub>v</sub> <0.25 FiO <sub>2</sub> >40% Ht <sub>v</sub> <0.40 FiO <sub>2</sub> <40% and <2 weeks of age Ht <sub>v</sub> <0.35 3-4 weeks of age Ht <sub>v</sub> <0.30 >4 weeks of age Ht <sub>v</sub> <0.25
Herman et al. <sup>6</sup>	FiO <sub>2</sub> >35-40% or MAP >6-9 cm H <sub>2</sub> O Ht ≤0.35-0.40 FiO <sub>2</sub> <35-40% or MAP <6-9 cm H <sub>2</sub> O Ht ≤0.28-0.30		Ht <20% SoA : Ht ≤0.28-0.30
Bell et al. <sup>38</sup>	Ht <0.46/0.34	Ht <0.38/0.28	Ht <0.30/0.22

Hb=hemoglobin; Hb<sub>c</sub>=capillary hemoglobin; Ht<sub>v</sub>=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.<sup>3-6</sup>

### **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.,<sup>43</sup> 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.<sup>44</sup> 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.<sup>45</sup> 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.<sup>46</sup>

### **Autologous cord blood transfusion**

Brune et al. took umbilical cord blood (UCB) from 290 term and premature newborns.<sup>47</sup> 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 allogenic RBC.<sup>47</sup> 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 allogenic products.<sup>47</sup> 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.<sup>48,49</sup>

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.<sup>50</sup>

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 allogenic 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.<sup>51</sup> 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.<sup>52</sup>

### **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.<sup>53-56</sup> 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.<sup>55,57,58</sup>

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.<sup>59</sup> 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  $100 \times 10^9/L$ .<sup>60</sup> 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.<sup>61</sup> 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.<sup>60,61</sup>

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.<sup>27</sup> Neonatal thrombocytopenia associated with maternal eclampsia and HELLP resolves spontaneously the first week after birth and needs no treatment.<sup>60</sup>

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.<sup>62</sup> 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.<sup>58</sup> 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.<sup>53</sup>

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.<sup>5,55,63</sup> 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.<sup>63</sup>

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.<sup>64</sup> Currently, aphaeresis devices can collect such highly concentrated platelets, which can be stored for 1–3 days.<sup>28</sup> Calhoun et al. advise giving 10–15 mL/kg CMV-negative standard platelet suspension (containing circa  $1 \times 10^9$  platelets/mL), which should raise the platelet count by approximately  $100 \times 10^9/\text{L}$ .<sup>63</sup>

In our centre, we transfuse platelet concentrates of 5 mL derived from a single donor (containing circa  $10 \times 10^9$  platelets/mL) and transfuse  $10 \times 10^9$  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.<sup>55,65</sup> 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. <sup>63</sup>	<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. <sup>55</sup> Murray et al. <sup>74</sup>	<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 10<sup>9</sup>/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.<sup>55</sup> 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.<sup>66</sup> 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.<sup>67</sup> 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.<sup>68</sup>

Granulocyte transfusions are used infrequently in neonatal sepsis<sup>66</sup> and there are no evidence-based studies on the use of granulocytes in newborns. A dose of 1–2 x 10<sup>10</sup>/L granulocytes per kg is suggested for effective treatment and this must be repeated daily or on alternative days for 1–2 weeks.<sup>69</sup> 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.<sup>70</sup>

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.<sup>71</sup> 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.<sup>72</sup>

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.<sup>73</sup>

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

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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