Abstract: Anaemia of prematurity will affect 90% of all very preterm infants, resulting in at least one red blood cell (RBC) transfusion. A significant proportion of preterm infants require multiple transfusions over the course of hospital admission. Growing evidence supports an association between transfusion exposure and adverse neonatal outcomes. In adults, transfusion-associated sepsis, transfusion-related acute lung injury and haemolytic reactions are the leading causes of transfusion-related morbidity and mortality; however, these are seldom recognised in newborns. The association between transfusion and adverse outcomes remains inconclusive. However, the evidence from preclinical studies demonstrates that RBC products can directly modulate immune cell function, a pathway termed transfusion-related immunomodulation (TRIM), which may provide a mechanism linking transfusion exposure with neonatal morbidities. Finally, we discuss the impact of TRIM on transfusion medicine, how we may address these issues and the emerging areas of research aimed at improving the safety of transfusions in this vulnerable population.

Key words: morbidity; mortality; preterm; red blood cell transfusion; transfusion-related immunomodulation.

The contribution of red blood cell transfusion to neonatal morbidity and mortality

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A decline in circulating red blood cells (RBCs) is observed in all newborns after birth, continuing throughout early post-natal life.1 The resultant decrease in haemoglobin (Hb) is termed ‘physiological anaemia of infancy’. In the preterm newborn, this fall is exaggerated and rapid, reaching a nadir at 4–6 weeks. This is due to a smaller circulating blood volume, shorter RBC life-span and an immature bone marrow rebound response to anaemia. Therefore, 90% of all extremely low birthweight (ELBW) premature infants develop anaemia of prematurity (AOP) requiring at least one RBC transfusion,1 with a significant proportion requiring multiple transfusions during their primary admission.2

Transfusion algorithms, based on either Hb concentration or haematocrit modified for chronological age and need for respiratory support,2,3 are generally used to determine when to transfuse an infant with AOP. However, adherence to either restrictive or liberal transfusion thresholds has failed to show clear clinical benefit.4

RBC transfusion improves oxygen-carrying capacity to tissues and may prevent apnoeic episodes and promote weight gain and growth in the preterm newborn.1 However, there is growing evidence of an association between transfusion exposure and adverse outcomes. Here, we review the evidence linking RBC transfusion exposure with mortality and neonatal morbidity and discuss the potential underlying mechanisms and emerging approaches to modify these responses.

Transfusion and Neonatal Mortality

Transfusion exposure increases the risk of death in critically ill adult patients,5 with the risk of both short- and long-term mortality increasing with total number of transfusions received.3–7 Despite preterm newborns representing one of the most heavily transfused patient groups,6 neonatal data linking transfusion exposure and increased mortality are limited. In a large retrospective study conducted in preterm neonates and controlling for confounding variables such as birthweight and lower gestational age, those who received any RBC transfusion had a 50% increase in mortality in the first 28 days of life. Furthermore, mortality rates after day 28 of life were almost two times greater in those who received more than two transfusions.8 Similarly, in a cohort study of ELBW newborns, the number of RBC transfusions in the first 7 days of life correlated with mortality before 1 month of age.9 However, a recent meta-analysis of the adverse outcomes
potentially associated with RBC transfusion exposure, particularly rates of mortality associated with restrictive versus liberal transfusion strategies, found no difference in mortality.11

Transfusion and Neonatal Morbidity

Extremely premature newborns are susceptible to numerous potentially fatal or severely disabling conditions, including necrotising enterocolitis (NEC), bronchopulmonary dysplasia (BPD), retinopathy of prematurity (ROP) and intraventricular haemorrhage (IVH). While a number of observational studies have raised the possibility of an association between RBC transfusion and adverse neonatal outcome, controversy remains about the contribution of transfusion exposure to their pathophysiology.11

Transfusion-associated NEC

NEC typically develops in a vulnerable preterm host with a hypoxic, ischemic or infectious bowel insult. In the 1980s, the association between RBC transfusion and NEC was thought to occur as a result of T-antigen activation, leading to the use of washed or anti-T blood products.12 Recently, the term TANEC has been used to describe transfusion-associated NEC. TANEC has been reported to be associated with approximately 30% of NEC cases, with these infants at the greatest risk of developing surgical NEC.13 While several mechanisms have been proposed to result in TANEC, it is thought to be secondary to an immune response to biological mediators contained within stored blood.13 Indeed, RBC transfusion exposure results in increases in pro-inflammatory cytokines associated with established NEC, including tumour necrosis factor, interleukin (IL)-1β, IL-6 and IL-8.14

Other investigators have focused on the temporal relationship between enteral feeding at the time of RBC transfusion and the development of NEC, demonstrating that the anticipated postprandial blood flow increase through the superior mesenteric artery is ablated in newborns receiving RBC transfusions. This suggests a subsequent postprandial period of intestinal hypoperfusion that may contribute to NEC development.15 Finally, it has been suggested that anaemia itself, rather than a transfusion, increases the risk of NEC.15 In a prospective observational study of very low birthweight preterm newborns, RBC transfusions in a given week were not significantly related to the rate of NEC. However, the rate of NEC was significantly increased in those infants with severe anaemia compared to those who did not.16

While a previous meta-analysis of 11 case-control studies and one cohort study suggested an association between RBC transfusion and NEC,17 the most recent meta-analysis of available observational data found no such effect.18 Furthermore, a recent systematic review of randomised trials on blood transfusion thresholds in ELBW infants failed to support an association between transfusion and NEC.19 This likely reflects how most studies investigating this association are affected by unadjusted differences in baseline patient characteristics and indication for transfusion, the lack of a specific definition of TRANEC and, most importantly, significant inconsistency in the direction of results.19

Bronchopulmonary dysplasia

In adults, acute respiratory decompensation is a well-recognised complication of RBC transfusion.20 This may be either transfusion-associated lung injury (TRALI), commonly occurring 6 h following exposure to RBCs21; transfusion-associated circulatory overload (TACO), defined as acute respiratory distress, tachycardia, elevated blood pressure, acute or worsening pulmonary oedema and evidence of positive fluid balance; or transfusion-associated dyspnoea, not meeting proposed diagnostic criteria for TRALI or TACO.22 TRALI is one of the leading causes of severe post-transfusion morbidity and acute mortality.23 However, TRALI is thought to be underreported and under-recognised in all patients but particularly in preterm infants.24 The incidence of TRALI has been reported to vary from 0.08 to 15% of adult patients receiving a transfusion,23 but the incidence in neonates is unknown. A two-event hypothesis has been postulated as central to the pathogenesis of TRALI.26 The first event is the patient’s underlying clinical condition, causing inflammation with priming of the pulmonary neutrophils. The second event is the transfusion product itself, bioactive molecules or antibodies that have accumulated during storage activate primed neutrophils.26 Although TRALI has previously been described in the paediatric population,24 there is a paucity of data in neonates.

BPD is among the most common and serious sequelae of preterm birth. This chronic condition is characterised by the need for supplemental oxygen at 36 corrected weeks, resulting from disrupted alveolar growth. BPD is inherently multifactorial; therefore, there is no consensus on the precise pathogenesis. Interestingly, the incidence of BPD is significantly higher in infants transfused during the neonatal period27 and increases with the total number of transfusions.27 There is growing recognition of the significant contribution of inflammation and oxidative stress to the pathogenesis of BPD, processes similar to those seen with TRALI.28 While rates of post-transfusion lung injury is reported to be as high as 10% in preterm neonates, failure to take into account very high rates of respiratory instability in this population limits the interpretation of these data.29 A retrospective study of 108 newborns transfused a total of 373 times reported a rate of post-transfusion respiratory deterioration in 8.3%.29 However, a meta-analysis of randomised trials comparing restrictive versus liberal transfusion thresholds fails to demonstrate increased incidence of chronic lung disease, with greater transfusion exposure.11 Both observational and randomised studies are limited by the lack of an accepted definition of TRALI in the preterm newborn and the difficulty in differentiating the contribution of underlying lung pathology versus a transfusion-related effect. As a result, prospective studies with significantly larger sample sizes are required to definitely prove or disprove any association.10

Retinopathy of prematurity

Red cell exposure and the development of ROP remain controversial. Several observational studies support an association with RBC exposure and ROP.31–33 Most report a significant association between both gestational age and transfusion frequency with the development of ROP.31,33 Others report a significant association between transfusion exposure and ROP following adjustment for
gestational age. Hesse et al. adjusted for gestational age and duration of mechanical ventilation in a population of ELBW newborns and found that the relative risk for ROP development was 6.4 (95% confidence interval 1.2–33.4) in those who received 16–45 mL/kg of RBCs, doubling for those receiving >45 mL/kg over their admission, an effect proposed to be associated with products of iron metabolism. Conversely, the sole randomised study of transfusion exposure with the incidence of ROP as the primary outcome failed to demonstrate significantly different risk for ROP in those babies allocated to the liberal transfusion threshold arm. However, this study is confounded by high attrition rate overall, variability in haematocrit in the liberal transfusion arm and a very small sample size for a study with a clinical outcome. Nonetheless, as seen for BPD, a meta-analysis of randomised trials comparing restrictive versus liberal transfusion thresholds fails to demonstrate increased incidence of ROP, with greater transfusion exposure.

Unlike NEC and BPD, the underlying pathogenesis of ROP is not primarily a consequence of inflammation. Exposure to biologically active substances from the transfusion pack may occur in redox reactions and oxidative damage, but whether these transient effects are significant enough to contribute to the pathogenesis of ROP remains unknown. Other potential mechanisms linking transfusions with ROP development include transfusion exposure lowering the ratio of HbF to adult Hb, shifting the oxygen dissociation curve to the right and increasing oxygen availability to the developing retina. Finally, anaemia itself may result in retinal hypoxia and, subsequently, retinal neovascularisation.

**Intraventricular haemorrhage**

An association with RBC transfusion exposure was first reported in 1998 with each subsequent RBC transfusion in the first week of life in ELBW newborns doubling the relative risk of severe IVH. Furthermore, in a retrospective study of 417 newborns already diagnosed with a grade 1 IVH, subsequent transfusion exposure, along with lower gestational age, was the most significant predictive factor in the 46 infants who subsequently developed grade III or IV haemorrhage. Similarly, in a prospective cohort of 4283 preterm newborns, those who received a transfusion had a 64% greater chance of diagnosis with grade III/IV haemorrhage. Following the implementation of a transfusion compliance programme, which was associated with a 23% reduction in the number of very low birthweight newborns transfused, the incidence of severe IVH similarly decreased from 17 to 8%. However, the interpretation of results from retrospective studies is limited by their failure to adequately deal with confounding variables and the inherently greater risk of bias. For instance, the association between transfusion and IVH is as likely related to the clinical reason the transfusion was ordered versus the transfusion itself. If the relationship between RBC transfusion exposure and subsequent IVH is truly causal, then any intervention that reduces or prevents the need for early RBC transfusion should be associated with a reduction in IVH occurrence. One such approach is delayed cord clamping, resulting in both reduced transfusion need and lower incidence of IVH. However, the results of the largest study investigating this intervention on neonatal outcome failed to show a significant reduction in IVH rates in the delayed cord-clamping group. As such, the latest meta-analysis of available studies (including the Australian Placental Transfusion Study (APTS) Collaborative Group randomised trial) reports no difference in the incidence of IVH despite a 10% reduction in transfusion exposure in newborns randomised to delayed cord clamping. Nonetheless, the contribution of transfusion exposure to the development of IVH is biologically plausible and likely only one part of the multifactorial pathogenesis of this neonatal morbidity along with environmental, developmental and genetic factors.

**Summary**

So, does RBC transfusion contribute to the development of neonatal morbidity? The majority of data supporting an association between RBC transfusion and adverse outcome comes from small retrospective and observational studies. Consequently, it is important to acknowledge the limitations of much of the primary evidence. This includes the failure to recognise confounding variables and limitations of the studies due to their susceptibility to bias. Therefore, while retrospective investigations may generate hypotheses and report associations, they rarely conclusively prove a causal relationship.

The most recent systematic review investigated clinical adverse effects and associations attributable to RBC exposure in the preterm newborn, including 61 randomised and non-randomised trials. Authors concluded that there was no evidence that mortality risk was greater between liberal or restrictive transfusion thresholds. However, Keir et al. cautioned that many studies were likely confounded by indication bias, with the more critically ill infants more likely to receive RBC transfusion. While approximately one-third of identified studies were randomised, the sample sizes in many were considered inadequate to address harm. Importantly, they conclude that there is a ‘pressing need for larger studies with clear definitions of adverse events to be conducted prospectively, so that uncertainty about the safety of transfusion can be addressed in a population of recipients characterised by prematurity and relative immunologic immaturity.’

**Does a Common Pathway Link RBC Exposure with Adverse Outcome?**

Numerous preclinical studies demonstrate that RBC products can directly modulate immune cell function. This may represent a common pathway linking RBC exposure to adverse outcomes, a process termed transfusion-related immunomodulation (TRIM). TRIM is proposed to be a ‘two-insult’ process. The immune system of the transfusion recipient is initially primed by an antecedent illness. The second insult is exposure to biological response mediators (BRMs) passively transfused with RBCs resulting in an excess inflammatory response coupled with immune suppression. This may be particularly relevant for the critically ill preterm population, increasing the risk of post-transfusion adverse outcomes. TRIM is thought to involve several processes, including priming of white blood cells (WBC), cytokine release and subsequent downstream effects including enhanced neutrophil chemotaxis and monocyte/macrophage activation. Immunosuppressive effects involve the suppression of lymphocyte proliferation, alterations in T lymphocyte ratios, impaired
natural killer cell function and decreased macrophage phagocytic function.49

The BRMs implicated in the second ‘hit’ include donor antibodies, bioactive lipids, free Hb, extracellular vesicles and WBC-derived mediators such as cytokines, which accumulate during storage.49 During normal storage, RBCs undergo structural and biochemical changes, resulting in outer membrane ‘shedding’, exacerbated as storage time increases.31 This has led to the adoption of new processing and storage approaches attempting to mitigate TRIM. Animal studies have reported that exposure to ‘fresh’ RBCs diminishes a pro-inflammatory response in the recipient.52 Likewise, small observational studies in adult populations demonstrate decreased inflammation and transfusion-related complications following transfusion with RBCs stored for shorter durations.7,55 However, recent meta-analyses of randomised trials in both adult paediatric and neonatal populations failed to show any benefit.53,54

The role of WBCs and/or soluble WBC-derived mediators in the development of several transfusion-associated consequences has led to the world-wide adoption of pre-storage leukoreduction. Randomised studies in cardiac surgery reported that transfusions with leukoreduced RBCs are associated with a reduced incidence of nosocomial infection in surgical patients53 and attenuated post-surgery systemic inflammatory response in patients.56 A large neonatal study reported that a transfusion of leukoreduced RBCs resulted in reduced rates of BPD, IVH and ROP57 but not NICU mortality. However, transfusion with leukoreduced RBCs still results in immune activation in the preterm newborn.14 This raises the following question: are there other modifications to RBC processing that may further reduce TRIM and the incidence of transfusion-related morbidities in preterm newborns?

Epidemiological and observational evidence supports an association between the transfusion of washed allogeneic RBCs and fewer post-transfusion cardiopulmonary complications,58 reduced transfusion-related adverse immunological effects and improved survival in patients with acute leukaemia.59 Data are available from a further two RCTs, which compare the effect of washed versus unwashed RBC transfusions on clinical and biochemical outcomes.60,61 Transfusion of washed allogeneic RBCs is associated with improved survival of adult patients with acute leukaemia60 and reduced transfusion related pro-inflammatory cytokine production in paediatric cardiac surgical patients (median age of 7 months).61 Implementing the use of washed RBCs has several limitations, including a restricted shelf life and extensive quality control, all resulting in an increase in manufacturing time and cost. Therefore, a single study of 36 newborns provides insufficient evidence to support or refute the clinical and economic value of transfusion of washed RBCs in this population.61

Conclusion

There is increasing awareness that the transfusion of blood products may be an independent predictor of adverse outcome; however, uncertainty remains regarding the risk of RBC transfusion exposure to the preterm neonate. In addition, few studies in this high-risk population have adequately investigated the mechanism proposed to link RBC transfusion exposure and adverse outcomes. It may be a direct response to donor RBCs, time-dependent accumulation of bioactive substances released from RBCs in the supernatant, a consequence or artificial additives designed to promote shelf life or a combination of these. Yet developments in transfusion medicine designed to specifically address these issues do not completely mitigate TRIM and its consequences. Therefore, it is imperative that future neonatal studies are prospective, use clear definitions of adverse events and are sufficiently powered to conclusively prove or disprove any association with RBC transfusion. We argue they should also consider the knowledge gap in our understanding of the underlying mechanistic pathways, potentially explaining an association between RBC transfusion exposure and neonatal mortality and significant neonatal morbidity.

Multiple Choice Questions

1 What clinical factor is not included in a transfusion algorithm:
   a) Haemoglobin concentration
   b) Respiratory support
   c) Haematocrit
   d) Weight
   e) Post-natal age

Answer: d. Haemoglobin concentration is used as a parameter in the PINT study conducted by Kirpalani et al., whereas the randomised trial conducted by Bell et al. used haematocrit as their transfusion guideline.

2 There is growing evidence of an association between transfusion exposure and neonatal morbidity and mortality. Which of the following statements is true?
   a) Exposure to a RBC transfusion in the first 28 days of life is not associated with intra-hospital mortality.
   b) Enteral feeding at the time of RBC transfusion is associated with increased postprandial blood flow and an increased risk of transfusion-associated necrotising enterocolitis.
   c) The association between transfusion and retinopathy of prematurity may be explained by a shift in the oxygen dissociation curve to the left, changing oxygen availability.
   d) Delayed cord clamping reduces the requirement for transfusion, incidence of major intraventricular haemorrhage and neonatal mortality.
   e) Transfusion-associated lung injury (TRALI) is the most common cause of post-transfusion morbidity occurring in up to 8% of high-risk transfused patients.

Answer: c. While TRALI is the most common identified transfusion morbidity in both the adult and paediatric populations, it is still thought to be underreported and recognised in the preterm population.

3 Transfusion-related immunomodulation (TRIM) is proposed to be a ‘two-insult’ process, with the recipient’s immune system initially primed by an antecedent illness. The processes implicated in the second ‘hit’ of TRIM include:
   a) White blood cell (WBC)-derived mediators
   b) Increased macrophage function
   c) Proliferation of natural killer (NK) cells
   d) Decrease in free haemoglobin
   e) Lymphocyte proliferation
Answer: a. WBC-derived mediators are thought to accumulate as the age of the pack increases. The immunosuppressive responses involved in the second hit are decreases in macrophage function, NK cells and lymphocytes.

References


58 Blumberg N, Heal JM, Gettings KF et al. An association between decreased cardiopulmonary complications (transfusion-related acute lung injury and transfusion-associated circulatory overload) and implementation of universal leukoreduction of blood transfusions. Transfusion 2010; 50: 2738–44.

